THE ROLE OF CERAMIDES IN DIABETES AND CARDIOVASCULAR DISEASE

EDITED BY: Scott A. Summers and William Louis Holland
PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-736-9
DOI 10.3389/978-2-88966-736-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

THE ROLE OF CERAMIDES IN DIABETES AND CARDIOVASCULAR DISEASE

Topic Editors:
Scott A. Summers, The University of Utah, United States
William Louis Holland, The University of Utah, United States

Citation: Summers, S. A., Holland, W. L., eds. (2021). The Role of Ceramides in Diabetes and Cardiovascular Disease. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88966-736-9

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 12 March 2021
doi: 10.3389/fendo.2021.667885

Editorial: The Role of Ceramides in Diabetes and Cardiovascular Disease

Scott A. Summers *

Department of Nutrition and Integrative Physiology, University of Utah, Salt Lake City, UT, United States

Keywords: ceramides, lipotoxicity, sphingolipids, diabetes, heart disease

Editorial on the Research Topic

The Role of Ceramides in Diabetes and Cardiovascular Disease

INTRODUCTION

The lipotoxicity hypothesis, first described by the late Roger Unger nearly 30 years ago (1, 2), posits that the excessive delivery of fatty acids to the heart, vasculature, liver, muscle, pancreas and adipose tissue gives rise to the tissue damage that underlies diabetes and cardiovascular disease. Under lipotoxic conditions, the quantity of fatty acids delivered to these tissues exceeds their energy needs and their storage capacity, leading to the aberrant formation of lipid metabolites that impair tissue function. This Research Topic of *Frontiers in Endocrinology* evaluates the role of ceramides and its metabolites in the tissue damage that drives these cardiometabolic disorders.

Ceramides are products of a biosynthetic pathway that adds fatty acids to a sphingoid scaffold derived from palmitoyl-CoA and serine (3). Quantitatively, ceramides and other sphingolipids are minor constituents of the cellular lipidome; they are present at far lower levels than the glycerolipids that comprise the bulk of lipid droplets and cellular membranes (4). The ceramides presumably accumulate when the glycerolipid pathway is saturated, and the residual fatty acids become available as substrates for the enzymes that produce sphingolipids.

In the first article of the series, entitled ***"Too Much of a Good Thing? An Evolutionary Theory to Explain the Role of Ceramides in NAFLD,"*** Poss and Summers present a conceptual framework that explains the evolutionary basis of the ceramide actions that elicit tissue dysfunction. We speculate that—at an early point in evolution—ceramides conferred upon cells and organisms a survival advantage by protecting membranes from detergent-like fatty acids. They do this by altering membrane properties and by changing metabolic programs; these adaptations facilitate the uptake and storage of fatty acids while decreasing utilization of glucose (**Figure 1**). As ceramide levels increase further, they induce apoptosis and fibrosis, which helps protect organisms from fragile cells that have become compromised by fatty acid overload. While these actions may provide a short-term advantage, the chronic changes in metabolism (e.g., decreased glucose utilization and increased fat deposition) elicit the insulin resistance and dyslipidemia that are early signs of disease progression. Moreover, the increased susceptibility to cell death elicits the terminal organ damage that drives cardiometabolic disease. This set of conserved actions would thus explain many of the key features of the diseases associated with dyslipidemia and obesity.

The other articles in this series provide evidentiary support for this hypothesis, describing in careful and thoughtful detail the means by which ceramides alter tissue function. Moreover, they highlight the numerous instances where inhibition of the enzymes required for ceramide biosynthesis ameliorate diabetes and heart disease.

OPEN ACCESS

Edited and reviewed by:
Jared Rutter,
The University of Utah, United States

***Correspondence:**
Scott A. Summers
scott.a.summers@health.utah.edu

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *15 February 2021*
Accepted: *17 February 2021*
Published: *12 March 2021*

Citation:
Summers SA (2021) Editorial: The Role of Ceramides in Diabetes and Cardiovascular Disease. Front. Endocrinol. 12:667885. doi: 10.3389/fendo.2021.667885

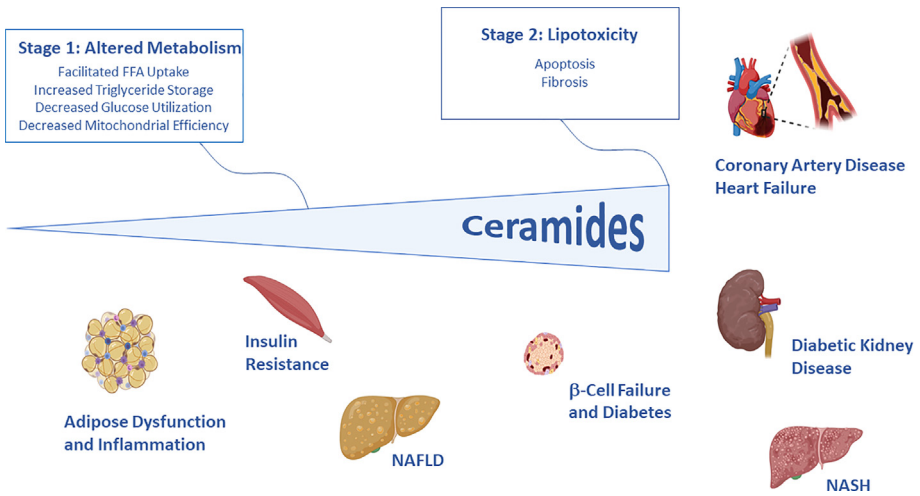

FIGURE 1 | Schematic depicting the two stages of ceramide-driven lipotoxicity. In initial stages, ceramides serve as signals of lipid excess that alter metabolic programs, enabling storage of fat and decreasing utilization of glucose. In the latter stages, ceramides trigger apoptosis and fibrosis, protecting the organism from compromised cells. These cellular events give rise to the key features of diabetes and cardiovascular disease. Image generated using Biorender.

In the article ***"Sphingolipid Metabolism and Signaling in Skeletal Muscle: From Physiology to Physiopathology,"*** Tan-Chen et al. discuss the ceramide actions that decrease insulin-stimulated glucose utilization in muscle. They also review the numerous interventional studies in rodents that reveal that lowering ceramides ameliorates insulin resistance.

In "***Ceramides in Adipose Tissue,"*** Li et al. discuss the studies indicating that ceramides inhibit glucose uptake, enhance lipid storage, and decrease mitochondrial efficiency of the adipose depots. Interestingly, they also note that ceramides inhibit phosphorylation of hormone-sensitive lipase, providing another mechanism by which ceramides work to lower cellular FFA levels.

In "***Sphingolipids in the Heart: From Cradle to Grave***," Kovilakath et al. discuss the contribution of sphingolipids to myocardial lipotoxicity and coronary artery disease, two of the key pathogenic features of major adverse cardiac events. The authors describe the key studies indicating that ceramides have deleterious actions on heart function, while its downstream metabolite sphingosine 1-phosphate is cardioprotective.

In ***"The Role of Ceramides in Diabetes and Cardiovascular Disease Regulation of Ceramides by Adipokines,"*** Field et al. review how the broad swath of anti-diabetic and cardioprotective actions of adiponectin—including its prevention of apoptosis of cardiomyocytes and pancreatic β-cells—result from the acceleration of ceramide degradation. Remarkably, they and others have found that the adiponectin receptors are ceramidases that are activated by ligand binding (4, 5).

Beyond these mechanistic studies on the tissue-specific roles of ceramides, additional articles discuss the potential therapeutic value of these studies on ceramides.

In their article ***"Ceramides and Ceramide Scores: Clinical Applications for Cardiometabolic Risk Stratification,"*** Hilvo et al. discuss the numerous studies showing that clinical risk scores based on serum ceramides demarcate patients that are at risk for cardiometabolic disorders.

Lastly, Raichur discusses work on ceramide-targeted therapies, concluding that "***Ceramide Synthases Are Attractive Drug Targets for Treating Metabolic Diseases.***"

Collectively, these studies describe the exciting body of evidence indicating that ceramides are drivers of diabetes and heart disease. They delineate the novel mechanisms by which ceramides influence metabolism and increase susceptibility to apoptosis and fibrosis. Moreover, they reveal the exciting potential of therapeutic strategies that block ceramide accumulation, which could emerge as treatments for a wide range of cardiometabolic pathologies.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* (1995) 44:863–70. doi: 10.2337/diabetes.44.8.863
2. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U.S.A.* (1994) 91:10878–82. doi: 10.1073/pnas.91.23.10878
3. Merrill AHJr. De novo sphingolipid biosynthesis: a necessary, but dangerous, pathway. *J Biol Chem* (2002) 277:25843–6. doi: 10.1074/jbc.R200009200
4. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* (2011) 17:55–63. doi: 10.1038/nm.2277

5. Vasiliauskaite-Brooks I, Sounier R, Rochaix P, Bellot G, Fortier M, Hoh F, et al. Structural insights into adiponectin receptors suggest ceramidase activity. *Nature* (2017) 544:120–3. doi: 10.1038/nature21714

Conflict of Interest: SS is a co-founder of Centaurus Therapeutics.

frontiers in Endocrinology

MINI REVIEW
published: 18 June 2020
doi: 10.3389/fendo.2020.00407

Ceramides in Adipose Tissue

Ying Li[1], *Chad Lamar Talbot*[1] *and Bhagirath Chaurasia*[1,2]*

[1] Department of Nutrition and Integrative Physiology, The Diabetes and Metabolism Research Center, University of Utah, Salt Lake City, UT, United States, [2] Division of Endocrinology, Department of Internal Medicine, Fraternal Order of Eagles Diabetes Research Center, Carver College of Medicine, University of Iowa, Iowa City, IA, United States

Adipose tissue is a key nutrient-sensing depot that regulates excess energy storage and consumption. Adipocytes, the key components of the adipose tissue, have unique ability to store excess energy in the form of triglycerides, sense systemic energy demands, and secrete factors (lipids, peptides, cytokines, and adipokines) to regulate other metabolic tissues. The presence of various types of adipocytes (white, brown, and beige) characterized by the number/size of lipid droplets, mitochondrial density, and thermogenic capacity, further highlights how intricate is the communication of these cell-types with other metabolic tissues to sense nutrients. In obesity the inherent capacity of adipose tissue to store and sense nutrients is compromised, causing spillover of the intermediate lipid metabolites into circulation and resulting in their ectopic deposition in tissues not suitable for lipid storage, a phenomenon known as lipotoxicity. This results in a spectrum of cellular dysfunction, that underlies various metabolic diseases. Of the numerous lipid classes implicated in eliciting lipotoxicity, sphingolipid: ceramides are among the most deleterious as they modulate signaling pathways involved in regulating glucose metabolism, triglyceride synthesis, apoptosis, and fibrosis. Notably, recent experimental studies have strongly implicated ceramides in the development of numerous metabolic diseases such as insulin resistance, diabetes, cardiomyopathy, hepatic-steatosis, and atherosclerosis. Herein we discuss and summarizes recent findings that implicate ceramides as a key contributor to adipocyte dysfunction underlying metabolic diseases and how depletion of ceramides can be exploited to improve metabolic health.

Keywords: metabolism, adipocytes, diabetes, insulin, ceramides

OPEN ACCESS

Edited by:
Jean-François Tanti, INSERM U1065 Centre Méditerranéen de Médecine Moléculaire, France

Reviewed by:
Eric Hajduch, Institut National de la Santé et de la Recherche Médicale (INSERM), France
Emilia Sokolowska, Medical University of Bialystok, Poland
Xavier Prieur, INSERM U1087 L'unité de recherche de l'institut du thorax, France

***Correspondence:**
Bhagirath Chaurasia
bchaurasia@healthcare.uiowa.edu

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *24 April 2020*
Accepted: *20 May 2020*
Published: *18 June 2020*

Citation:
Li Y, Talbot CL and Chaurasia B (2020) Ceramides in Adipose Tissue. Front. Endocrinol. 11:407. doi: 10.3389/fendo.2020.00407

INTRODUCTION

Metabolic diseases represent a significant health burden that impacts millions of households worldwide. According to the World Health Organization (WHO) global report, ∼422 million adults were living with diabetes in 2014 (1), and that cardiovascular diseases account for 31% mortality worldwide (2). The epidemic of obesity is one of the major causes of these metabolic disorders. Accumulation of neutral lipids, such as triglycerides, in key insulin target tissues, has been postulated to inhibit metabolic functions, however, they are less likely to be deleterious. On the other hand, there is growing evidence for the involvement of other lipid metabolites in inducing this metabolic outburst (3–5). Notably, recent studies suggest that the accumulation of sphingolipids, namely ceramides and it's metabolites, play essential roles in the development of insulin resistance in tissues such as skeletal muscle, liver and, adipose tissue in obese rodents, and humans (6–20). In mice, blocking ceramide production improves insulin sensitivity, prevents β-cell failure, resolves hepatic steatosis, hypertriglyceridemia, and prevents atherosclerosis, and heart failure (6–22).

Enhancing ceramide degradation also endows these metabolic benefits, and adiponectin exerts their antidiabetic, cardioprotective, and insulin-sensitizing actions through activating its receptors, which are ligand-activated ceramidases (23). In humans, ceramides predict the occurrence of major adverse cardiac events, such that the numerous clinics have started to offer serum ceramide tests as prognostic measures of cardiovascular risk (24, 25).

In this review, we intend to provide a perspective on ceramides and ceramide metabolites in the maintenance of adipose tissue homeostasis and how adipose tissue ceramides contribute to the development of metabolic diseases.

CLINICAL ASSOCIATION OF ADIPOSE CERAMIDE CONTENT WITH OBESITY AND INSULIN RESISTANCE

As it is apparent in this series, numerous studies have shown that manipulation of ceramide synthesis or degradation pathways in rodents through pharmacologic and genetic interventions have profound effects on insulin sensitivity (6, 12, 26, 27). Despite the lack of these interventions in humans, clinical studies highlight a strong association between serum/plasma ceramides and adverse outcomes in metabolic and cardiovascular diseases (28–33). In adipose tissue, ceramide content has been associated with the development of insulin resistance in numerous human studies consisting of small cohorts. In one of these studies, Yki-Jävinen et al. profiled adipose tissue from 20 individuals of Finish descent and demonstrated that adipose tissue ceramides are elevated in individuals with insulin resistance independent of obesity (34). Consistent with this observation, we found that in a small cohort of individuals with Asian descent (18 individuals) various ceramide species are elevated in the adipose tissues of subjects with type 2 diabetes independent of obesity (35). Furthermore, Brüning et al. profiled 20 individuals of European descent and found that various ceramide species are elevated in the adipose tissues of obese individuals (20). Brüning et al. went on to further demonstrate that in a larger cohort of similar descent (439 individuals), mRNA expression of *CERS6* in adipose tissue positively correlates with Body Mass Index, body fat content, and hyperglycemia while negatively correlating with glucose infusion rate during euglycemic-hyperinsulinemic clamps (20).

REGULATION OF CERAMIDE SYNTHESIS IN ADIPOSE TISSUE

Overnutrition is the major cause of obesity that results in the increased supply of macronutrients into the living body. They are digested, absorbed, and broken down into numerous small molecules like palmitate and serine, which are the key determinant of the elevated ceramide levels (4). Moreover, consumption of high levels of saturated fat but not unsaturated fat increases ceramide accumulation (36), while limiting the cellular serine pool reduces ceramide accumulation (37). Palmitate in addition to its role as a major substrate for ceramide synthesis also induces expression of genes involved in sphingolipid biosynthesis and metabolism (38). Although, palmitate increases ceramide accumulation in most cell types, in cultured 3T3-L1 adipocytes they do not stimulate ceramide accumulation (39). In contrast, numerous studies in rodents and humans have demonstrated an increased accumulation of ceramides in adipose tissue under conditions of nutrient excess (i.e., obesity) (20, 34, 35). Although it is not clear how nutrient excess or lipotoxic conditions increases adipose tissue ceramide content; it could potentially be due to increased trafficking of ceramides from circulation into the adipocyte. Despite this, recent studies implicate ceramides in adipose tissue act as an important secondary messenger that sense changes in nutrient status and regulates the whole-body metabolic homeostasis (35, 40). To optimally regulate nutrient homeostasis, ceramides level in the adipose tissue is tightly regulated by various systemic or intracellular signaling pathways that include a variety of hormonal factors associated with obesity and metabolic diseases independent of the dietary content (4, 6, 23, 35, 38). Here we summarize these key hormonal regulators of adipose tissue ceramide content (**Figure 1**).

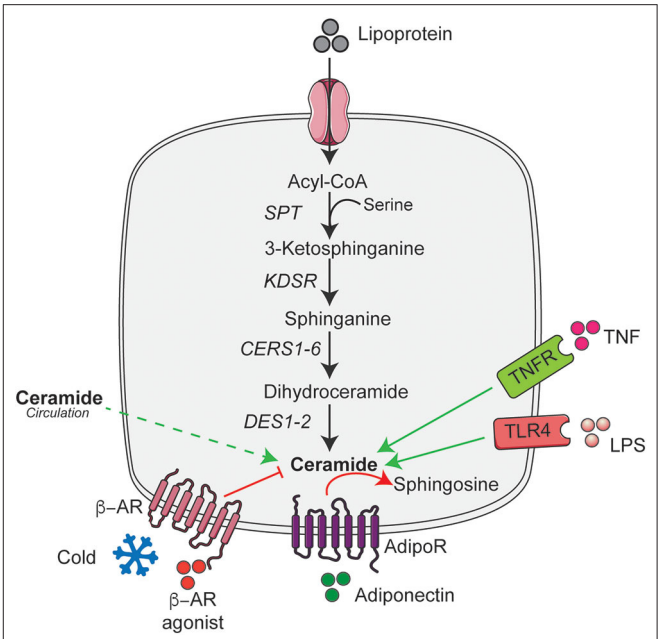

FIGURE 1 | *De novo* ceramide synthesis pathway and its hormonal regulators in adipose tissue. Schematic of the *de novo* ceramide synthesis pathway and regulators of ceramide synthesis discussed in the text. Black arrow indicates the key steps in *de novo* ceramide synthesis; solid green arrow indicates the factors that stimulate ceramide synthesis; dashed green arrow indicate the putative uptake and red arrows and lines indicate factors that reduce or metabolize ceramide. AdipoR, Adiponectin receptor; ASAH1, Acid ceramidase 1; β-AR, β-adrenergic receptor; CERS1-6, Ceramide synthase 1-6; DES1-2, Dihydroceramide desaturase 1-2; KDSR, 3-ketodihydrosphingosine reductase; LPS, Lipopolysaccharide; SPT, Serine palmitoyltransferase; TLR4, Toll-like receptor-4; TNFR, Tumor necrosis factor receptor. This figure was drawn using the Servier Art.

Ceramide and Inflammation

Adipose tissue inflammation is a hallmark of obesity characterized by the increased recruitment and activation

of macrophages to adipose tissue (41). This results in increased expression and secretion of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukins, and chemokines which increase levels of ceramides without affecting glycerolipids such as diacylglycerol (42). The excess nutrient supply in conjunction with various triggers of inflammation is a key determinant of ceramide biosynthesis and exhibits a tight association with ceramides and insulin resistance (42, 43). Moreover, saturated fatty acids that accrue in obesity induce the activation of toll-like receptors-4 (TLR4), resulting in increased inflammation and augmented mRNA expression of various enzymes involved in *de novo* ceramide biosynthesis (44, 45). Mechanistically, saturated fatty acids do not bind directly to the TLR4 receptors, but require TLR-dependent priming to induce inflammatory signaling (46). In contrast, mice lacking systemic TLR4 exhibit reduced accumulation of ceramides in response to saturated fatty acids in numerous tissues (38). These data indicate TLR4 is an essential component linking saturated fats to modulation of ceramide synthesis (47–49). However, future studies are warranted to delineate if these consequences were due to autonomous effects in adipocytes, immune cells or due to cross-talk among these cells. Interestingly in one of the earlier seminal studies, inflammatory cytokine TNF-α was also found to induce ceramide accumulation via coordinated changes in the ceramide generating (e.g., SPT) and metabolizing enzymes (e.g., sphingomyelinase) that induce hydrolysis of sphingomyelin (50–52). Mechanistically, ceramides elicit inflammation-induced insulin resistance at least in part by activation of the Nod-like receptor (Nlrp3) inflammasome that induce caspase-1 cleavage in macrophages and adipose tissue, which thereon inhibits Akt/PKB activation and results in the development of insulin resistance (53).

Ceramides and β-adrenergic Agonists

The induction of the thermogenic program as a means to increase energy expenditure has gained notoriety in recent years given its high therapeutic potential to combat obesity. Cold exposure or β-adrenergic agonists (e.g., norepinephrine, isoproterenol, etc.), that activates β-adrenergic receptors in adipocytes, increases the thermogenic capacity of adipocytes by elevating the expression of thermogenic genes such as *Ucp1*, *Pgc1a*, and *Prdm16* (54). We recently found that exposing mice to cold temperatures for 5-days reduced ceramides, dihydroceramides, and sphinganine in the adipose tissue while also reduced expression of the ceramide biosynthetic genes *Sptlc2* and *CerS6* (35). We further demonstrated that systemic inhibition of ceramide biosynthesis or adipocyte-specific ablation of *Sptlc2* increased the recruitment of beige adipocytes in the adipose tissue and improved mitochondrial function (35).

In parallel with these findings, Jiang et al. demonstrated that ceramides inhibit the browning of beige adipocytes cultured *ex vivo*, suggesting that endogenous ceramides could be autonomous regulators of adipocyte function (55). Indeed, using a similar *ex vivo* assay, we found that pharmacological manipulation of endogenous ceramides content modulates beige adipocytes' function (35). More recently, using a newly developed flux assay to monitor rates of ceramide production, we found that β-adrenergic agonists rapidly and completely shut down ceramide biosynthesis in primary adipocytes by inhibiting activation of hormone sensitive lipase (HSL) (6). Collectively, the work shows that the β-adrenergic's actions on the adipocyte were cell-autonomous and driven by ceramides, but not other sphingolipids.

Ceramides and Adiponectin

Adiponectin, the antidiabetic and cardioprotective adipokine is predominantly produced and secreted from metabolically healthy adipocytes and regulates glucose and lipid homeostasis by exerting pleiotropic effects on numerous tissues such as the liver, pancreatic β-cells, kidney, heart, bone, and immune cells (56–59). Mechanistically, these effects of adiponectin were initially thought to be mediated by AMPK, a serine/threonine kinase (59). Owing to the sequence homology between adiponectin receptors (AdipoR) and other progestin and adipoQ receptor family members (PAQR) with ceramidases, and ability of human AdipoRs to promote ceramidase activity in ceramidase deficient yeast, the Scherer group hypothesized that the adiponectin might elicit its broad spectrum of actions by deacylating ceramides (23). Herein, the Scherer group recently demonstrated that the activation of adiponectin receptors AdipoR1 and 2 via adiponectin stimulates deacylation of ceramides yielding sphingosine that can be converted to sphingosine 1-phosphate (S1P) by sphingosine kinase, an effect that is dependent on a critical residue in the predicted ceramidase motif in AdipoRs (23). In a subsequent study, the Scherer group found that overexpression of AdipoRs in the adipose tissue or liver is sufficient to reduce ceramide accumulation in these depots owing to their increased ceramidase activity (60). Conversely, acute inhibition of adiponectin in adipose tissue increased accumulation of the most abundant ceramide species ($C_{16:0}$) in adipose tissue and reduced sphingosine content, further highlighting the presence of ceramidase activity in AdipoRs that requires adiponectin (61). Consistent with this, Tanabe et al. initially showed that crystal structures of human AdipoRs possess a hydrophobic binding pocket potentially resembling that of the ceramidases (62). Using x-ray crystallography, Vasiliauskaite-Brooks et al. recently showed that purified adiponectin receptors possess inherent ceramidase enzymatic activity (63). They further went on to solve the crystal structure of adiponectin receptors in the presence of ceramide, obtaining a final entity bound to a fatty acid product of the reaction (63). From the clinical perspective, increased ceramide accumulation in plasma and tissues is inversely correlated with adiponectin in obese and insulin resistant subjects (64, 65).

LOWERING CERAMIDE ACCUMULATION IMPROVES ADIPOCYTE FUNCTION

We recently found that pharmacological inhibition of ceramide biosynthesis in obese mice, using myriocin (a selective inhibitor of SPT, the first rate-limiting enzyme in the ceramide synthetic pathway), induced profound changes in the adipose tissue. Importantly, this intervention significantly reduced adipocyte

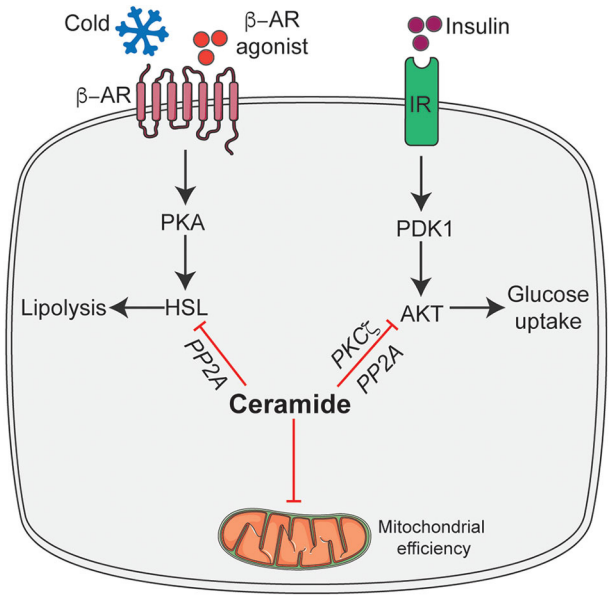

FIGURE 2 | Schematic of ceramide dependent molecular mechanisms that modulate adipocyte function. Ceramides gauge adipocyte energy stores via the following mechanisms: inhibit insulin-stimulated Akt/PKB thus inhibiting glucose uptake and metabolism; inhibit HSL activation in response to β-adrenergic receptor activation thereby attenuating lipolysis and release of free fatty acid and inhibit mitochondrial efficiency. β-AR, β-adrenergic receptor; IR, Insulin receptor; HSL, Hormone sensitive lipase; Akt/PKB, Protein kinase B; PDK1, phosphoinositide dependent kinase-1; PKA, Protein kinase A; PP2A, Protein phosphatase 2A; PKCζ, protein kinase Cζ. This figure was drawn using the Servier Art.

size, increased recruitment of M2 macrophages, and elevated numbers of brown/beige adipocytes in white adipose tissue, particularly in the subcutaneous depot (35). We further found that the effects observed following myriocin treatment could be recapitulated by ablation of *Sptlc2,* specifically in the adipocyte, including the improvement in insulin sensitivity and glucose tolerance, resolution of hepatic steatosis, increased recruitment of M2 macrophages, recruitment of beige adipocytes in the adipose tissue, and improvement in mitochondrial respiration (35). Interestingly, these adipocyte-specific but not macrophage-specific changes were sufficient to increase whole-body energy expenditure and resolve hepatic steatosis. The increased recruitment of M2 macrophages into adipose tissue accompanied by *Sptlc2* inhibition in the adipocyte, further highlight the cross-talk between adipocytes and macrophages to maintain adipose tissue homeostasis. However, future studies determining how adipocyte ceramides modulate M2 macrophages recruitment either via expression and secretion of an adipokine/cytokine or ceramide are warranted. In a subsequent study, we demonstrated that these newly identified ceramide effects were driven in part by PP2A dependent inhibition of hormone sensitive lipase (HSL) (6) (**Figure 2**). Based on these findings, we proposed that ceramides act as nutrient signals that direct the adipocyte into a diminished metabolic state, rather than an active thermogenic state.

More recently, we found that inhibition of DES1 in adipose tissue improved glucose metabolism and resolved hepatic steatosis. Interestingly, these observed improvements were independent of browning/beiging of adipose tissue (6). Using an alternative approach to selectively reduce adipose ceramides by overexpressing ASAH1 in adipose tissue, Scherer et al. found that ceramide reduction, as early as within 3-days, resolved hepatic steatosis and improved glucose tolerance (40). Again, these effects were shown to be independent of adipose tissue browning/beiging as there were no changes observed in body weight (40).

Recent papers from the two independent laboratories found that ablation of either *Sptlc1* or *2,* respectively, in adipose tissue impaired adipose differentiation and exhibited lipodystrophy (66, 67). These differentiated effects between the two studies might arise due to the difference in animal models that were used. Specifically, the aforementioned studies used an adiponectin-Cre-recombinase line from Jackson Laboratories (68) that expresses the recombinase earlier in development, compared to the adiponectin-Cre-recombinase line used in our study that is expressed late during adipocyte differentiation and was obtained from Scherer laboratory (69). We hypothesize that this accounts for the differences in phenotypes observed in these studies. In support of this, our studies in primary pre-adipocyte show that myriocin is a potent inhibitor of adipocyte differentiation whereas inhibition of ceramide synthesis in fully differentiated adipocytes promote browning/beiging (35).

C_{16} Ceramide Is the Deleterious Species Causing Adipocyte Dysfunction

To understand which ceramide species account for the adipose tissue dysfunction, Brüning et al. profiled various ceramide species in adipose tissue isolated from rodents (20). They found that C_{16}-ceramides species were highly enriched in adipose tissue, which was supported by the finding of *CerS6,* the enzyme essential for synthesizing C_{16}-ceramides species, robustly elevated in various rodent models of obesity. This finding was reinforced by the fact that *CERS6* expression is dramatically increased in obese individuals (20). Brüning et al. went on to generate mice lacking *CerS6* in brown adipose tissue and demonstrated that these mice exhibited resolution in hepatic steatosis, improved glucose tolerance, and enhanced mitochondrial β-oxidation and energy expenditure (20). These studies further highlight the importance of ceramide accumulation in BAT in regulating systemic metabolic homeostasis. Using the alternate approach, Hoch et al. found that mice lacking *CerS5*, another enzyme that produces C_{16}-ceramides, presented with reduced weight gain and improved systemic metabolic health, including glucose tolerance and white adipose tissue inflammation after high fat diet feeding (70).

CERAMIDE CENTRIC MOLECULAR MECHANISMS THAT IMPAIR ADIPOCYTE FUNCTION

The broad spectrum of changes observed in adipocyte function due to ceramide intervention results from a series of cell-autonomous ceramide action. We hypothesize that these

mechanisms are part of an evolutionarily-conserved pathway that were originally intended to protect cells from excess accumulation of detergent-like fatty acids during times of fuel surplus. Specifically, these activities would reduce mitochondrial efficiency, decrease availability of glucose-and thus increase reliance on fatty acids-for energy production, and block the release of fatty acids from lipid droplets (**Figure 2**).

Ceramides Regulate Adipose Tissue Glucose Uptake and Metabolism

In cultured adipocytes, ceramides inhibit insulin-stimulated glucose uptake by blocking the translocation of GLUT4 (71). Consistent with these initial findings, pharmacological inhibition of ceramide biosynthesis via myriocin, adipose tissue-specific reductions in ceramide accumulation via inhibition of SPT2, and DES1 or overexpression of ASAH1 increases adipose tissue glucose uptake and metabolism (6, 35, 40). This result from ceramide's ability to attenuate activation of Akt/PKB, that is obligate for insulin-stimulated glucose uptake (4). Ceramides regulate Akt/PKB by at least two known mechanisms in numerous cell types: First, ceramides activate atypical protein kinase C (PKCζ) which in turn phosphorylates a key residue in the plekstrin homology domain of Akt/PKB, preventing it from being recruited and activated at the plasma membrane in response to insulin (72–74). The second mechanism involves the dephosphorylation of Akt/PKB by protein phosphatase 2A(PP2A). Ceramides may activate PP2A either directly (75), or indirectly by displacing the PP2A inhibitory protein I2PP2A (76). Inhibition of PP2A, with either inhibitor (e.g., okadaic acid) or by overexpressing the SV40 Small T antigen (which blocks access of PP2A to its substrates), negate the effect of ceramides in Akt/PKB in numerous cell types (**Figure 2**) (77–80).

Of these two known mechanisms, Hajduch et al. demonstrated that in adipocytes the predominant mechanism via which ceramide inhibit Akt/PKB is exclusively mediated by PKCζ (81) as inhibition of PP2A (with okadaic acid) did not prevent ceramide induced insulin action. Moreover, these authors went on to show that adipocytes tend to favor this mechanism due to the preferential sub-localization of ceramide in caveolin enriched membranes (81).

Ceramides Regulate Adipose Tissue Thermogenic Program

The emerging studies, as discussed above, have highlighted the role of adipose ceramide in the modulation of energy homeostasis. We recently found that ceramide actions on energy homeostasis were due to its ability to selectively impair non-shivering thermogenesis by modulating browning/beiging of the adipose tissue (35). Mechanistically, ceramide analogs were shown to attenuate the expression of key thermogenic genes (e.g., *Ucp1*, *Pgc1a*, *Prdm16*, etc.) in beige adipocytes *ex vivo* (35, 55). Conversely, pharmacological intervention that reduced ceramide synthesis in beige adipocytes *ex vivo* increased expression of various thermogenic genes in both rodents and humans. This effect seems to be mediated, at least in part, by ceramides' ability to inhibit lipolysis by blocking the activation of HSL via PP2A (6) (**Figure 2**).

Ceramides Regulate Adipose Tissue Mitochondrial Bioenergetics

Ceramides impair mitochondrial function and respiratory capacity by inhibiting oxidative phosphorylation and promoting mitochondrial fragmentation in numerous cell-types including adipocytes (19, 20, 82) (**Figure 2**). In adipocytes, short-chain ceramide analogs acutely disrupt components of the electron transport chain and β-oxidation (20, 35). Inhibition of genes encoding for SPTLC2, CERS6, and DEGS1 in adipose tissue, results in reductions in adipose tissue ceramide and leads to improvements in mitochondrial energetics (6, 20, 35).

OUTLOOK AND FUTURE DIRECTION

The data described in this review and others in this series, identify ceramides as critical lipid metabolite that modulate adipose tissue function, homeostasis, and contribute to metabolic disease. Moreover, interventions that reduce ceramide synthesis in adipose tissue, delay, or prevent various comorbidities of obesity, such as insulin resistance and liver steatosis. These discoveries, while exciting, raise several essential questions to be answered to validate ceramide as a potential therapeutic target.

First, can additional mechanisms be identified to explain ceramide action? Although initial studies identified a couple of key mechanisms (i.e., regulation of Akt) for ceramide actions, the numerous effects (e.g., regulation of thermogenic program, mitochondrial function) elicited by ceramide seems unlikely to be fully explained solely by PP2A-Akt axis or PKCζ-Akt axis. Therefore, identifying additional molecular mechanisms will be crucial for understanding the roles of ceramides such that the therapeutic strategy could be developed accordingly.

Second, what is the role of immune-cell ceramide content in maintaining adipose tissue homeostasis? How do ceramides regulate these cell populations and how do they interact with adipocytes to gauge nutrient content?

Third, how do β-adrenergic's inhibit ceramide synthesis in adipose tissue? Delineation of the molecular targets downstream of the β-adrenergic receptor may offer additional interventional strategies to target ceramide synthesis.

Fourth, are there additional regulators (nutrients/enzymes) that modulate intracellular ceramide content? The recent development of the ceramide flux assays coupled with secondary biochemical assays might favor the identification of more regulators and lead to additional strategies to safely target ceramide synthesis for its therapeutic use.

Fifth, how various enzymes in sphingolipids synthesis and degradation pathways coordinate to maintain lipid homeostasis, particularly in response to various local and systemic stimuli.

The future research elucidating these important queries holds great promise in not only understanding how ceramides modulate nutrient sensing that underlies metabolic disease processes but also potentially identifying new therapeutic targets to combat metabolic diseases epidemic.

AUTHOR CONTRIBUTIONS

YL and BC concieved and wrote the manuscript with inputs from CT. All authors approved it for publication.

FUNDING

The authors received research support from the National Institutes of Health (DK124326 to BC) and the United States Department of Agriculture (2019-67018-29250 to BC).

REFERENCES

1. World Health Organization. *Global Report on Diabetes*. Geneva: World Health Organization (2016).
2. World Health Organization. *Cardovascular Diseases*. Geneva: World Health Organization (2019).
3. Unger RH. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends Endocrinol Metab*. (2003) 14:398–403. doi: 10.1016/j.tem.2003.09.008
4. Chaurasia B, Summers SA. Ceramides - lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab*. (2015) 26:538–50. doi: 10.1016/j.tem.2015.07.006
5. Petersen MC, Shulman GI. Roles of diacylglycerols and ceramides in hepatic insulin resistance. *Trends Pharmacol Sci*. (2017) 38:649–65. doi: 10.1016/j.tips.2017.04.004
6. Chaurasia B, Tippetts TS, Mayoral Monibas R, Liu J, Li Y, Wang L, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science*. (2019) 365:386–92. doi: 10.1126/science.aav3722
7. Chen TC, Lee RA, Tsai SL, Kanamaluru D, Gray NE, Yiv N, et al. An ANGPTL4-ceramide-protein kinase Czeta axis mediates chronic glucocorticoid exposure-induced hepatic steatosis and hypertriglyceridemia in mice. *J Biol Chem*. (2019) 294:9213–24. doi: 10.1074/jbc.RA118.006259
8. Correnti JM, Juskeviciute E, Swarup A, Hoek JB. Pharmacological ceramide reduction alleviates alcohol-induced steatosis and hepatomegaly in adiponectin knockout mice. *Am J Physiol Gastrointest Liver Physiol*. (2014) 306:G959–73. doi: 10.1152/ajpgi.00395.2013
9. Dekker MJ, Baker C, Naples M, Samsoondar J, Zhang R, Qiu W, et al. Inhibition of sphingolipid synthesis improves dyslipidemia in the diet-induced hamster model of insulin resistance: evidence for the role of sphingosine and sphinganine in hepatic VLDL-apoB100 overproduction. *Atherosclerosis*. (2013) 228:98–109. doi: 10.1016/j.atherosclerosis.2013.01.041
10. Glaros EN, Kim WS, Quinn CM, Jessup W, Rye KA, Garner B. Myriocin slows the progression of established atherosclerotic lesions in apolipoprotein E gene knockout mice. *J Lipid Res*. (2008) 49:324–31. doi: 10.1194/jlr.M700261-JLR200
11. Glaros EN, Kim WS, Wu BJ, Suarna C, Quinn CM, Rye KA, et al. Inhibition of atherosclerosis by the serine palmitoyl transferase inhibitor myriocin is associated with reduced plasma glycosphingolipid concentration. *Biochem Pharmacol*. (2007) 73:1340–6. doi: 10.1016/j.bcp.2006.12.023
12. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab*. (2007) 5:167–79. doi: 10.1016/j.cmet.2007.01.002
13. Ji R, Akashi H, Drosatos K, Liao X, Jiang H, Kennel PJ, et al. Increased *de novo* ceramide synthesis and accumulation in failing myocardium. *JCI Insight*. (2017) 2:e82922. doi: 10.1172/jci.insight.82922
14. Kasumov T, Li L, Li M, Gulshan K, Kirwan JP, Liu X, et al. Ceramide as a mediator of non-alcoholic fatty liver disease and associated atherosclerosis. *PLoS ONE*. (2015) 10:e0126910. doi: 10.1371/journal.pone.0126910
15. Kurek K, Piotrowska DM, Wiesiolek-Kurek P, Lukaszuk B, Chabowski A, Gorski J, et al. Inhibition of ceramide de novo synthesis reduces liver lipid accumulation in rats with nonalcoholic fatty liver disease. *Liver Int*. (2014) 34:1074–83. doi: 10.1111/liv.12331
16. Park TS, Panek RL, Mueller SB, Hanselman JC, Rosebury WS, Robertson AW, et al. Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation*. (2004) 110:3465–71. doi: 10.1161/01.CIR.0000148370.60535.22
17. Park TS, Panek RL, Rekhter MD, Mueller SB, Rosebury WS, Robertson A, et al. Modulation of lipoprotein metabolism by inhibition of sphingomyelin synthesis in ApoE knockout mice. *Atherosclerosis*. (2006) 189:264–72. doi: 10.1016/j.atherosclerosis.2005.12.029
18. Park TS, Rosebury W, Kindt EK, Kowala MC, Panek RL. Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic ApoE-deficient mice. *Pharmacol Res*. (2008) 58:45–51. doi: 10.1016/j.phrs.2008.06.005
19. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab*. (2014) 20:687–95. doi: 10.1016/j.cmet.2014.10.007
20. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab*. (2014) 20:678–86. doi: 10.1016/j.cmet.2014.08.002
21. Hojjati MR, Li Z, Zhou H, Tang S, Huan C, Ooi E, et al. Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J Biol Chem*. (2005) 280:10284–9. doi: 10.1074/jbc.M412348200
22. Park TS, Hu Y, Noh HL, Drosatos K, Okajima K, Buchanan J, et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res*. (2008) 49:2101–12. doi: 10.1194/jlr.M800147-JLR200
23. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med*. (2011) 17:55–63. doi: 10.1038/nm.2277
24. Meeusen JW, Donato LJ, Bryant SC, Baudhuin LM, Berger PB, Jaffe AS. Plasma ceramides. *Arterioscler Thromb Vasc Biol*. (2018) 38:1933–9. doi: 10.1161/ATVBAHA.118.311199
25. Westra B. *Ceramides. Plasma [A Test in Focus]*. Mayo Clinic. Mayo Medical Laboratories 2017 (2016).
26. Yang G, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol Endocrinol Metab*. (2009) 297:E211–24. doi: 10.1152/ajpendo.91014.2008
27. Ussher JR, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, et al. Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. *Diabetes*. (2010) 59:2453–64. doi: 10.2337/db09-1293
28. Jensen PN, Fretts AM, Yu C, Hoofnagle AN, Umans JG, Howard BV, et al. Circulating sphingolipids, fasting glucose, and impaired fasting glucose: the strong heart family study. *EBioMedicine*. (2019) 41:44–49. doi: 10.1016/j.ebiom.2018.12.046
29. Lemaitre RN, Yu C, Hoofnagle A, Hari N, Jensen PN, Fretts AM, et al. Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: the strong heart family study. *Diabetes*. (2018) 67:1663–72. doi: 10.2337/db17-1449
30. Huynh K, Barlow CK, Jayawardana KS, Weir JM, Mellett NA, Cinel M, et al. High-throughput plasma lipidomics: detailed mapping of the associations with cardiometabolic risk factors. *Cell Chem Biol*. (2019) 26:71–84.e4. doi: 10.1016/j.chembiol.2018.10.008
31. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, et al. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 Cohort. *Arterioscler Thromb Vasc Biol*. (2016) 36:2424–30. doi: 10.1161/ATVBAHA.116.307497
32. Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur Heart J*. (2016) 37:1967–76. doi: 10.1093/eurheartj/ehw148
33. Poss AM, Maschek JA, Cox JE, Hauner BJ, Hopkins PN, Hunt SC, et al. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. *J Clin Invest*. (2019) 130:1363–76. doi: 10.1172/JCI131838

34. Kolak M, Westerbacka J, Velagapudi VR, Wagsater D, Yetukuri L, Makkonen J, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes*. (2007) 56:1960–8. doi: 10.2337/db07-0111
35. Chaurasia B, Kaddai VA, Lancaster GI, Henstridge DC, Sriram S, Galam DL, et al. Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism. *Cell Metab*. (2016) 24:820–34. doi: 10.1016/j.cmet.2016.10.002
36. Luukkonen PK, Sadevirta S, Zhou Y, Kayser B, Ali A, Ahonen L, et al. Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars. *Diabetes Care*. (2018) 41:1732–9. doi: 10.2337/dc18-0071
37. Xia W, Pessentheiner AR, Hofer DC, Amor M, Schreiber R, Schoiswohl G, et al. Loss of ABHD15 impairs the anti-lipolytic action of insulin by altering PDE3B stability and contributes to insulin resistance. *Cell Rep*. (2018) 23:1948–61. doi: 10.1016/j.celrep.2018.04.055
38. Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J Clin Invest*. (2011) 121:1858–70. doi: 10.1172/JCI43378
39. Chavez JA, Summers SA. Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch Biochem Biophys*. (2003) 419:101–9. doi: 10.1016/j.abb.2003.08.020
40. Xia JY, Holland WL, Kusminski CM, Sun K, Sharma AX, Pearson MJ, et al. Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab*. (2015) 22:266–78. doi: 10.1016/j.cmet.2015.06.007
41. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. (2006) 444:860–7. doi: 10.1038/nature05485
42. de Mello VD, Lankinen M, Schwab U, Kolehmainen M, Lehto S, Seppanen-Laakso T, et al. Link between plasma ceramides, inflammation and insulin resistance: association with serum IL-6 concentration in patients with coronary heart disease. *Diabetologia*. (2009) 52:2612–5. doi: 10.1007/s00125-009-1482-9
43. Majumdar I, Mastrandrea LD. Serum sphingolipids and inflammatory mediators in adolescents at risk for metabolic syndrome. *Endocrine*. (2012) 41:442–9. doi: 10.1007/s12020-011-9589-4
44. Schilling JD, Machkovech HM, He L, Sidhu R, Fujiwara H, Weber K, et al. Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. *J Biol Chem*. (2013) 288:2923–32. doi: 10.1074/jbc.M112.419978
45. Sims K, Haynes CA, Kelly S, Allegood JC, Wang E, Momin A Jr, et al. Kdo2-lipid A. a TLR4-specific agonist, induces de novo sphingolipid biosynthesis in RAW264.7 macrophages, which is essential for induction of autophagy. *J Biol Chem*. (2010) 285:38568–79. doi: 10.1074/jbc.M110.170621
46. Lancaster GI, Langley KG, Berglund NA, Kammoun HL, Reibe S, Estevez E, et al. Evidence that TLR4 Is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism. *Cell Metab*. (2018) 27:1096–110.e5. doi: 10.1016/j.cmet.2018.03.014
47. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med*. (2005) 11:183–90. doi: 10.1038/nm1166
48. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, et al. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest*. (2001) 108:437–46. doi: 10.1172/JCI11559
49. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest*. (2006) 116:3015–25. doi: 10.1172/JCI28898
50. Hannun YA, Obeid LM. Ceramide: an intracellular signal for apoptosis. *Trends Biochem Sci*. (1995) 20:73–7. doi: 10.1016/S0968-0004(00)88961-6
51. Kolesnick R. Ceramide: a novel second messenger. *Trends Cell Biol*. (1992) 2:232–6. doi: 10.1016/0962-8924(92)90310-J
52. Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. *Science*. (1993) 259:1769–71. doi: 10.1126/science.8456305
53. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med*. (2011) 17:179–88. doi: 10.1038/nm.2279
54. Ikeda K, Maretich P, Kajimura S. The common and distinct features of brown and beige adipocytes. *Trends Endocrinol Metab*. (2018) 29:191–200. doi: 10.1016/j.tem.2018.01.001
55. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat Commun*. (2015) 6:10166. doi: 10.1038/ncomms10166
56. Straub L, Scherer P. Metabolic messengers: adiponectin. *Nat Metab*. (2019) 1:334–39. doi: 10.1038/s42255-019-0041-z
57. Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett*. (2008) 582:74–80. doi: 10.1016/j.febslet.2007.11.070
58. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation*. (2000) 102:1296–301. doi: 10.1161/01.CIR.102.11.1296
59. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*. (2002) 8:1288–95. doi: 10.1038/nm788
60. Holland WL, Xia JY, Johnson JA, Sun K, Pearson MJ, Sharma AX, et al. Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced ceramidase signaling in lipid and glucose homeostasis. *Mol Metab*. (2017) 6:267–75. doi: 10.1016/j.molmet.2017.01.002
61. Xia JY, Sun K, Hepler C, Ghaben AL, Gupta RK, An YA, et al. Acute loss of adipose tissue-derived adiponectin triggers immediate metabolic deterioration in mice. *Diabetologia*. (2018) 61:932–41. doi: 10.1007/s00125-017-4516-8
62. Tanabe H, Fujii Y, Okada-Iwabu M, Iwabu M, Nakamura Y, Hosaka T, et al. Crystal structures of the human adiponectin receptors. *Nature*. (2015) 520:312–16. doi: 10.1038/nature14301
63. Vasiliauskaite-Brooks I, Sounier R, Rochaix P, Bellot G, Fortier M, Hoh F, et al. Structural insights into adiponectin receptors suggest ceramidase activity. *Nature*. (2017) 544:120–23. doi: 10.1038/nature21714
64. Blachnio-Zabielska AU, Koutsari C, Tchkonia T, Jensen MD. Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance. *Obesity*. (2012) 20:2341–7. doi: 10.1038/oby.2012.126
65. Lopez X, Goldfine AB, Holland WL, Gordillo R, Scherer PE. Plasma ceramides are elevated in female children and adolescents with type 2 diabetes. *J Pediatr Endocrinol Metab*. (2013) 26:995–8. doi: 10.1515/jpem-2012-0407
66. Alexaki A, Clarke BA, Gavrilova O, Ma Y, Zhu H, Ma X, et al. *De novo* sphingolipid biosynthesis is required for adipocyte survival and metabolic homeostasis. *J Biol Chem*. (2017) 292:3929–39. doi: 10.1074/jbc.M116.756460
67. Lee SY, Lee HY, Song JH, Kim GT, Jeon S, Song YJ, et al. Adipocyte-specific deficiency of *de novo* sphingolipid biosynthesis leads to lipodystrophy and insulin resistance. *Diabetes*. (2017) 66:2596–609. doi: 10.2337/db16-1232
68. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, et al. Transcriptional control of adipose lipid handling by IRF4. *Cell Metab*. (2011) 13:249–59. doi: 10.1016/j.cmet.2011.02.005
69. Wang ZV, Deng Y, Wang QA, Sun K, Scherer PE. Identification and characterization of a promoter cassette conferring adipocyte-specific gene expression. *Endocrinology*. (2010) 151:2933–9. doi: 10.1210/en.2010-0136
70. Gosejacob D, Jager PS, Vom Dorp K, Frejno M, Carstensen AC, Kohnke M, et al. Ceramide synthase 5 is essential to maintain C16:0-ceramide pools and contributes to the development of diet-induced obesity. *J Biol Chem*. (2016) 291:6989–7003. doi: 10.1074/jbc.M115.691212
71. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol*. (1998) 18:5457–64. doi: 10.1128/MCB.18.9.5457
72. Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol*. (2003) 23:7794–808. doi: 10.1128/MCB.23.21.7794-7808.2003
73. Powell DJ, Turban S, Gray A, Hajduch E, Hundal HS. Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in

palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J.* (2004) 382:619–29. doi: 10.1042/BJ20040139
74. Stratford S, DeWald DB, Summers SA. Ceramide dissociates 3'-phosphoinositide production from pleckstrin homology domain translocation. *Biochem J.* (2001) 354:359–68. doi: 10.1042/bj3540359
75. Dobrowsky RT, Kamibayashi C, Mumby MC, Hannun YA. Ceramide activates heterotrimeric protein phosphatase 2A. *J Biol Chem*. (1993) 268:15523–30.
76. Bharath LP, Ruan T, Li Y, Ravindran A, Wan X, Nhan JK, et al. Ceramide-initiated protein phosphatase 2A activation contributes to arterial dysfunction *in vivo*. *Diabetes*. (2015) 64:3914–26. doi: 10.2337/db15-0244
77. Chavez JA, Knotts TA, Wang LP, Li G, Dobrowsky RT, Florant GL, et al. A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J Biol Chem*. (2003) 13:10297–303. doi: 10.1074/jbc.M212307200
78. Salinas M, Lopez-Valdaliso R, Martin D, Alvarez A, Cuadrado A. Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide- activated protein phosphatase in PC12 cells. *Mol Cell Neurosci*. (2000) 15:156–69. doi: 10.1006/mcne.1999.0813
79. Teruel T, Hernandez R, Lorenzo M. Ceramide mediates insulin resistance by tumor necrosis factor-alpha in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. *Diabetes*. (2001) 50:2563–71. doi: 10.2337/diabetes.50.11.2563
80. Zinda MJ, Vlahos CJ, Lai MT. Ceramide induces the dephosphorylation and inhibition of constitutively activated Akt in PTEN negative U87mg cells. *Biochem Biophys Res Commun*. (2001) 280:1107–15. doi: 10.1006/bbrc.2000.4248
81. Blouin CM, Prado C, Takane KK, Lasnier F, Garcia-Ocana A, Ferre P, et al. Plasma membrane subdomain compartmentalization contributes to distinct mechanisms of ceramide action on insulin signaling. *Diabetes*. (2010) 59:600–10. doi: 10.2337/db09-0897
82. Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, et al. CerS6-derived sphingolipids interact with Mff and promote mitochondrial fragmentation in obesity. *Cell*. (2019) 177:1536–52.e23. doi: 10.1016/j.cell.2019.05.008

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 29 July 2020
doi: 10.3389/fendo.2020.00483

Ceramide Synthases Are Attractive Drug Targets for Treating Metabolic Diseases

*Suryaprakash Raichur**

Evotec International GmbH, Göttingen, Germany

Ceramide synthases (CerS) are central enzymes required for the *de-novo* synthesis of ceramides and other sphingolipids. They catalyze the addition of different acyl-chains to a sphingoid base, and thus account for much of the rich diversity in the sphingolipid family. Recent studies have demonstrated that the acyl-chain is an important determinant of ceramide function, such that a small subset of ceramides (e.g., those containing the C16 or C18 acyl-chain) alter metabolism by inhibiting insulin signaling or inducing mitochondrial fragmentation. Herein I discuss the therapeutic potential of targeting certain ceramide synthase isoforms for the treatment of obesity, insulin resistance, steatohepatitis, and other metabolic disorders.

Keywords: ceramides, sphingolipids, C16 ceramide, insulin resistance, obesity, NAFLD, NASH and mitochondrial dysfunction

OPEN ACCESS

Edited by:
Scott A. Summers,
The University of Utah, United States

Reviewed by:
Carsten Schmitz-Peiffer,
Garvan Institute of Medical Research, Australia
Yael Pewzner-Jung,
Weizmann Institute of Science, Israel

***Correspondence:**
Suryaprakash Raichur
surya.prakash@evotec.com

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *11 May 2020*
Accepted: *22 June 2020*
Published: *29 July 2020*

Citation:
Raichur S (2020) Ceramide Synthases Are Attractive Drug Targets for Treating Metabolic Diseases. Front. Endocrinol. 11:483. doi: 10.3389/fendo.2020.00483

INTRODUCTION

The increasing burden of metabolic diseases such as diabetes and heart disease is alarming, not only in developed countries, but throughout the world. Some prevalent metabolic disorders, such as non-alcoholic steatohepatitis (NASH), have no approved pharmacotherapies (1, 2). Unhealthy lifestyles fuel these pathologies, as combinations of sedentary lifestyles and poor dietary habits promote the delivery of excess saturated fatty acids and carbohydrates into non-adipose tissues such as liver and skeletal muscle, impairing their function (3, 4). Numerous studies reveal that the conversion of these excess fuels into sphingolipids such as ceramides is a critical event that leads to the cellular defects that accompany obesity (5, 6).

Clinical studies confirm that excess saturated fatty acid intake elevates levels of serum and tissue ceramides (7). Contrastingly, polyunsaturated fat intake reduces serum ceramides (7). In rodents, inhibiting ceramide biosynthesis using genetic and pharmacological approaches ameliorates atherosclerosis, hepatic steatosis, insulin resistance and obesity (8).

Two primary pathways produce ceramides in cells (**Figure 1**): (a) a *de novo* synthesis enzyme cascade that starts with the condensation of a saturated acyl-CoA (typically palmitoyl-CoA) and amino acid (typically serine) to produce the sphingoid backbone; and (b) a salvage pathway that involves the re-acylation of sphingosine. In both cases, ceramides (or dihydroceramides, in the case of the *de novo* synthesis pathway) are produced by ceramide synthases (CerS) through N-acylation of the sphingoid base. Mammalian CerS exists in six isoforms (CerS1-6) with differing preferences for specific fatty acid chain lengths. CerS1 attaches C18 fatty acyl CoA to the sphingoid base; CerS2 attaches very long fatty acyl CoAs such as C22–C24; CerS3 attaches C26–C34 acyl CoA; CerS4 attaches C18–C20 fatty acyl CoA; and CerS5 and 6 have specificity for C14–C16 fatty acyl CoA. Thus, the CerS enzymes determine the acyl-chain composition of ceramides. CerS

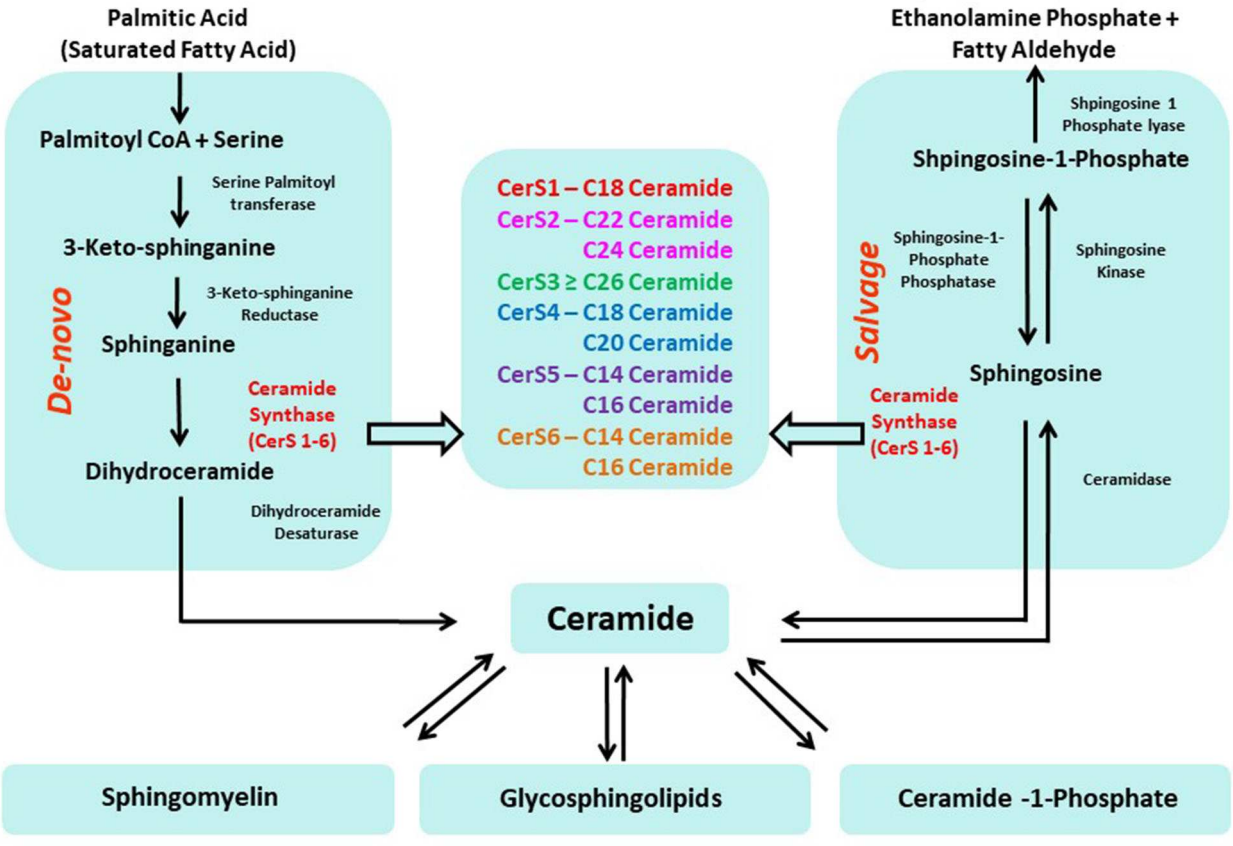

FIGURE 1 | Two primary pathways produce ceramides in cells: (a) *de novo* synthesis (b) re-acylation of sphingosine (salvage pathway).

expression, structure, localization, knock-out phenotypes, and association with other diseases have been reviewed comprehensively (9).

Ceramides can be further modified by the addition of different head groups, and the number of estimated bioactive sphingolipids range from 4,000 to 60,000 (10). The ceramides themselves have emerged as important signaling molecules that contribute to cellular stress responses (11). A primary mechanism through which ceramide promotes insulin resistance is by decreasing the activity of Akt/PKB, which is an essential facilitator of cellular glucose uptake. Ceramide blocks the activity of Akt/PKB by independent mechanisms: by enhancing Akt dephosphorylation via protein phosphatase 2A (PP2A) and by blocking the translocation of Akt via PKCz (12). Other ceramide actions have been identified that contribute to triglyceride production, mitochondrial dysfunction, and ultimately apoptosis (13).

Several genetic and pharmacological studies targeting *de novo* ceramide biosynthesis focused on the first enzyme in the pathway: serine palmitoyltransferase (SPT). Inhibiting this enzyme in rodents reduces global ceramide levels and ameliorates many forms of metabolic diseases (14). However, SPT is not a viable therapeutic target, owing to safety issues that result from the extreme diminution of all sphingolipids (15, 16). Therefore, therapeutic approaches focusing on a more limited subset of detrimental ceramides subspecies may represent a new strategy for therapeutic intervention. Fortunately, two recent reports demonstrated that the sphingolipid acylation patterns influences disease progression (17, 18), thus suggesting that the CerS enzymes might be viable targets.

CLINICAL CORRELATIONS BETWEEN SPECIFIC CERAMIDES AND THE METABOLIC SYNDROME IN HUMANS

Studies in clinical cohorts reveal striking relationships between serum or tissue ceramides and various measures of cardiometabolic disease. However, considerable variability exists regarding the precise sphingolipids that are most commonly elevated in a diseased individual. In the following paragraphs, I will focus on the larger studies that reveal specific roles for certain ceramides in diabetes and NASH.

Insulin Resistance and Diabetes

Insulin resistance, a condition typically defined as an inability of insulin to appropriately clear glucose from the bloodstream,

is a risk factor for diabetes, heart disease, and NASH. Clinical observations reveal that circulating ceramides packaged in LDL (low-density lipoprotein) negatively associate with insulin sensitivity (19). The authors also demonstrated that infusion of LDL-containing ceramides into healthy mice attenuated whole body glucose clearance and increased levels of inflammatory markers (19), thus confirming that circulating lipids can be taken into tissues to alter metabolism. These authors also demonstrated that LDLs containing C16:0 and C24:0 ceramides reduced glucose uptake in cultured myotubes by inhibiting insulin signaling and decreasing translocation of the GLUT4 glucose transporter (19). In the Dallas Heart Study, consisting of 1,557 participants without type 2 diabetes assessed for metabolic biomarkers, fat depots and plasma ceramides over a period of 7 years, the saturated C16 and C18 ceramides correlated with insulin resistance (i.e., homeostatic model assessment of insulin resistance, HOMA-IR), total body fat and visceral adipose tissue (20). In contrast, healthier metabolic profiles were associated with longer-chain polyunsaturated fatty acid ceramides C24:2, C30:10, and C32:11 (20). Similarly, an evaluation of plasma sphingolipids in a large cohort of Chinese individuals in Singapore revealed that C16, C18, and C20 ceramides containing a d16:1 backbone correlated positively with body mass index (BMI) and HOMA-IR (21). By comparison, hexosylceramides and ceramides with a d18:2 backbone negatively correlated with HOMA-IR and BMI (21). Off note, this study comprehensively assessed both the d16:1 and d18:2 backbone of sphingolipid species, whereas most of the studies generally focus on most abundant d18:2 backbone (21). Another interesting clinical study investigated ceramide subspecies and their ratios to determine the best predictors of diabetes (22). The authors identified C18/16 ratios as an independent marker for risk of diabetes incidence (22). Moreover, this ratio decreased in individuals following weight loss of 5% or more (22). A prospective study has revealed significant reductions in circulating very long chain ceramides (C20, C20:1, C22.1, C24, C26, C26:1) in type 1 diabetic patients that also associated with the development of nephropathy (23).

Liver Disease

A clinical study consisting of 406 patients with chronic viral hepatitis revealed that levels of sphingosine, sphinganine, and certain ceramides significantly associated with the severity of liver fibrosis in HCV-infected patients (compared to HBV-infected patients) (24). Moreover, Apostolopoulou et al. demonstrated that elevation of various sphingolipids species in the liver and serum of NASH patients compared to non-alcoholic fatty liver and control subjects (25). Specifically, in NASH, hepatic dihydroceramides (16:0, 22:0, and 24:1) and lactosylceramides increased significantly. Total serum dihydroceramides and hepaic dihydroceramides (22:0 and 24:1) increased significantly in NASH and strongly associated with whole body insulin-resistance (25). Additional analysis shows that sphingolipid species correlated with hepatic oxidative stress and inflammation (25). In a prospective study consisting of 31 children diagnosed with non-alcoholic fatty liver disease (NAFLD), Wasilewska et al. demonstrated significant, positive correlation between total serum concentration of ceramides with insulin and also with HOMA-IR (26). Additionally, this study reveals that total ceramide concentration and specific (saturated fatty acyl) subspecies of ceramides such as C14, C16, C16:1, C18, and C18:1 were significantly higher in children with NAFLD compared to controls (26). Lastly, a randomized clinical trial conducted to determine the influence of dietary saturated and polyunsaturated fat on fatty liver development found that saturated fat markedly induces liver fat and serum ceramides (27). The effect was pronounced on C16 ceramides, whereas dietary polyunsaturated fat prevents liver fat accumulation and associated with reduced total ceramides (27).

Bariatric Surgery

The benefits of bariatric surgery on obesity and metabolic disease are well-established, including an almost immediate remission of type 2 diabetes and hyperlipidemia. In this context, surgically induced weight loss, which was associated with the improvement in insulin sensitivity and a decrease in proinflammatory cytokines, was shown to decrease plasma ceramide levels. Numerous serum ceramides, including the C16 subspecies, decreased in a time-dependent manner post-surgery (28). Subsequently, another study reveals a significant, time-dependent reduction of serum C22 and C24 ceramides after laparoscopic sleeve gastrectomy (29).

Taken together, these clinical observations suggest that increases of saturated fatty acyl C16-18 ceramides, as well as several other sphingolipid species, are apparent in obese, fatty liver and insulin resistant individuals. The heterogeneity in species is a result of the dysregulated changes in ceramide biosynthesis in different organs. In this context, several mechanistic studies have found that the supply of saturated fat is sufficient to induce ceramides and cause metabolic dysfunction. For example, oversupply of saturated fatty acids by lipid infusion or diet promotes ceramide accumulation and activates inflammatory pathways, induces insulin resistance, impairs mitochondrial function, and stimulates endoplasmic stress and lipotoxicity. Blocking ceramide synthesis negates these saturated fatty acid actions (6, 8). Beyond saturated fatty acids, other factors, such as inflammatory signaling pathways, increase the rate of ceramide synthesis.

The above human lipodomic data (**Table 1**) also suggest that altered ratios of ceramide subspecies are associated with the comorbidities of diabetes and obesity. In this context, recent comprehensive sphingolipid analysis in rodent metabolic disease mouse models suggests that the ratio of long-chain ceramide species to very long-chain ceramide species in liver is a key marker of metabolic disease (32). Below I will discuss interventional studies in rodents suggest that accumulation of toxic species of ceramides such as C16 may play a role during progression of simple steatosis to NASH in humans.

CERS ENZYMES AS THERAPEUTIC TARGETS

The studies described above suggest that therapeutic interventions that reduce ceramides could have utility for

TABLE 1 | Altered ceramide ratios in insulin resistance, diabetes, and fatty liver disease.

Altered circulating/tissue long and very long chain ceramide ratios associated with insulin resistance and diabetes	
Neeland et al. (20)	Increased C16 and C18 ceramides in serum correlated with insulin resistance, total body fat, and visceral fat tissue Elevated levels of C24:2, C30:10, and C32:11 ceramides in serum associated with healthier metabolic profiles
Chew et al. (21)	Increased C16, C18, and C20 ceramides in serum correlated positively with body mass index (BMI) and HOMA-IR
Hilvo et al. (22)	Increased C18 ceramide in serum shows the strongest association with incident diabetes. Study identifies C18/16 ratios as an independent marker for risk of incidence of diabetes
Klein et al. (23)	Very long chain ceramides (C20, C20:1, C22.1, C24, C26, and C26:1) are significantly reduced in serum of type 1 diabetic
Bergman et al. (30)	Higher levels of C18 ceramide in skeletal muscle association with insulin resistance and inflammation
Perreault et al. (31)	C18-ceramide levels increased in the skeletal muscle cells isolated from individuals with type 2 diabetes
Increased dihydroceramides/long chain ceramide with fatty liver diseases	
Apostolopoulou et al. (25)	Total serum dihydroceramides and hepatic dihydroceramides (16:0, 22:0, and 24:1) increased in NASH Hepatic dihydroceramides (22:0 and 24:1) increased significantly in NASH and strongly associated with whole body insulin-resistance
Wasilewska et al. (26)	Serum saturated ceramides species such as C14, C16, C16:1, C18, and C18:1 significantly higher in children with NAFLD
Rosqvist et al. (27)	Dietary saturated fat markedly induces the fatty liver development, associated with increase serum total ceramides specifically pronounced effect observed in C16 ceramides Dietary polyunsaturated fat prevents fatty liver development associated with reduced serum total ceramides

treating insulin resistance and fatty liver disease. More recent efforts have interrogated the value of targeting the CerS enzymes to alter the acyl-composition of ceramides to treat the comorbidities of obesity. These interventions are producing promising results.

CerS1 and Muscle Insulin Resistance

Skeletal muscle is the primary organ responsible for insulin-mediated glucose uptake and utilization. During obesity, excess energy is delivered to muscle in the form of non-esterified fatty acids (NEFA) or lipoprotein-bound triglycerides. Excess delivery of these fatty acids to skeletal muscle causes insulin resistance, as the muscles adapt to the use of more plentiful energy source. This condition increases one's risk for type 2 diabetes and cardiovascular disease (33). Mechanistic studies have suggested that diacylglycerols (34) and ceramides (6) may contribute to skeletal muscle insulin resistance development.

CerS1 is the predominant isoform in muscle and the C18 ceramides that it produces are the major ceramide subspecies found in the tissue (9). Recent clinical observations identified higher levels of C18 ceramide in association with insulin resistance and inflammation in skeletal muscle (30). Another study found that C18-ceramide levels increased in skeletal muscle cells isolated from individuals with type 2 diabetes (31). To study the role of CerS1 in metabolic pathologies, researchers turned to rodent loss-of-function models (35). In these studies, high fat induced obesity led to an increase in C18 ceramides in muscle (35). Global ablation of CerS1 protected mice from high fat diet-induced weight gain. Moreover, the intervention increased energy expenditure, reduced adiposity, and improved insulin and glucose tolerance (35). Subsequently, the authors developed skeletal muscle-specific CerS1 knockout mice, demonstrating that tissue-specific ablation of the gene also improved glucose tolerance and insulin sensitivity (35). These observations suggest key roles for skeletal muscle C18 ceramides in the pathophysiology of obesity associated insulin resistance [**Figure 2**; (35)].

Turner et al. evaluated CerS1 as a potential drug target using novel small molecule inhibitors of the enzyme. The researchers developed P053, a specific inhibitor of CerS1 that displays nanomolar potency (36). PO53 displayed selectivity for CerS1 in mice by reducing C18 ceramide concentrations in skeletal muscle without changing other subspecies of ceramides (36). PO53-treated animals showed improved skeletal muscle mitochondrial beta-oxidation relative to vehicle treated group (36). However, PO53 treated animals did not show any improvement in glucose tolerance and insulin sensitivity, despite having decreased intramuscular C18 ceramides (by ∼50%) (36). This is in apparent contrast to the studies using genetic knockouts, which markedly altered glucose disposal. The contradictory observations could relate to differences in the diet induced obese mouse models used, the duration of the study or the degree of CerS1 inhibition that was achieved: (a) C57BL/6N mice were used in the knockout study, while the PO53 study used smaller C57BL/6J mice; (b) the knockout animals were fed for 12 weeks while the PO53 study were treated for only 4–6 weeks; and (c) the PO53 pharmacological treatment had a more modest effect on ceramides, reducing them by ∼50%, compared to >90% reduction in skeletal muscle of the knockout animals (35, 36). Therefore, further investigation of CerS1 inhibitors is warranted.

CerS6 and NASH

NASH, characterized by hepatocellular lipid accumulation (steatosis) along with inflammation and varying degrees of fibrosis, is a serious condition affecting 1.5–6.45% of the population (1). Once afflicted, ∼10–15% of NASH patients progress to cirrhosis (37). The estimated annual economic burden of NASH in the USA is $103 billion (38). NASH has no FDA-approved therapy, prompting many companies to race for approval of the first NASH-targeted drugs (38). Current disease management is primarily focused on promoting weight loss through lifestyle interventions, weight loss medication, and/or bariatric surgery (39). Limited prospective data are available on these options (39).

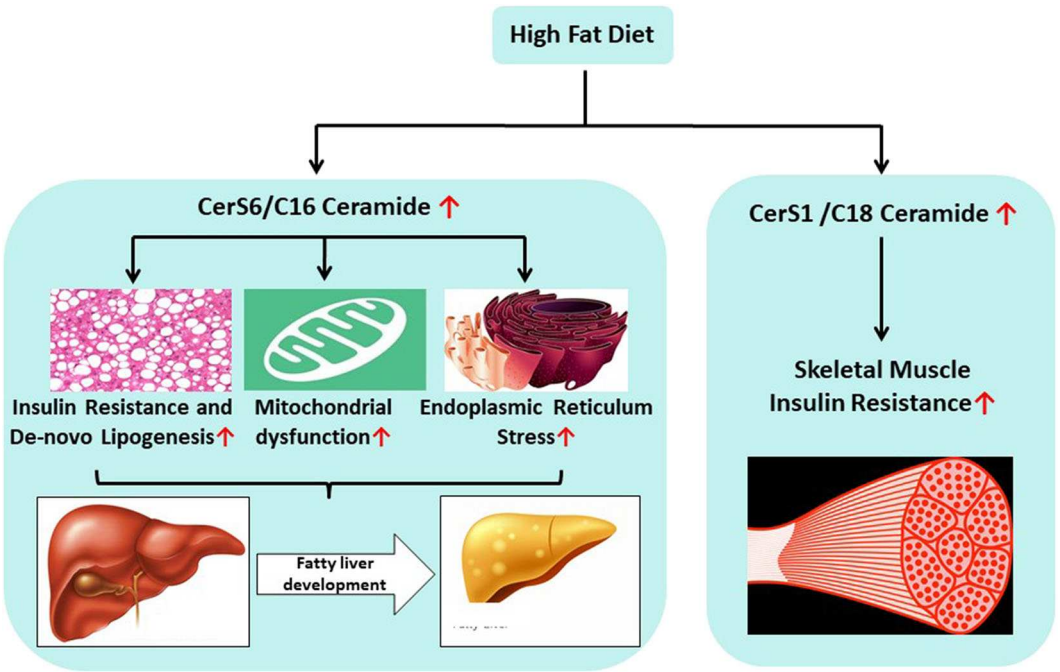

FIGURE 2 | Unhealthy food habits promote the delivery of excess saturated fatty acids and carbohydrates that lead to increased biosynthesis of C16 and C18 ceramides in the liver and skeletal muscle, respectively. CerS6 mediated C16 ceramide plays critical role in the fatty liver development via (a) increased insulin resistance (b) increased *de-novo* lipogenesis, (c) mitochondrial dysfunction, and (d) increased endoplasmic reticulum stress. CerS1 mediated C18 ceramide is involved in the development of skeletal muscle insulin resistance.

The pathology was originally interpreted to result from "dual-hits." The first hit, the steatosis that characterizes NAFLD, predisposes individuals to the second hits that include inflammation (40). More recently, complex "multiple-hit" hypotheses have been proposed, as numerous other factors including oxidative stress, mitochondrial dysfunction, and other insults are implicated in the pathology (41). Each stage is defined by specific risk factors and pathological mechanisms (42). As noted above, a number of studies indicate that sphingolipids such as ceramides, which can induce each of these elements, including inflammation, oxidative stress, and mitochondrial dysfunction, to drive NASH (13). Myriocin, a potent inhibitor of SPT, inhibits NASH progression in rodents (43, 44).

Several studies suggest that altering ceramide acylation patterns through CerS inhibition could also alter disease progression. Mice lacking both copies of the CerS2 gene display numerous liver abnormalities such as hepatocyte death and chronic apoptosis and regeneration (45, 46). The animals develop hepatoadenoma at 3–4 months of age, markedly reduced body weight, and hepatocarcinoma (around age 1 year) (45, 46). Furthermore, homozygous CerS2 knockout mice display decreased lipid accumulation and uptake in the liver (47). These animals displayed the expected reduction in very long chain acyl ceramides; however, they had a compensatory increases in C16-ceramides (45, 46). This was interesting, as the C16-ceramides had been associated with hepatic insulin resistance (48) and disruption of the mitochondrial respiratory chain (49).

We subsequently studied heterozygous CerS2 knockout mice, which show a more modest pathology than their homozygous counterparts. The heterozygous CerS2 knockout mice show normal lifespan and no liver phenotype on normal chow diet (17) but phenotypes emerged when they were challenged with an obesogenic high fat diet (HFD). On the HFD, they displayed increased liver weight, triglycerides, macrophage infiltration, circulating liver enzymes, and plasma cholesterol, all of which are indicative of liver damage (17). These mice also displayed slightly impaired glucose tolerance, high fasting, and fed insulin levels, increased glucose mediated insulin secretion, reduced insulin sensitivity, decreased ambulatory activity, and increased fat to lean mass (17). As in the aforementioned study of the homozygous animals, the liver abnormalities were associated with reduced levels of very long chain ceramides coupled with the compensatory increase in C16-ceramides (17). In these studies, the increase in C16-ceramides could be explained by increased expression of CerS6. No changes were apparent on the normal chow diet. Mechanistic studies in primary hepatocytes obtained from the heterozygous CerS2 knockout mice revealed that the alteration in ceramide acylation attenuated insulin signaling and induced mitochondrial dysfunction (17). To recapitulate the compensatory increase of CerS6 expression, we overexpressed CerS6 in wild-type primary mouse hepatocytes. This intervention increased accumulation of C16 ceramides and compromised mitochondrial function, increased triglyceride accumulation and attenuated insulin signaling (17).

Additional studies in mice have shown that diet induced NAFLD is associated with liver ceramide acylation patterns that paralleled the profile of the CerS2 knockouts. In general, high fat diets lead to increases in CerS6 expression and a concomitant elevation of C16-ceramides (50). Of note, in one study, overexpression of CerS2 was protective, presumably because it prevented the induction of CerS6 (50). In that study, CerS6 was shown to induce sterol regulatory element binding protein-1 (SREBP-1) cleavage and decrease levels of INSIG-1, leading to increased *de-novo* lipogenesis (DNL) (50). SREBP-1 is a master regulator of DNL in the liver and one of the primary insults that is dysregulated in the NASH pathophysiology (42). These authors reproduced our work (17), showing that CerS2 heterozygotes were susceptible to diet-induced steatohepatitis, exhibiting a pronounced endoplasmic reticulum (ER) stress response (50). The involvement of ER-stress was novel, but not surprising; Cinar et al. previously demonstrated that HFD-induced hepatic insulin resistance was associated with increased ER stress that was associated with elevated hepatic C16 and C18 ceramides (51).

Bruning et al. obtained the strongest evidence to date that CerS6 was pathogenic. They demonstrated that levels of CerS6 were increased in visceral and subcutaneous adipose tissue of obese humans, correlating positively with BMI and insulin resistance (18). These authors also found that the CerS6 product C16 ceramides were elevated in the visceral adipose of obese humans (18). They subsequently created CerS6 null mice, which allowed for a precise determination of the role of the enzyme in the development of obesity and fatty liver disease. The CerS6 null mice had the expected reduction in C16 ceramides. On a non-obesogenic chow diet, they had no obvious phenotype. However, the CerS6 null mice were protected from HFD-induced obesity (18). The change in body mass was completely explained by a reduction in adiposity, including a decrease in adipocyte size, serum leptin, adipose macrophage infiltration, and adipose pro-inflammatory gene expression (18). Additionally, the CerS6 null mice displayed improved insulin sensitivity and glucose tolerance (18). Turpin et al. also produced tissue-specific knockout mice, excising the gene from macrophages, brown adipocytes, and liver (18). Deletion of CerS6 from the brown adipose depots reduced diet induced obesity and improved mitochondrial beta oxidation, leading to elevations in energy expenditure (18). Liver-specific CerS6 null mice displayed a partial protection from diet induced obesity but a robust protection from glucose intolerance and insulin resistance explained by enhanced insulin signaling relative to wild type animals (18). Deletion of CerS6 from macrophages had no effect (18).

Brüning's group later identified a novel molecular mechanism through which CerS6 derived C16:0 ceramides alter mitochondrial dynamics, determining that the lipids interacted with mitochondrial fission factor (52). Deficiency of either CerS6 or mitochondrial fission factor encoded by the Mff gene protected mice from fatty acid-induced mitochondrial fragmentation *in vitro*. Moreover, the two proteins genetically interacted *in vivo*, participating in a linear pathway that accounted for obesity-induced mitochondrial fragmentation (52). Of note, an independent study reported that germline CerS6 knockout mice suffer neurobehavioral defects (53). This developmental defect was not observed by Brüning's group, which did not report any abnormal behavioral side effects of CerS6 deletion (18). These differences could be possibly explained by different knockout mouse generation strategies that were used (18, 53). However, from the safety pharmacology prospective it may be advisable to restrict compound use during pregnancy and/or to develop compounds that spares central nerves systems (CNS).

To develop therapeutic approaches to inhibit CerS6, we tested whether selective ablation of CerS6 using antisense oligonucleotides (ASO) was sufficient to reverse metabolic abnormalities in mice that were made obese by high fat diet (DIO mice) or leptin deficiency (ob/ob mice) (54). Delivery of the CerS6 ASO selectively reduced CerS6 expression by 90%, predominantly in the liver. CerS6 knockdown reduced C16:0 ceramides by about 50% in both liver and plasma (54). CerS6 ASO treatment efficiently lowered body weight gain and reduced body fat and fed (and fasted) blood glucose levels (1% reduction in HbA1c). Moreover, CerS6 inhibition improved oral glucose tolerance and insulin sensitivity (54). Both genetic knockout and ASO mediated genetic knock-down studies clearly demonstrated that inhibition of CerS6 activity ameliorated metabolic diseases including insulin resistance, type 2 diabetes and obesity [**Figure 2**; (17, 18, 54)]. Therefore, further discovery of selective small molecule therapeutics targeting CerS6 inhibition is warranted.

CerS5 and Obesity

Gosejacob et al. developed CerS5 knockout mice and demonstrated that CerS5 null mice are viable and fertile and do not show any obvious morphological and phenotypic alterations on normal chow diet (55). However, when challenged with high fat diet CerS5 knockout animals protected against diet induced obesity and associated with reduced levels of leptin relative to wild type animals (55). Additionally, on diet induced obesity CerS5 knockout animals displayed improved glucose tolerance, insulin sensitivity, and reduced white adipose inflammation compared to wild type animals (55). This was also in contrast to the Brüning study, which showed no effect of CerS5 depletion (52).

CONCLUSIONS

Clinical observations and experimental studies in rodents suggest that specific ceramides have distinct roles in the pathophysiology of various human diseases. Studies reveal that altering sphingolipid acylation patterns impacts hepatic steatosis, adiposity, adipocyte size, adipokine secretion, macrophage infiltration, inflammation, insulin sensitivity, mitochondrial dysfunction, and ER stress. In particular, the ratio of long-chain ceramides (e.g., C16 and C18) to very long-chain ceramides (e.g., C24:0 or C24:1) appears to be a key factor in the development of metabolic disease. Therefore, inhibition of CerS6, and perhaps

CerS1 and CerS5, may serve as an attractive therapeutic approach for treating insulin resistance, obesity, fatty liver, and NASH.

AUTHOR CONTRIBUTIONS

SR researched the literature and wrote the manuscript.

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. (2016) 64:73–84. doi: 10.1002/hep.28431
2. Mittermayer F, Caveney E, De Oliveira C, Alexander Fleming G, Gourgiotis L, Puri M, et al. Addressing unmet medical needs in type 1 diabetes: a review of drugs under development. *Curr Diabetes Rev*. (2016) 13:300–14. doi: 10.2174/1573399812666160413115655
3. Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. *Nat Rev Endocrinol*. (2017) 13:79–91. doi: 10.1038/nrendo.2016.169
4. Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J Clin Invest*. (2011) 121:1858–70. doi: 10.1172/JCI43378
5. Bikman BT, Summers SA. Ceramides as modulators of cellular and whole-body metabolism. *J Clin Invest*. (2011) 121:4222–30. doi: 10.1172/JCI 57144
6. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. *Cell Metab*. (2012) 15:585–94. doi: 10.1016/j.cmet.2012.04.002
7. Rosqvist F, Kullberg J, Ståhlman M, Cedernaes J, Heurling K, Johansson HE, et al. Overeating saturated fat promotes fatty liver and ceramides compared with polyunsaturated fat: a randomized trial. *J Clin Endocrinol Metab*. (2019) 104:6207–19. doi: 10.1210/jc.2019-00160
8. Chaurasia B, Summers SA. Ceramides - lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab*. (2015) 26:538–50. doi: 10.1016/j.tem.2015.07.006
9. Park WJ, Park JW. The effect of altered sphingolipid acyl chain length on various disease models. *Biol Chem*. (2015) 396:693–705. doi: 10.1515/hsz-2014-0310
10. Merrill AH. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem Rev*. (2011) 111:6387–422. doi: 10.1021/cr2002917
11. Hannun YA, Obeid LM. Ceramide and the eukaryotic stress response. *Biochem Soc Trans*. (2000) 10:73–80. doi: 10.1016/s0962-8924(99)01694-3
12. Stratford S, Hoehn KL, Liu F, Summers SA. Regulation of insulin action by ceramide: Dual mechanisms linking ceramide accumulation to the inhibition of Akt/protein kinase B. *J Biol Chem*. (2004) 279:36608–15. doi: 10.1074/jbc.M406499200
13. Summers SA, Chaurasia B, Holland WL. Metabolic messengers: ceramides. *Nat Metab*. (2019) 1:1051–58. doi: 10.1038/s42255-019-0134-8
14. Schmitz-Peiffer C. Targeting ceramide synthesis to reverse insulin resistance. *Diabetes*. (2010) 59:2351–53. doi: 10.2337/db10-0912
15. Ohta E, Ohira T, Matsue K, Ikeda Y, Fujii K, Ohwaki K, et al. Analysis of development of lesions in mice with serine palmitoyltransferase (SPT) deficiency - Sptlc2 conditional knockout mice. *Exp Anim*. (2009) 58:515–24. doi: 10.1538/expanim.58.515
16. Hojjati MR, Li Z, Jiang XC. Serine palmitoyl-CoA transferase (SPT) deficiency and sphingolipid levels in mice. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2005) 1737:44–51. doi: 10.1016/j.bbalip.2005.08.006
17. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. CerS2 haploinsufficiency inhibits β-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab*. (2014) 20:687–95. doi: 10.1016/j.cmet.2014.10.007
18. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab*. (2014) 20:678–86. doi: 10.1016/j.cmet.2014.08.002
19. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, et al. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes*. (2013) 62:401–10. doi: 10.2337/db12-0686
20. Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, et al. Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the dallas heart study. *Diabetologia*. (2018) 61:2570–79. doi: 10.1007/s00125-018-4720-1
21. Chew WS, Torta F, Ji S, Choi H, Begum H, Sim X, et al. Large-scale lipidomics identifies associations between plasma sphingolipids and T2DM incidence. *JCI Insight*. (2019) 5:e126925. doi: 10.1172/jci.insight.126925
22. Hilvo M, Salonurmi T, Havulinna AS, Kauhanen D, Pedersen ER, Tell GS, et al. Ceramide stearic to palmitic acid ratio predicts incident diabetes. *Diabetologia*. (2018) 61:1424–34. doi: 10.1007/s00125-018-4590-6
23. Klein RL, Hammad SM, Baker NL, Hunt KJ, Al Gadban MM, Cleary PA, et al. Decreased plasma levels of select very long chain ceramide species are associated with the development of nephropathy in type 1 diabetes. *Metabolism*. (2014) 63:1287–95. doi: 10.1016/j.metabol.2014.07.001
24. Grammatikos G, Ferreiros N, Bon D, Schwalm S, Dietz J, Berkowski C, et al. Variations in serum sphingolipid levels associate with liver fibrosis progression and poor treatment outcome in hepatitis C virus but not hepatitis B virus infection. *Hepatology*. (2015) 61:812–22. doi: 10.1002/hep.27587
25. Apostolopoulou M, Gordillo R, Koliaki C, Gancheva S, Jelenik T, De Filippo E, et al. Specific hepatic sphingolipids relate to insulin resistance, oxidative stress, and inflammation in nonalcoholic steato hepatitis. *Diabetes Care*. 41:1235–43. doi: 10.2337/dc17-1318
26. Wasilewska N, Bobrus-Chociej A, Harasim-Symbor E, Tarasów E, Wojtkowska M, Chabowski A, et al. Increased serum concentration of ceramides in obese children with nonalcoholic fatty liver disease. *Lipids Health Dis*. (2018) 17:216. doi: 10.1186/s12944-018-0855-9
27. Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson HE, Larsson A, et al. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes*. (2014) 63:2356–68. doi: 10.2337/db13-1622
28. Huang H, Kasumov T, Gatmaitan P, Heneghan HM, Kashyap SR, Schauer PR, et al. Gastric bypass surgery reduces plasma ceramide subspecies and improves insulin sensitivity in severely obese patients. *Obesity*. (2011) 19:2235–40. doi: 10.1038/oby.2011.107
29. Özer H, Aslan I, Oruç MT, Çöpelci Y, Afşar E, Kaya S, Aslan M. Early postoperative changes of sphingomyelins and ceramides after laparoscopic sleeve gastrectomy. *Lipids Health Dis*. (2018) 17:269. doi: 10.1186/s12944-018-0917-z
30. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, et al. Muscle sphingolipids during rest and exercise: a C18:0 signature for insulin resistance in humans. *Diabetologia*. (2016) 59:785–98. doi: 10.1007/s00125-015-3850-y
31. Perreault L, Newsom SA, Strauss A, Kerege A, Kahn DE, Harrison KA, et al. Intracellular localization of diacylglycerols and sphingolipids influences insulin sensitivity and mitochondrial function in human skeletal muscle. *JCI insight*. (2018) 3:e96805. doi: 10.1172/jci.insight.96805
32. Montgomery MK, Brown SHJ, Lim XY, Fiveash CE, Osborne B, Bentley NL, et al. Regulation of glucose homeostasis and insulin action by ceramide acyl-chain length: a beneficial role for very long-chain sphingolipid species. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2016) 1861:1828–39. doi: 10.1016/j.bbalip.2016.08.016
33. Meex RCR, Blaak EE, van Loon LJC. Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes. *Obes Rev*. (2019) 20:1205–17. doi: 10.1111/obr.12862
34. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev*. (2018) 98:2133–2223. doi: 10.1152/physrev.00063.2017
35. Turpin-Nolan SM, Hammerschmidt P, Chen W, Jais A, Timper K, Awazawa M, et al. CerS1-derived C18:0 ceramide in skeletal muscle promotes obesity-induced insulin resistance. *Cell Rep*. (2019) 26:1–10.e7. doi: 10.1016/j.celrep.2018.12.031

36. Turner N, Lim XY, Toop HD, Osborne B, Brandon AE, Taylor EN, et al. A selective inhibitor of ceramide synthase 1 reveals a novel role in fat metabolism. *Nat Commun*. (2018) 9:3165. doi: 10.1038/s41467-018-05613-7
37. Rinella ME. Nonalcoholic fatty liver disease a systematic review. *JAMA J Am Med Assoc*. (2015) 313:2263–73. doi: 10.1001/jama.2015.5370
38. Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology*. (2016) 64:1577–86. doi: 10.1002/hep.28785
39. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. *Hepatology*. (2012) 55:2005–23. doi: 10.1002/hep.25762
40. Day CP, James OFW. Steatohepatitis: a tale of two "Hits"? *Gastroenterology*. (1998) 114:842–5. doi: 10.1016/S0016-5085(98)70599-2
41. Rosso N, Chavez-Tapia NC, Tiribelli C, Bellentani S. Translational approaches: from fatty liver to non-alcoholic steatohepatitis. *World J Gastroenterol*. (2014) 20:9038–49. doi: 10.3748/wjg.v20.i27.9038
42. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. *Clin Exp Gastroenterol*. (2014) 7:221–39. doi: 10.2147/CEG.S62831
43. Jiang M, Li C, Liu Q, Wang A, Lei M. Inhibiting ceramide synthesis attenuates hepatic steatosis and fibrosis in rats with non-alcoholic fatty liver disease. *Front Endocrinol*. (2019) 10:665. doi: 10.3389/fendo.2019.00665
44. Yang RX, Pan Q, Liu XL, Zhou D, Xin FZ, Zhao ZH, et al. Therapeutic effect and autophagy regulation of myriocin in nonalcoholic steatohepatitis. *Lipids Health Dis*. (2019) 18:179. doi: 10.1186/s12944-019-1118-0
45. Pewzner-Jung Y, Brenner O, Braun S, Laviad EL, Ben-Dor S, Feldmesser E, et al. A critical role for ceramide synthase 2 in liver homeostasis II. Insights into molecular changes leading to hepatopathy. *J Biol Chem*. (2010) 285:10911–23. doi: 10.1074/jbc.M109.077610
46. Pewzner-Jung Y, Park H, Laviad EL, Silva LC, Lahiri S, Stiban J, et al. A critical role for ceramide synthase 2 in liver homeostasis: I. Alterations in lipid metabolic pathways. *J Biol Chem*. (2010) 285:10902–10. doi: 10.1074/jbc.M109.077594
47. Park WJ, Park JW, Merrill AH, Storch J, Pewzner-Jung Y, Futerman AH. Hepatic fatty acid uptake is regulated by the sphingolipid acyl chain length. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2014) 1841:1754–66. doi: 10.1016/j.bbalip.2014.09.009
48. Park JW, Park WJ, Kuperman Y, Boura-Halfon S, Pewzner-Jung Y, Futerman AH. Ablation of very long acyl chain sphingolipids causes hepatic insulin resistance in mice due to altered detergent-resistant membranes. *Hepatology*. (2013) 57:525–32. doi: 10.1002/hep.26015
49. Zigdon H, Kogot-Levin A, Park JW, Goldschmidt R, Kelly S, Merrill AH, et al. Ablation of ceramide synthase 2 causes chronic oxidative stress due to disruption of the mitochondrial respiratory chain. *J Biol Chem*. (2013) 288:4947–56. doi: 10.1074/jbc.M112.402719
50. Kim YR, Lee EJ, Shin KO, Kim MH, Pewzner-Jung Y, Lee YM, et al. Hepatic triglyceride accumulation via endoplasmic reticulum stress-induced SREBP-1 activation is regulated by ceramide synthases. *Exp Mol Med*. (2019) 51:1–16. doi: 10.1038/s12276-019-0340-1
51. Cinar R, Godlewski G, Liu J, Tam J, Jourdan T, Mukhopadhyay B, et al. Hepatic cannabinoid-1 receptors mediate diet-induced insulin resistance by increasing *de novo* synthesis of long-chain ceramides. *Hepatology*. (2014) 59:143–53. doi: 10.1002/hep.26606
52. Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, et al. CerS6-derived sphingolipids interact with mff and promote mitochondrial fragmentation in obesity. *Cell*. (2019) 177:1536–52.e23. doi: 10.1016/j.cell.2019.05.008
53. Ebel P, Vom Dorp K, Petrasch-Parwez E, Zlomuzica A, Kinugawa K, Mariani J, et al. Inactivation of ceramide synthase 6 in mice results in an altered sphingolipid metabolism and behavioral abnormalities. *J Biol Chem*. (2013) 288:21433–47. doi: 10.1074/jbc.M113.479907
54. Raichur S, Brunner B, Bielohuby M, Hansen G, Pfenninger A, Wang B, et al. The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Mol Metab*. (2019) 21:36–50. doi: 10.1016/j.molmet.2018.12.008
55. Gosejacob D, Jäger PS, Dorp K Vom, Frejno M, Carstensen AC, Köhnke M, et al. Ceramide synthase 5 is essential to maintain C16:0-ceramide pools and contributes to the development of diet-induced obesity. *J Biol Chem*. (2016) 291:6989–7003. doi: 10.1074/jbc.M115.691212

Conflict of Interest: SR is an employee of Evotec International GmbH, Germany.

frontiers in Endocrinology

MINI REVIEW
published: 31 July 2020
doi: 10.3389/fendo.2020.00505

Too Much of a Good Thing? An Evolutionary Theory to Explain the Role of Ceramides in NAFLD

*Annelise M. Poss and Scott A. Summers**

Department of Nutrition and Integrative Physiology, University of Utah, Salt Lake City, UT, United States

Non-alcoholic fatty liver disease (NAFLD), which ranges from the relatively benign and reversible fatty liver (NAFL) to the more advanced and deadly steatohepatitis (NASH), affects a remarkably high percentage of adults in the population. Depending upon severity, NAFLD can increase one's risk for diabetes, cardiovascular disease, and hepatocellular carcinoma. Though the dominant histological feature of all forms of the disease is the accumulation of liver triglycerides, these molecules are likely not pathogenic, but rather serve to protect the liver from the damaging consequences of overnutrition. We propose herein that the less abundant ceramides, through evolutionarily-conserved actions intended to help organisms adapt to nutrient excess, drive the cellular events that define NAFL/NASH. In early stages of the disease process, they promote lipid uptake and storage, whilst inhibiting utilization of glucose. In later stages, they stimulate hepatocyte apoptosis and fibrosis. In rodents, blocking ceramide synthesis ameliorates all stages of NAFLD. In humans, serum and liver ceramides correlate with the severity of NAFLD and its comorbidities diabetes and heart disease. These studies identify key roles for ceramides in these hepatic manifestations of the metabolic syndrome.

Keywords: fatty liver, ceramide, NASH, NAFL, NAFLD (non alcoholic fatty liver disease)

OPEN ACCESS

Edited by:
Ondrej Šeda,
Charles University, Czechia

Reviewed by:
Christa Buechler,
University Medical Center Regensburg, Germany
Giovanni Solinas,
University of Gothenburg, Sweden

***Correspondence:**
Scott A. Summers
scott.a.summers@health.utah.edu

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *11 May 2020*
Accepted: *24 June 2020*
Published: *31 July 2020*

Citation:
Poss AM and Summers SA (2020) Too Much of a Good Thing? An Evolutionary Theory to Explain the Role of Ceramides in NAFLD. Front. Endocrinol. 11:505. doi: 10.3389/fendo.2020.00505

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is reaching epidemic proportions, affecting 25% of the adult population worldwide (1, 2). It consists of two disease states of varying severity: the relatively benign simple fatty liver termed non-alcoholic fatty liver (NAFL) and the more severe non-alcoholic steatohepatitis (NASH). NASH can lead to hepatic scarring (cirrhosis) and fibrosis, which contribute to hepatocellular carcinoma (HCC), the most common liver cancer and the third leading cause of cancer-related death in the USA. Moreover, NAFLD increases one's risk for cardiometabolic disorders including type-2 diabetes and coronary artery disease. These interrelated cardiometabolic disorders account for a sizable proportion of global deaths and overall health expenditures.

The defining feature of NAFL is the accumulation of large lipid droplets in hepatocytes. NASH is a worsened condition characterized by additional hepatocellular ballooning, lobular inflammation, and fibrosis. These histological characteristics of NASH reveal serious tissue damage that can result in organ failure. Though the most striking histological feature of NAFLD across all stages is the accumulation of triglycerides, these lipids are likely inert markers of the condition, rather than being bioactive drivers of the pathology. Instead, studies have identified

roles for sphingolipids such as ceramides in each stage of NAFLD and in many features of NASH: fat accumulation, insulin resistance, mitochondrial dysfunction, apoptosis, and fibrosis. The purpose of this review is to discuss the relevant evidence in both human and animal studies for the role of ceramides in each element of these liver pathologies. Moreover, we propose a unifying, evolutionary theory to explain why ceramides initiate these deleterious actions.

SPHINGOLIPID BIOSYNTHESIS AND DEGRADATION

Sphingolipids are a richly diverse lipid class, comprising over 4,000 distinct species that serve a wide variety of biological roles. Their *de novo* synthesis starts in the endoplasmic reticulum with the condensation of fatty and amino acids to produce the basic sphingoid scaffold. A series of enzymatic reactions follow that produce ceramides, which are the precursors for the more abundant sphingolipids (e.g., sphingomyelins, gangliosides). Sphingolipids play integral roles in membrane structure and fluidity as well as cellular growth and function, including initiation of a coordinated stress response and ultimately apoptosis (3). Circulating factors associated with metabolic disorders including saturated fats, inflammatory cytokines, and glucocorticoids invariably stimulate biosynthesis of ceramides (4, 5).

The first enzyme in the biosynthetic pathway is serine palmitoyl transferase (SPT), which condenses amino (e.g., serine) and fatty acids (e.g., palmitoyl-CoA) to produce the sphingoid backbone (6). The third step in the pathway, catalyzed by one of six different (dihydro)ceramide synthases (CERS1-6), accounts for much of the diversity in sphingolipids by adding acyl chains to the sphingoid scaffold (7). These enzymes show variable substrate specificities and tissue distributions. CERS2 is the primary isoform in the liver, adding the C24 and C24:1 acyl-chains. These very-long-chain ceramides appear benign. CERS5 and 6 add the C16-acyl chains, producing the ceramides that are most strongly implicated in cardiometabolic diseases. CERS6 produces C16-ceramides that contribute to NAFLD and adipose tissue dysfunction (8, 9). CERS5 appears to have a deleterious role in the heart (10). Other articles in this review series will offer a more in-depth analysis of the unique roles and distributions of distinct ceramide species.

The product of the CERS reactions, during *de novo* biosynthesis, are the dihydroceramides. Dihydroceramide desaturases introduce a double bond into the d4 position of the sphingoid backbone of dihydroceramides to produce the more abundant ceramides (11). Despite the structural similarities between ceramides and dihydroceramides, differing by only a double bond, the two molecules exhibit radically different functional roles in cellular signaling and metabolism (12, 13). Mammals contain two desaturases: a ubiquitously expressed DES1 and a skin- and gut-specific DES2 that inserts an additional hydroxyl group in the d4 position to generate phytoceramides.

Following their synthesis in the ER, ceramides and dihydroceramides traffic to the Golgi apparatus where they are converted into complex sphingolipids through the addition of various head groups (e.g., phosphocholine to produce sphingomyelin; sugar moieties to produce the glucosylceramides and gangliosides; phosphate to produce ceramide-1-phosphate; or acyl-CoAs to produce the 1-O-acylceramides). Ceramides can be re-formed by the hydrolysis of the choline head group from sphingomyelins or through a salvage pathway that involves the re-acylation of sphingosine (via CERS enzymes) produced by ceramidase-mediated ceramide degradation (14, 15). Receptors for adiponectin, an anti-diabetic and cardioprotective adipokine, are ligand-activated ceramidases (16, 17). Studies in rodents have demonstrated hepatoprotective effects of adiponectin in multiple modes of liver injury. Moreover, adiponectin is an independent risk factor for NAFLD in diverse clinical cohorts (18–22). The rate of adiponectin-stimulated ceramide degradation thus appears to influence the progression of NAFLD and other cardiometabolic diseases.

Experimental manipulations of the enzymes that control ceramide synthesis or metabolism have produced a wealth of data about the role of ceramides in in the progressive stages of NAFL and NASH, as well as associated comorbidities including insulin resistance and cardiovascular disease. These will be discussed in depth below.

CERAMIDES IN NAFL

NAFL, or simple fatty liver, is characterized in humans by ≥5% macrovesicular fat accumulation in hepatocytes without substantial inflammation or hepatocellular damage (1). NAFL is considered relatively benign and is asymptomatic. Studies demonstrate no direct relationship between the extent of steatosis and survival in humans (23). In the current progressive model of NAFLD development, however, NAFL precedes the more severe liver disease states such as NASH, cirrhosis and HCC.

Though increased hepatic triglyceride deposition precedes NASH, triglycerides are likely inert storage molecules that do not directly elicit the cellular dysfunction that causes NASH (24, 25). In accordance with this theory, inhibiting triglyceride synthesis at the level of DGAT2 reduces steatosis, but enhances fibrosis, oxidative stress, and lipid peroxidation (26, 27). The bioactive ceramides are more likely to drive the pathology. In accordance with this theory, a number of studies have identified correlations between ceramides and different measures of NAFLD/NASH in humans (28–37).

Interventional studies in rodents invariably demonstrate that ceramides are necessary for NAFL development. One such study was performed using mice that allow for conditional depletion of the *Degs1* gene that encodes DES1. Deletion of the gene allows the investigator to acutely replace the ceramides in tissues with dihydroceramides lacking the key double bond (13). Deleting *Degs1* from adult mice either prevented or reversed hepatic steatosis in *ob/ob* mice by blunting lipid uptake (via the fatty acid translocase CD36) and decreasing expression of lipogenic genes (e.g., *Srebf1*). Similarly, liver-specific ablation of the *Degs1* gene encoding DES1 (or liver-targeted knockdown of *Degs1* using shRNA) reversed high fat diet-induced hepatic steatosis

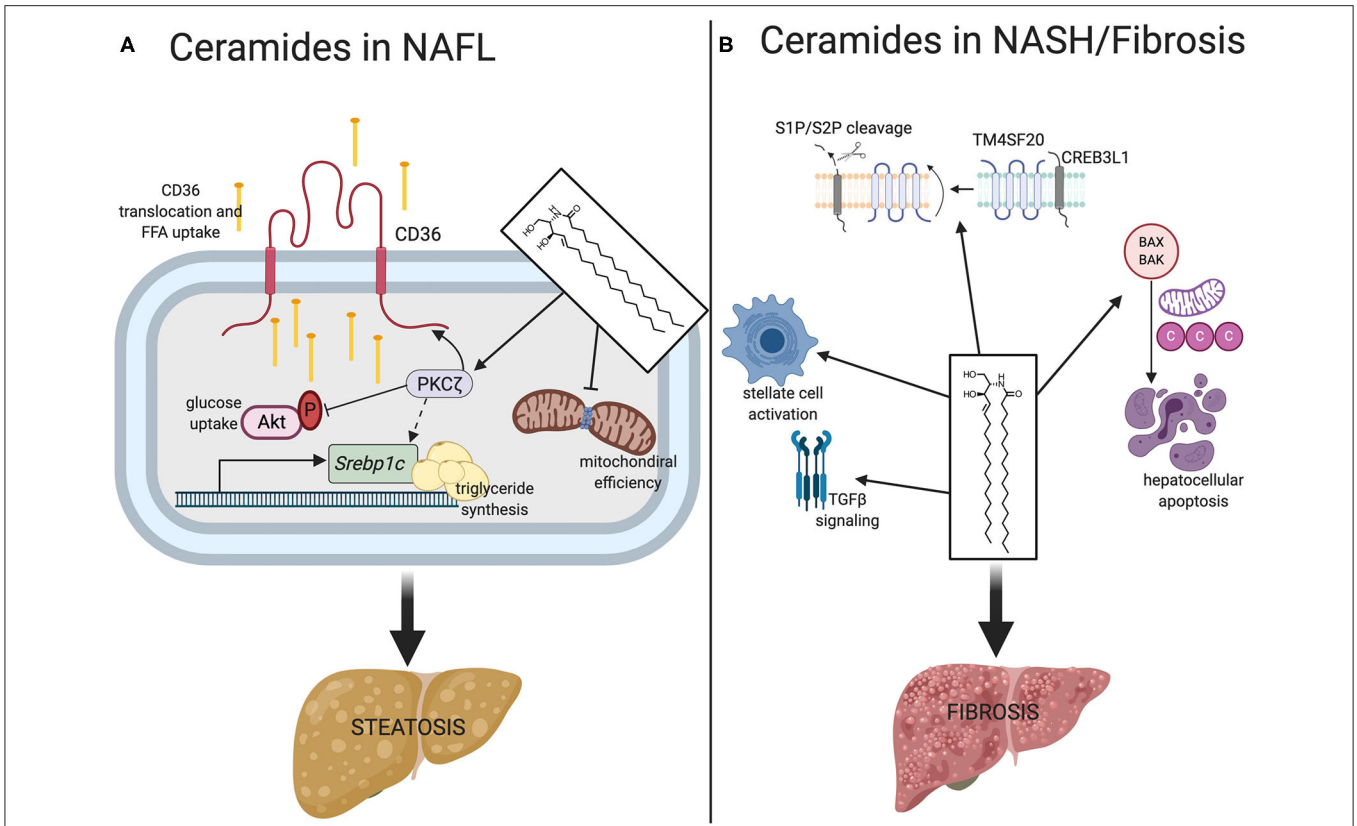

FIGURE 1 | Mechanisms of ceramides contribute to NALF and NASH/fibrosis. **(A)** In NAFL, ceramides promote lipid accumulation ultimately resulting in hepatosteatosis. In promoting steatosis, ceramides stimulate the translocation of CD36 to its active site on the plasma membrane via obligate intermediate PKCζ where it facilitates uptake and esterification of free fatty acids. Ceramide action on PKCζ also upregulates transcription of *Srebpc1* and inhibits Akt. Ceramides also reduce mitochondrial efficiency, promoting fat as a preferred substrate. **(B)** Ceramides promote fibrosis through TGF-β signaling, CREB3L1 proteolysis, stellate cell activation, and hepatocellular apoptosis. *Srebpc1*, sterol regulatory element binding protein 1; S1P/S2P, site 1 protease, site 2 protease; TM4SF20, transmembrane 4 super family 20; CREB3L1, CAMP responsive element binding protein 3. Figure was created with BioRender.com.

(13). Similar findings were reported using mice that allow for inducible, liver-specific overexpression of acid ceramidase (38) or liver-specific deletion of CERS6 (9), which lower ceramides, normalize lipid uptake and metabolism, and resolve diet-induced hepatic steatosis. Thus, depleting ceramides from the liver, by either blocking their synthesis or stimulating their degradation, resolves NAFL. In each of these studies, the interventions also resolved other features of the metabolic syndrome including insulin resistance and serum hypertriglyceridemia.

Though the studies in the preceding paragraph have demonstrated that ceramides are necessary for NAFL, relatively few have evaluated whether they are sufficient to drive the pathology using gain-of-function approaches. *In vivo*, the closest has been studies by the Gonzalez laboratory, which found that intestinal Farnesoid X receptors induced ceramides which traveled to the liver to stimulate steatosis. In these studies, they administered C16-ceramides to antibiotic-treated mice, finding that they induced steatosis and increased expression of the lipogenic gene *Srebp1c* and other transcriptional markers of steatosis (e.g., *Cidea*, *Fasn*, etc.) (39). *In vitro*, overexpression of CERS6 overexpression in primary hepatocytes was shown to be sufficient to increase levels of C16-ceramides, induce triglyceride accumulation, impair mitochondrial respiration, and antagonize insulin signaling (40).

CERAMIDES, LIPID UPTAKE, AND TRIGLYCERIDE SYNTHESIS

Several mechanisms explain the ceramide-induction of hepatic steatosis (**Figure 1A**).

First, ceramides increase lipid uptake into the liver, at least in part by stimulating the translocation of the CD36 fatty acid translocase to the plasma membrane (13, 38). This event involves the atypical protein kinase C zeta (PKCζ) (38), a ceramide effector that has several roles in lipid and glucose metabolism. Addition of exogenous short-chain ceramide analogs activates PKCζ in H4IIe hepatocytes and overexpression of dominant negative PKCζ negates ceramide-induced CD36 translocation. In some tissues, this ceramide-PKCζ axis also impairs glucose utilization through diminished activation of Akt (protein kinase B) (41).

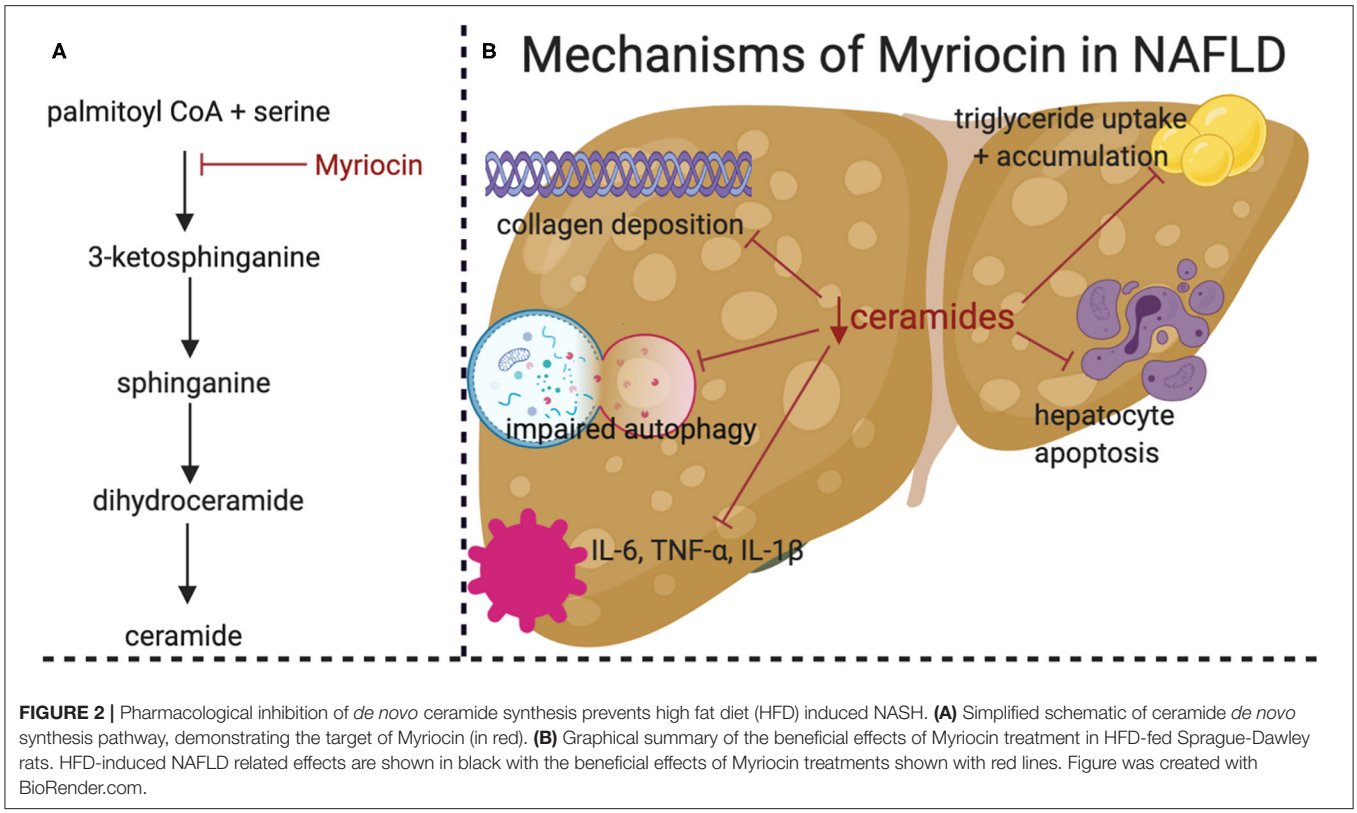

FIGURE 2 | Pharmacological inhibition of *de novo* ceramide synthesis prevents high fat diet (HFD) induced NASH. **(A)** Simplified schematic of ceramide *de novo* synthesis pathway, demonstrating the target of Myriocin (in red). **(B)** Graphical summary of the beneficial effects of Myriocin treatment in HFD-fed Sprague-Dawley rats. HFD-induced NAFLD related effects are shown in black with the beneficial effects of Myriocin treatments shown with red lines. Figure was created with BioRender.com.

Second, ceramides activate signaling pathways that induce triglyceride synthesis, in part by inducing the master transcriptional regulator sterol response element binding protein (SREBP). These are potently induced by ceramides and during NAFLD pathogenesis (39). In fact, gene variants and polymorphisms of *Srebpf1* are associated with increased risk of NAFLD development (42). *In vitro,* ceramides are sufficient to stimulate hepatic *Srebpf1* gene expression, activating a number of downstream targets that facilitate triglyceride production and fatty acid elongation (43). This phenomenon is independent of intracellular cholesterol levels, mediated by ceramides regulating posttranscriptional physiological processing of *Srebpf1*. Similarly, liver specific DES1 depletion in mice displays a striking downregulation of SREBPs (13). This mechanism is likely driven through the aforementioned ceramide-PKCζ axis, which is established as a contributor to hypertriglyceridemia and hepatic steatosis in mice (44). Indeed, several studies have shown that PKCζ regulates SREBP-mediated triglyceride synthesis.

NAFL TO NASH TRANSITION/NASH

The transition from NAFL to NASH worsens an individual's prognosis, as NASH is considerably more likely to progress to HCC and cirrhosis, and ultimately mortality, than the relatively benign NAFL (1, 45). Meta-analyses have also identified fibrosis grade as an independent risk factor for liver and cardiovascular-related mortality (46). Understanding this transition is essential for developing clinical interventions to prevent end stage liver disease and improve patient outcomes. NASH differs from NAFL in that steatosis is accompanied by lobular inflammation, hepatocellular ballooning degeneration, and fibrosis. NAFLD has a complicated bidirectional relationship with insulin resistance, which is considered a driving factor for progression to more severe liver disease states (47).

NASH patients have elevated liver ceramides and increased hepatic expression of ceramide synthesizing genes (29, 30, 32, 48, 49). Ceramides have also been observed as a signature of the NASH disease state in disparate mouse models of the condition, including those that are not obese. For example, an unbiased comparison of two diet-mediated mouse NASH models, the methionine choline deficient and the atherogenic diets, identified alterations in sphingolipid metabolism as a common feature in both (34). This study included a thorough exploration of histological, transcriptional, and lipidomic changes in both models. Despite considerable differences in the mechanisms of these two NASH models, both exhibited decreases in cholesterogenesis, transcriptional upregulation of ceramide synthases, and increased *de novo* sphingolipid synthesis resulting in elevated ceramides, dihydroceramides, and glucosylceramides. In accordance, NASH patients that undergo a weight loss-based lifestyle intervention that reverses the condition show a reduction in circulating ceramides and hepatic expression of pro-ceramide synthesizing genes (32).

In rodents, several studies have shown that pharmacological reduction of ceramides prevents NASH onset (**Figure 2**). The majority have utilized myriocin, which is a potent and irreversible inhibitor of serine palmitoyltransferase. One such

study used high fat diet feeding in adult Sprague-Dawley rats and demonstrated that myriocin prevented steatosis and fibrosis (50). In this experiment, ceramide depletion led to inactivation of signaling by c-Jun N-terminal kinase (JNK), a kinase implicated in inflammatory responses and the regulation of apoptosis. Moreover, myriocin normalized levels of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. A similar study, also conducted in Sprague-Dawley rats fed a high fat diet, demonstrated that myriocin normalized body weight, serum transaminase, and serum triglycerides (28). Studies in cultured hepatocytes showed that myriocin normalized expression profiles of genes involved in fatty acid metabolism and restored autophagy. A final study performed in Wistar rats demonstrated that myriocin treatment for 7 days following establishment of high fat diet induce NAFLD was sufficient to lower ceramides, improve whole body insulin tolerance, and reduce hepatic steatosis (51). Ceramides are also elevated in livers of mice exposed to carbon tetrachloride, a toxin that is commonly used to mimic NASH, fibrosis, and cirrhosis (52).

Unfortunately, no studies involving direct manipulation of ceramide synthesizing genes to increase or decrease ceramides in the context of NASH have been published to date.

CERAMIDES, CELL DEATH, AND FIBROSIS

Apoptosis is an essential element in the cascade of events that cause NASH. In both *in vitro* and *in vivo* models, administration of pan-caspase inhibitors or targeted genetic ablation of specific caspases suppresses apoptosis, and fibrosis (53). Ceramides have long-been known to induce apoptosis, as they increase mitochondrial outer membrane permeability to cytochrome C, leading to caspase activation (54). Blocking ceramide production negates the pro-apoptotic effects of many different cellular insults (55), including the saturated fatty acids and cytokines implicated in NASH. Ceramides have also been demonstrated to induce hepatocellular necrosis through induction of mitochondrial failure (56).

Ceramides alter mitochondrial membrane permeability by several different mechanisms. They recruit the pro-apoptotic protein BAX to mitochondria, where it oligomerizes and increases membrane permeability (57). Ceramides bind voltage-dependent anion channel 2, which further increases mitochondrial outer membrane permeability (58). Ceramides, through extensive hydrogen bonding with themselves, are capable of forming channels in membranes that allow passage of small proteins (59–62). And, ceramides inhibit pro-survival signaling by the protein kinase Akt/PKB (63). This latter effect on Akt/PKB accounts for the ability of ceramides to induce insulin resistance (63, 64).

Ceramides may also directly participate in the fibrosis signaling pathways that control collagen deposition (**Figure 1B**). In fact, hepatic stellate cells, the major extracellular matrix producing cells of the liver, exhibit increased ceramide concentrations and ceramide synthesizing gene transcripts upon activation in culture (65). This suggests that bioactive lipids such as ceramides may affect hepatic stellate cell activation and hepatic collagen deposition during NASH. Though the precise mechanisms remain elusive, ceramides are positive regulators of signaling by TGFβ, a major pro-fibrogenic cytokine (66–68). In addition, ceramides enhance cleavage of plasma membrane resident CREB3L1 by site-1 or site-2 proteases, a process that liberates a protein fragment that enters the nucleus to bind Smad4 and activate transcription of genes required for assembly of collagen-containing extracellular matrix (69, 70). This process, termed regulated intramembrane proteolysis, results from ceramides altering the orientation of TM4SF20, a protein that, in the absence of ceramides, blocks access of the proteases to CREB3L1 (70, 71).

CERAMIDES IN NAFLD ASSOCIATED COMORBIDITIES

NALFD is the hepatic manifestation of the metabolic syndrome. Ranging from a simple fatty liver to the far more severe NASH, the conditions increase risk for diabetes, cardiovascular disease, hepatocellular carcinoma and liver failure. In fact, cardiovascular disease is the most common cause of death in NAFLD patients, accounting for more than twice the number of deaths than progressive liver related complications (1, 72). Even in the absence of metabolic syndrome, NAFLD patients experience increased risk of cardiovascular disease (73).

Studies with knockout mice reveal that the liver provides >50% of serum ceramides (13). Interestingly, measurement of ceramides and related sphingolipids in serum has proven to have clinical utility, serving as potent, cholesterol-independent prognostic biomarkers of cardiovascular disease incidence and mortality (74–79). Circulating ceramides have also been associated with insulin resistance and type 2 diabetes in large clinical cohorts (80–82). Though the literature is largely devoid of large studies relating circulating ceramides to NAFLD, the noted relationships between serum and hepatic ceramides as with established NAFLD and as a distinction between NAFL and NASH strongly suggests that their measurement could have utility for diagnosing the condition (30, 32, 33).

A large proportion of patients with NAFL express a common gene variant (i.e., rs738409) in Patatin-like phospholipase domain-containing protein 3 (PNPLA3). The mutation in this triglyceride lipase gene leads to marked changes in liver fat. Unlike individuals with the "metabolic NAFLD" described in this review, those with PNPLA3-induced NAFLD often remain metabolically healthy, with less insulin resistance, diabetes, and cardiovascular disease (83, 84). Interestingly, ceramide concentrations are not elevated in individuals with "PNPLA3 NAFLD." The authors attributed the lack of hepatic ceramide in subjects with the PNPLA3 variant to their ability to effectively store fat as triglycerides, rather than letting it be diverted into the sphingolipid pathway. Notably, PNPLA3 mutant patients can still develop severe fibrosis (85), suggesting ceramide-independent modes of fibrogenesis exist, in addition to ceramide-dependent ones.

Collectively, these studies indicate that ceramides are markers of NAFLD that play causative roles in several disease features.

While more work is needed to determine the precise role of ceramides in NASH pathogenesis, the data thus far strongly support development of ceramide-lowering strategies to combat these liver pathologies.

AN EVOLUTIONARY BASIS FOR THE ROLE OF CERAMIDES IN NAFLD

We've discussed a number of ceramide-driven mechanisms that influence the health of the hepatocyte under conditions of nutrient overload. We speculate that ceramides serve as nutritional signals, accumulating when the triglyceride stores are saturated and the cellular energy needs are met. The cells respond to the increasing ceramides by initiating actions which protect them from detergent-like FFAs (86). Indeed, each of the ceramide mechanisms we have identified protect cellular membranes from the destabilizing actions of these lytic fatty acids.

In the early stages of disease progressions (e.g., NAFL), ceramides initiate actions that promote the safe uptake of fatty acids through the cellular membrane and facilitate their storage as triglycerides. For example, they increase the allotment of fatty acid translocases (e.g., CD36 or fatty acid binding proteins) on the cellular membrane, which facilitates safe passage of FFAs through the bilayer and speeds their esterification. In parallel, ceramides induce SREBP and its target genes (e.g., DGATs) to enable the incorporation of fatty acids into triglycerides. Ceramides also reduce glucose utilization by inhibiting Akt/PKB, thus allowing cells to switch to fatty acids as a preferred energy source. Lastly, they decrease mitochondrial efficiency, thus maximizing the number of fatty acids that can be oxidized while minimizing the effect on mitochondrial membrane potential. All of these actions, which were at one time likely intended to protect the cell from fatty acid toxicity, underlie NAFL.

As ceramide levels continue to rise, they initiate actions to protect the organism from the compromised, lipid-laden cells. For example, we hypothesize that ceramide-mediated apoptosis and fibrosis are extensions of the aforementioned signaling mechanisms that protect the organism when cells are compromised. For example, ceramide induction of apoptosis allows for a controlled cell death, preventing the release of cytosolic content into the extracellular space. Similarly, the induction of fibrosis allows the organism to protect itself from a damaged region of tissue. Thus, ceramides protect the organism from damage resulting from uncontrolled membrane lysis.

This theory—that ceramides signal cellular events to combat the burden of toxic levels of free fatty acids—provides a unifying framework and testable hypotheses about their role in disease.

CONCLUSIONS

The published data thus far clearly establish roles for ceramides as drivers of different features NAFLD. Nonetheless, additional work is needed. First, more comprehensive assessments of the roles of ceramides in the terminal steps of NASH (e.g., apoptosis and fibrosis) in rodent models are warranted. These should include investigations of whether ceramide-lowering is sufficient to reverse the pathology at the later stages of disease progression. Moreover, it should include genetic manipulations to discern which effects are due to autonomous actions within the hepatocyte. Second, additional clinical verifications, including lipidomics of large liver biobanks and genomic determinations of genes that influence ceramides, would support efforts to develop this therapeutic approach. Despite these areas of need, the evidence is strong that ceramides contribute to NAFLD. Therapeutic approaches that selectively lower ceramides could show enormous progress as a means of combating NAFLD. Ceramides merit a sustained and rigorous evaluation from the scientific community.

AUTHOR CONTRIBUTIONS

AP and SS drafted the manuscript in collaboration with one another. AP generated the figures. All authors contributed to the article and approved the submitted version.

FUNDING

The authors received research support from the National Institutes of Health (DK115824, DK116888, and DK116450 to SS), the Juvenile Diabetes Research Foundation (JDRF 3-SRA-2019-768-A-B to SS), the American Diabetes Association (to SS), the American Heart Association (to SS), and the Margolis Foundation (to SS).

REFERENCES

1. Tesfay M, Goldkamp WJ, Neuschwander-Tetri BA. NASH: the emerging most common form of chronic liver disease. *Mo Med.* (2018) 115:225–9.
2. Ye Q, Zou B, Yeo YH, Li J, Huang DQ, Wu Y, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *The lancet. Gastroenterology & Hepatology* (2020). doi: 10.1016/s2468-1253(20)30077-7
3. Nikolova-Karakashian MN, Rozenova KA. Ceramide in stress response. *Adv Exp Med Biol.* (2010) 688:86–108. doi: 10.1007/978-1-4419-6741-1_6
4. Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res.* (2006) 45:42–72. doi: 10.1016/j.plipres.2005.11.002
5. Summers SA, Nelson DH. A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X, Cushing's syndrome. *Diabetes.* (2005) 54:591–602. doi: 10.2337/diabetes.54.3.591
6. Merrill AH Jr. *De novo* sphingolipid biosynthesis: a necessary, but dangerous, pathway. *J Biol Chem.* (2002) 277:25843–6. doi: 10.1074/jbc.R200009200
7. Cingolani F, Futerman AH, Casas J. Ceramide synthases in biomedical research. *Chem Phys Lipids.* (2016) 197:25–32. doi: 10.1016/j.chemphyslip.2015.07.026
8. Raichur S, Brunner B, Bielohuby M, Hansen G, Pfenninger A, Wang B, et al. The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Mol Metab.* (2019) 21:36–50. doi: 10.1016/j.molmet.2018.12.008
9. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* (2014) 20:678–86. doi: 10.1016/j.cmet.2014.08.002
10. Russo SB, Baicu CF, Van Laer A, Geng T, Kasiganesan H, Zile MR, et al. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. *J Clin Investig.* (2012) 122:3919–30. doi: 10.1172/JCI63888
11. Rodriguez-Cuenca S, Barbarroja N, Vidal-Puig A. Dihydroceramide desaturase 1, the gatekeeper of ceramide induced lipotoxicity. *Biochim Biophys Acta.* (2015) 1851:40–50. doi: 10.1016/j.bbalip.2014.09.021

12. Siddique MM, Li Y, Chaurasia B, Kaddai VA, Summers SA. Dihydroceramides: from bit players to lead actors. *J Biol Chem.* (2015) 290:15371–9. doi: 10.1074/jbc.R115.653204
13. Chaurasia B, Tippetts TS, Monibas RM, Liu J, Li Y, Wang L, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science*. (2019) 365:386–92. doi: 10.1126/science.aav3722
14. Kitatani K, Sheldon K, Rajagopalan V, Anelli V, Jenkins RW, Sun Y, et al. Involvement of acid beta-glucosidase 1 in the salvage pathway of ceramide formation. *J Biol Chem.* (2009) 284:12972–8. doi: 10.1074/jbc.M802790200
15. Claus RA, Dorer MJ, Bunck AC, Deigner HP. Inhibition of sphingomyelin hydrolysis: targeting the lipid mediator ceramide as a key regulator of cellular fate. *Curr Med Chem.* (2009) 16:1978–2000. doi: 10.2174/092986709788682182
16. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med.* (2011) 17:55–63. doi: 10.1038/nm.2277
17. Holland WL, Xia JY, Johnson JA, Sun K, Pearson MJ, Sharma AX, et al. Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced ceramidase signaling in lipid and glucose homeostasis. *Mol Metab.* (2017) 6:267–75. doi: 10.1016/j.molmet.2017.01.002
18. Buechler C, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol.* (2011) 17:2801–11. doi: 10.3748/wjg.v17.i23.2801
19. Lemoine M, Ratziu V, Kim M, Maachi M, Wendum D, Paye F, et al. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. *Liver Int.* (2009) 29:1431–8. doi: 10.1111/j.1478-3231.2009.02022.x
20. Matsuzawa Y. Adiponectin: a key player in obesity related disorders. *Curr Pharm Des.* (2010) 16:1896–901. doi: 10.2174/138161210791208893
21. Fukushima J, Kamada Y, Matsumoto H, Yoshida Y, Ezaki H, Takemura T, et al. Adiponectin prevents progression of steatohepatitis in mice by regulating oxidative stress and Kupffer cell phenotype polarization. *Hepatol Res.* (2009) 39:724–38. doi: 10.1111/j.1872-034X.2009.00509.x
22. Matsumoto H, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A, et al. Adiponectin deficiency exacerbates lipopolysaccharide/D-galactosamine-induced liver injury in mice. *World J Gastroenterol.* (2006) 12:3352–8. doi: 10.3748/wjg.v12.i21.3352
23. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2015) 149:389–97.e10. doi: 10.1053/j.gastro.2015.04.043
24. Postic C, Girard J. Contribution of *de novo* fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Investig.* (2008) 118:829–38. doi: 10.1172/JCI34275
25. Choi SS, Diehl AM. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Curr Opin Lipidol.* (2008) 19:295–300. doi: 10.1097/MOL.0b013e3282ff5e55
26. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology.* (2007) 45:1366–74. doi: 10.1002/hep.21655
27. Li ZZ, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. *J Biol Chem.* (2009) 284:5637–44. doi: 10.1074/jbc.M807616200
28. Yang RX, Pan Q, Liu XL, Zhou D, Xin FZ, Zhao ZH, et al. Therapeutic effect and autophagy regulation of myriocin in nonalcoholic steatohepatitis. *Lipids Health Dis.* (2019) 18:179. doi: 10.1186/s12944-019-1118-0
29. Alkhouri N, Dixon LJ, Feldstein AE. Lipotoxicity in nonalcoholic fatty liver disease: not all lipids are created equal. *Exp Rev Gastroenterol Hepatol.* (2009) 3:445–51. doi: 10.1586/egh.09.32
30. Luukkonen PK, Zhou Y, Sadevirta S, Leivonen M, Arola J, Oresic M, et al. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J Hepatol.* (2016) 64:1167–75. doi: 10.1016/j.jhep.2016.01.002
31. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology.* (2010) 52:774–88. doi: 10.1002/hep.23719
32. Promrat K, Longato L, Wands JR, de la Monte SM. Weight loss amelioration of non-alcoholic steatohepatitis linked to shifts in hepatic ceramide expression and serum ceramide levels. *Hepatol Res.* (2011) 41:754–62. doi: 10.1111/j.1872-034X.2011.00815.x
33. Wasilewska N, Bobrus-Chociej A, Harasim-Symbor E, Tarasow E, Wojtkowska M, Chabowski A, et al. Increased serum concentration of ceramides in obese children with nonalcoholic fatty liver disease. *Lipids Health Dis.* (2018) 17:216. doi: 10.1186/s12944-018-0855-9
34. Montandon SA, Somm E, Loizides-Mangold U, de Vito C, Dibner C, Jornayvaz FR. Multi-technique comparison of atherogenic and MCD NASH models highlights changes in sphingolipid metabolism. *Sci Rep.* (2019) 9:16810. doi: 10.1038/s41598-019-53346-4
35. Simon J, Ouro A, Ala-Ibanibo L, Presa N, Delgado TC, Martinez-Chantar ML. Sphingolipids in non-alcoholic fatty liver disease and hepatocellular carcinoma: ceramide turnover. *Int J Mol Sci.* (2019) 21:40. doi: 10.3390/ijms21010040
36. Montefusco DJ, Allegood JC, Spiegel S, Cowart LA. Non-alcoholic fatty liver disease: insights from sphingolipidomics. *Biochem Biophys Res Commun.* (2018) 504:608–16. doi: 10.1016/j.bbrc.2018.05.078
37. Luukkonen PK, Sadevirta S, Zhou Y, Kayser B, Ali A, Ahonen L, et al. Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars. *Diabetes Care.* (2018) 41:1732–9. doi: 10.2337/dc18-0071
38. Xia JY, Holland WL, Kusminski CM, Sun K, Sharma AX, Pearson MJ, et al. Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab.* (2015) 22:266–78. doi: 10.1016/j.cmet.2015.06.007
39. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Investig.* (2015) 125:386–402. doi: 10.1172/JCI76738
40. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab.* (2014) 20:687–95. doi: 10.1016/j.cmet.2014.09.015
41. Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol.* (2003) 23:7794–808. doi: 10.1128/MCB.23.21.7794-7808.2003
42. Moslehi A, Hamidi-Zad Z. Role of SREBPs in liver diseases: a mini-review. *J Clin Transl Hepatol.* (2018) 6:332–8. doi: 10.14218/JCTH.2017.00061
43. Worgall TS, Juliano RA, Seo T, Deckelbaum RJ. Ceramide synthesis correlates with the posttranscriptional regulation of the sterol-regulatory element-binding protein. *Arterioscler Thromb Vasc Biol.* (2004) 24:943–8. doi: 10.1161/01.atv.0000125703.20434.4d
44. Chen TC, Lee RA, Tsai SL, Kanamaluru D, Gray NE, Yiv N, et al. An ANGPTL4-ceramide-protein kinase Czeta axis mediates chronic glucocorticoid exposure-induced hepatic steatosis and hypertriglyceridemia in mice. *J Biol Chem.* (2019) 294:9213–24. doi: 10.1074/jbc.RA118.006259
45. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. *BMC Med.* (2017) 15:45. doi: 10.1186/s12916-017-0806-8
46. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology.* (2017) 65:1557–65. doi: 10.1002/hep.29085
47. Manco M. Insulin resistance and NAFLD: a dangerous liaison beyond the genetics. *Children.* (2017) 4:74. doi: 10.3390/children4080074
48. Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology.* (2009) 50:1827–38. doi: 10.1002/hep.23229
49. Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology.* (2007) 46:1081–90. doi: 10.1002/hep.21763
50. Jiang M, Li C, Liu Q, Wang A, Lei M. Inhibiting ceramide synthesis attenuates hepatic steatosis and fibrosis in rats with non-alcoholic fatty liver disease. *Front Endocrinol.* (2019) 10:665. doi: 10.3389/fendo.2019.00665
51. Kurek K, Piotrowska DM, Wiesiolek-Kurek P, Lukaszuk B, Chabowski A, Gorski J, et al. Inhibition of ceramide *de novo* synthesis reduces liver lipid

accumulation in rats with nonalcoholic fatty liver disease. *Liver Int.* (2014) 34:1074–83. doi: 10.1111/liv.12331
52. Ichi I, Nakahara K, Fujii K, Iida C, Miyashita Y, Kojo S. Increase of ceramide in the liver and plasma after carbon tetrachloride intoxication in the rat. *J Nutr Sci Vitaminol.* (2007) 53:53–6. doi: 10.3177/jnsv.53.53
53. Akazawa Y, Nakao K. To die or not to die: death signaling in nonalcoholic fatty liver disease. *J Gastroenterol.* (2018) 53:893–906. doi: 10.1007/s00535-018-1451-5
54. Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. *Science.* (1993) 259:1769–71. doi: 10.1126/science.8456305
55. Hernandez-Corbacho MJ, Salama MF, Canals D, Senkal CE, Obeid LM. Sphingolipids in mitochondria. *Biochim Biophys Acta.* (2017) 1862:56–68. doi: 10.1016/j.bbalip.2016.09.019
56. Arora AS, Jones BJ, Patel TC, Bronk SF, Gores GJ. Ceramide induces hepatocyte cell death through disruption of mitochondrial function in the rat. *Hepatology.* (1997) 25:958–63. doi: 10.1002/hep.510250428
57. Ganesan V, Perera MN, Colombini D, Datskovskiy D, Chadha K, Colombini M. Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis.* (2010) 15:553–62. doi: 10.1007/s10495-009-0449-0
58. Dadsena S, Bockelmann S, Mina JGM, Hassan DG, Korneev S, Razzera G, et al. Ceramides bind VDAC2 to trigger mitochondrial apoptosis. *Nat Commun.* (2019) 10:1832. doi: 10.1038/s41467-019-09654-4
59. Siskind LJ, Kolesnick RN, Colombini M. Ceramide forms channels in mitochondrial outer membranes at physiologically relevant concentrations. *Mitochondrion.* (2006) 6:118–25. doi: 10.1016/j.mito.2006.03.002
60. Siskind LJ, Davoody A, Lewin N, Marshall S, Colombini M. Enlargement and contracture of C2-ceramide channels. *Biophys J.* (2003) 85:1560–75. doi: 10.1016/S0006-3495(03)74588-3
61. Siskind LJ, Kolesnick RN, Colombini M. Ceramide channels increase the permeability of the mitochondrial outer membrane to small proteins. *J Biol Chem.* (2002) 277:26796–803. doi: 10.1074/jbc.M200754200
62. Siskind LJ, Colombini M. The lipids C2- and C16-ceramide form large stable channels. Implications for apoptosis. *J Biol Chem.* (2000) 275:38640–4. doi: 10.1074/jbc.C000587200
63. Zhou H, Summers SA, Birnbaum MJ, Pittman RN. Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. *J Biol Chem.* (1998) 273:16568–75. doi: 10.1074/jbc.273.26.16568
64. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol.* (1998) 18:5457–64. doi: 10.1128/MCB.18.9.5457
65. Shmarakov IO, Jiang H, Liu J, Fernandez EJ, Blaner WS. Hepatic stellate cell activation: a source for bioactive lipids. *Biochim Biophys Acta.* (2019) 1864:629–42. doi: 10.1016/j.bbalip.2019.02.004
66. Nikolova-Karakashian M. Sphingolipids at the crossroads of NAFLD and senescence. *Adv Cancer Res.* (2018) 140:155–90. doi: 10.1016/bs.acr.2018.05.002
67. Shea BS, Tager AM. Sphingolipid regulation of tissue fibrosis. *Open Rheumatol J.* (2012) 6:123–9. doi: 10.2174/1874312901206010123
68. Sato M, Markiewicz M, Yamanaka M, Bielawska A, Mao C, Obeid LM, et al. Modulation of transforming growth factor-beta (TGF-beta) signaling by endogenous sphingolipid mediators. *J Biol Chem.* (2003) 278:9276–82. doi: 10.1074/jbc.M211529200
69. Chen Q, Lee CE, Denard B, Ye J. Sustained induction of collagen synthesis by TGF-beta requires regulated intramembrane proteolysis of CREB3L1. *PLoS ONE.* (2014) 9:e108528. doi: 10.1371/journal.pone.0108528
70. Chen Q, Denard B, Lee CE, Han S, Ye JS, Ye J. Inverting the topology of a transmembrane protein by regulating the translocation of the first transmembrane helix. *Mol Cell.* (2016) 63:567–78. doi: 10.1016/j.molcel.2016.06.032
71. Denard B, Lee C, Ye J. Doxorubicin blocks proliferation of cancer cells through proteolytic activation of CREB3L1. *Elife.* (2012) 1:e00090. doi: 10.7554/eLife.00090
72. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: a systematic review and meta-analysis. *Sci Rep.* (2016) 6:33386. doi: 10.1038/srep33386
73. Tana C, Ballestri S, Ricci F, Di Vincenzo A, Ticinesi A, Gallina S, et al. Cardiovascular risk in non-alcoholic fatty liver disease: mechanisms and therapeutic implications. *Int J Environ Res Public Health.* (2019) 16:3104. doi: 10.3390/ijerph16173104
74. Anroedh S, Hilvo M, Akkerhuis KM, Kauhanen D, Koistinen K, Oemrawsingh R, et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J Lipid Res.* (2018) 59:1729–37. doi: 10.1194/jlr.P081281
75. de Carvalho LP, Tan SH, Ow GS, Tang Z, Ching J, Kovalik JP, et al. Plasma ceramides as prognostic biomarkers and their arterial and myocardial tissue correlates in acute myocardial infarction. *JACC Basic Transl Sci.* (2018) 3:163–75. doi: 10.1016/j.jacbts.2017.12.005
76. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, et al. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol.* (2016) 36:2424–30. doi: 10.1161/ATVBAHA.116.307497
77. Hilvo M, Meikle PJ, Pedersen ER, Tell GS, Dhar I, Brenner H, et al. Development and validation of a ceramide- and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. *Eur Heart J.* (2019) 41:371–80. doi: 10.1093/eurheartj/ehz387
78. Poss AM, Maschek JA, Cox JE, Hauner BJ, Hopkins PN, Hunt SC, et al. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. *J Clin Invest.* (2019) 130:1363–76. doi: 10.1172/JCI131838
79. Tarasov K, Ekroos K, Suoniemi M, Kauhanen D, Sylvanne T, Hurme R, et al. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J Clin Endocrinol Metab.* (2014) 99:E45–52. doi: 10.1210/jc.2013-2559
80. Lemaitre RN, Yu C, Hoofnagle A, Hari N, Jensen PN, Fretts AM, et al. Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: the strong heart family study. *Diabetes.* (2018) 67:1663–72. doi: 10.2337/db17-1449
81. Thorens B, Rodriguez A, Cruciani-Guglielmacci C, Wigger L, Ibberson M, Magnan C. Use of preclinical models to identify markers of type 2 diabetes susceptibility and novel regulators of insulin secretion - A step towards precision medicine. *Mol Metab.* (2019) 27S:S147–54. doi: 10.1016/j.molmet.2019.06.008
82. Wigger L, Cruciani-Guglielmacci C, Nicolas A, Denom J, Fernandez N, Fumeron F, et al. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep.* (2017) 18:2269–79. doi: 10.1016/j.celrep.2017.02.019
83. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nat Genet.* (2017) 49:1758–66. doi: 10.1038/ng.3977
84. Simons N, Isaacs A, Koek GH, Kuc S, Schaper NC. Brouwers PNPLA3 M, TM6SF2, and MBOAT7 genotypes and coronary artery disease. *Gastroenterology.* (2017) 152:912–3. doi: 10.1053/j.gastro.2016.12.020
85. Chen L-Z, Xin Y-N, Geng N, Jiang M, Zhang D-D, Xuan S-Y. PNPLA3 I148M variant in nonalcoholic fatty liver disease: demographic and ethnic characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. *World J Gastroenterol.* (2015) 21:794–802. doi: 10.3748/wjg.v21.i3.794
86. Summers SA. Ceramides: nutrient signals that drive hepatosteatosis. *J Lipid Atheroscler.* (2020) 9:50–65. doi: 10.12997/jla.2020.9.1.50

Conflict of Interest: SS is a consultant and shareholder with Centaurus Therapeutics.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 07 August 2020
doi: 10.3389/fendo.2020.00491

Sphingolipid Metabolism and Signaling in Skeletal Muscle: From Physiology to Physiopathology

*Sophie Tan-Chen[1,2], Jeanne Guitton[3], Olivier Bourron[1,2,4], Hervé Le Stunff[3] and Eric Hajduch[1,2]**

[1] Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, Université de Paris, Paris, France, [2] Institut Hospitalo-Universitaire ICAN, Paris, France, [3] Université Saclay, CNRS UMR 9197, Institut des Neurosciences Paris-Saclay, Orsay, France, [4] Assistance Publique-Hôpitaux de Paris, Département de Diabétologie et Maladies Métaboliques, Hôpital Pitié-Salpêtrière, Paris, France

Sphingolipids represent one of the major classes of eukaryotic lipids. They play an essential structural role, especially in cell membranes where they also possess signaling properties and are capable of modulating multiple cell functions, such as apoptosis, cell proliferation, differentiation, and inflammation. Many sphingolipid derivatives, such as ceramide, sphingosine-1-phosphate, and ganglioside, have been shown to play many crucial roles in muscle under physiological and pathological conditions. This review will summarize our knowledge of sphingolipids and their effects on muscle fate, highlighting the role of this class of lipids in modulating muscle cell differentiation, regeneration, aging, response to insulin, and contraction. We show that modulating sphingolipid metabolism may be a novel and interesting way for preventing and/or treating several muscle-related diseases.

Keywords: ceramide, insulin, diabetes, obesity, sphingosine-1-phosphate, glycosphingolipids, sphingomyelin

OPEN ACCESS

Edited by:
Scott A. Summers,
The University of Utah, United States

Reviewed by:
Bryan Bergman,
University of Colorado, United States
Clinton Bruce,
Deakin University, Australia

***Correspondence:**
Eric Hajduch
eric.hajduch@crc.jussieu.fr

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *30 April 2020*
Accepted: *22 June 2020*
Published: *07 August 2020*

Citation:
Tan-Chen S, Guitton J, Bourron O, Le Stunff H and Hajduch E (2020) Sphingolipid Metabolism and Signaling in Skeletal Muscle: From Physiology to Physiopathology. Front. Endocrinol. 11:491. doi: 10.3389/fendo.2020.00491

INTRODUCTION

Sphingolipids (SLs) have been studied and described for several years as modulators of many functions in cells, including proliferation, differentiation, mobility, and survival. Skeletal muscle represents by far the most important tissue for maintaining posture/vertical position, movement, and locomotion of the human body. It is also the major tissue where insulin stimulates glucose uptake, and to store it into glycogen, making it a key organ in carbohydrate homeostasis maintenance (1, 2). Since abundant literature shows that SLs regulate positively or negatively many of biological functions, such as cell proliferation and differentiation, contraction, and insulin response, the extensive study of the function of these lipids in muscle cells is very important because they could represent pharmacological targets to counteract several important diseases, such as muscular dystrophies and diabetes.

OVERVIEW OF SPHINGOLIPID METABOLISM

SLs represent one of the major classes of eukaryotic lipids. They were originally described as intermediate lipids for the synthesis of other lipids. They mainly play a structural role, especially in cell membranes where they exert signaling properties and are capable of modulating multiple cell functions, such as apoptosis, cell proliferation, and inflammation (3). This is a class of lipids defined

by their sphingoid skeleton made up of 18 carbons with an amine and two alcohol groups (4). This lipid class includes many lipids with various structures and functions.

Ceramides, central lipids for SL biosynthesis, are mainly synthesized via three metabolic pathways (**Figure 1**):

- The *de-novo* synthesis pathway from saturated fatty acid (FA) that takes place in the endoplasmic reticulum (ER).
- The sphingomyelinase (SMase) pathway that uses SMase to convert sphingomyelin (SM) present in the cell membrane to give ceramide.
- The salvage pathway in the lysosome that produces sphingosine, and then ceramide after breakdown of complex SL.

De-novo Ceramide Synthesis Pathway

SL biosynthesis is induced at the cytosolic leaflet membrane of the ER where ceramide is synthesized after several enzymatic reactions (5, 6). First, condensation of palmitate and serine forms 3-keto-dihydrosphingosine. This reaction is catalyzed by serine palmitoyl transferase (SPT) and is rate-limiting for the pathway. It should be noted that SPT can also use both glycine and alanine to make atypical 1-deoxysphingolipids (7). Functions of 1-deoxysphyngolipids remain still unclear, but these lipids appear to be significantly elevated in plasma from diabetic (8) and non-alcoholic fatty liver disease patients (9). In addition, 1-deoxysphingolipids have been shown to be toxic to β-cells and neurons (10, 11). SPT can also use myristate instead of palmitate, resulting in the production of a d16:0 sphingoid base (10). Interestingly, d16 SL promoted cell death of cardiomyocytes (12). Then, 3-keto-dihydrosphingosine (KDSR) is reduced to dihydrosphingosine, which is acylated by ceramide synthases (CerS) to produce dihydroceramide (Dcer). In mammals, six CerS isoforms (CerS 1 to 6) are expressed. They perform identical chemical reaction but produce ceramide species with different acyl-CoA chain length. Eventually, Dcer desaturase-1 (DES1) desaturates Dcer to give ceramide (**Figure 1**).

Abbreviations: ACER, Alkaline ceramidases; ApoM, Apolipoprotein M; ASAH1, Acid ceramidase; ASAH2, Neutral ceramidase; aSMase, Acid sphingomyelinase; C1P, Ceramide-1-phosphate; CDase, Ceramidase; CEM, Cavolin Enriched Microdomains; CerK, Ceramide kinase; CerS, Ceramide synthases; CERT, CERamide Transporter; CGT, Galactosyl transferase; CPT1, Carnitine palmitoyltransferase 1; Dcer, Dihydroceramide; DES1, Dcer desaturase-1; DES2, C4-hydroxylase / Δ4-desaturase; ER, Endoplasmic reticulum; ERK1/2, Signal-regulated kinase ½; GCS, Glucosylceramide synthase; GBA, Acid β-glucosidase; GLUT, Glucose transporter; GM3, Ganglioside monosialo 3; FA, Fatty acids; FABPm, Plasma membrane-associated FA-binding protein; FAT/CD36, FA translocase/cluster of differentiation; Fgf21, Fibroblast Growth Factor 21; HFD, High fat diet; IRS, Insulin receptor substrates; JNK, c-Jun kinase; KDSR, 3-keto-dihydrosphingosine; KO, Knock-out; LDL, Low-density lipoproteins; LPP, Phosphohydrolases; mTORC2, Mammalian target of rapamycin-2; nSMase, Neutral sphingomyelinase; PDK1, Phosphoinositide-dependent kinase 1; PI3K, Phosphoinositide-3- kinase; PKCζ, Protein kinase C ζ; PKR, Double-stranded RNA-dependent protein kinase; PP2A, Protein phosphatase 2A; Prep1, Pbx regulating protein 1; S1P, Sphingosine-1-phosphate; S1PR, S1P receptors; SFA, Saturated FA; SMS, SM synthases; SL, Sphingolipids; SM, Sphingomyelin; SMase, Sphingomyelinases; SMS, Sphingomyelin synthase; SphK, Sphingosine kinases; SPL, S1P-lyase; SPT, Serine palmitoyl transferase; T2D, Type 2 diabetes; TAG, Triacylglycerol; TNFα, Tumor necrosis factor α; WAT, White adipose tissue.

During the conversion of Dcer into ceramide, an alternative product can be synthesized, 4-hydroxyceramide, or phytoceramide (13), and it is the second member of the DES family, C4-hydroxylase/Δ4-desaturase (DES2) that catalyses the formation of phytoceramide from Dcer (13). DES2 is highly expressed in fungi, plants, but also in the intestine, kidney, and skin where phytoceramide is present in large quantity (14).

ER-synthesized ceramide is then immediately transported into the Golgi apparatus to generate other SL. This translocation is mediated by two types of ceramide transport. The first, and the most characterized ceramide transporter, is the non-vesicular transporter CERamide Transporter (CERT). CERT specifically transports ceramide from the ER and displays small activity toward other SL. CERT efficiently mediates the transfer of ceramide-containing C14–C20 FAs to the Golgi in order to be transformed into SM. The second type of ceramide transporter is a vesicular transporter (15). This transporter ensures the transport of long-chain ceramide (>C20) into the Golgi to generate glucosylceramides. Unlike CERT, this vesicular transporter has not been well-characterized and is known to be dependent on phosphoinositide-3-kinase (PI3K) activity (4).

Once in the Golgi apparatus, ceramide can be further metabolized to SM and complex glycosphingolipids.

SM, one of the major components of cell membranes, is synthesized through the transfer of a phosphorylcholine head group from phosphatidylcholine to ceramide. This reaction is catalyzed by SM synthases (SMS) (5). Two isoforms of SMS exist: SMS1 and 2. Both are present in the Golgi, but SMS2 is also localized at the plasma membrane, maintaining plasma membrane SM content (**Figure 1**) (16).

Ceramide can also be transformed into glycosylphingolipids, such as glucosylceramide (in the Golgi apparatus), or galactosylceramide (in the ER). Glucosylceramide is the precursor of gangliosides, whereas galactosylceramide will be transformed into sulfatides (**Figure 1**) (17). Glucosylceramides are synthesized from ceramide and UDP-glucose. This reaction is catalyzed by glucosylceramide synthase (GCS), which is localized on the cis side of the Golgi (17). On the other hand, synthesis of galactosylceramide takes place on the lumenal surface of the ER under the control of the galactosyl transferase (CGT) (4, 18).

Glucosylceramides are SL that play an essential role in mammal development and survival, as for instance, in cell recognition processes (19). Galactosylceramides are essential to myelin structure and function, and they are involved in oligodendrocyte function (4, 20).

In the Golgi, ceramides can also be phosphorylated by the ceramide kinase (CerK), thus generating ceramide-1-phosphate (C1P). C1P acts as a docking site for the cytosolic phospholipase A2 and enhances arachidonic acid release. C1P is involved in cell growth, anti-apoptosis, and inflammation in numerous cell types (**Figure 1**) (21, 22).

Sphingomyelinase and Ceramidase Pathways

Hydrolysis of SM to give ceramide is catalyzed by SMases. SMases catalyze the cleavage of the phosphocholine head

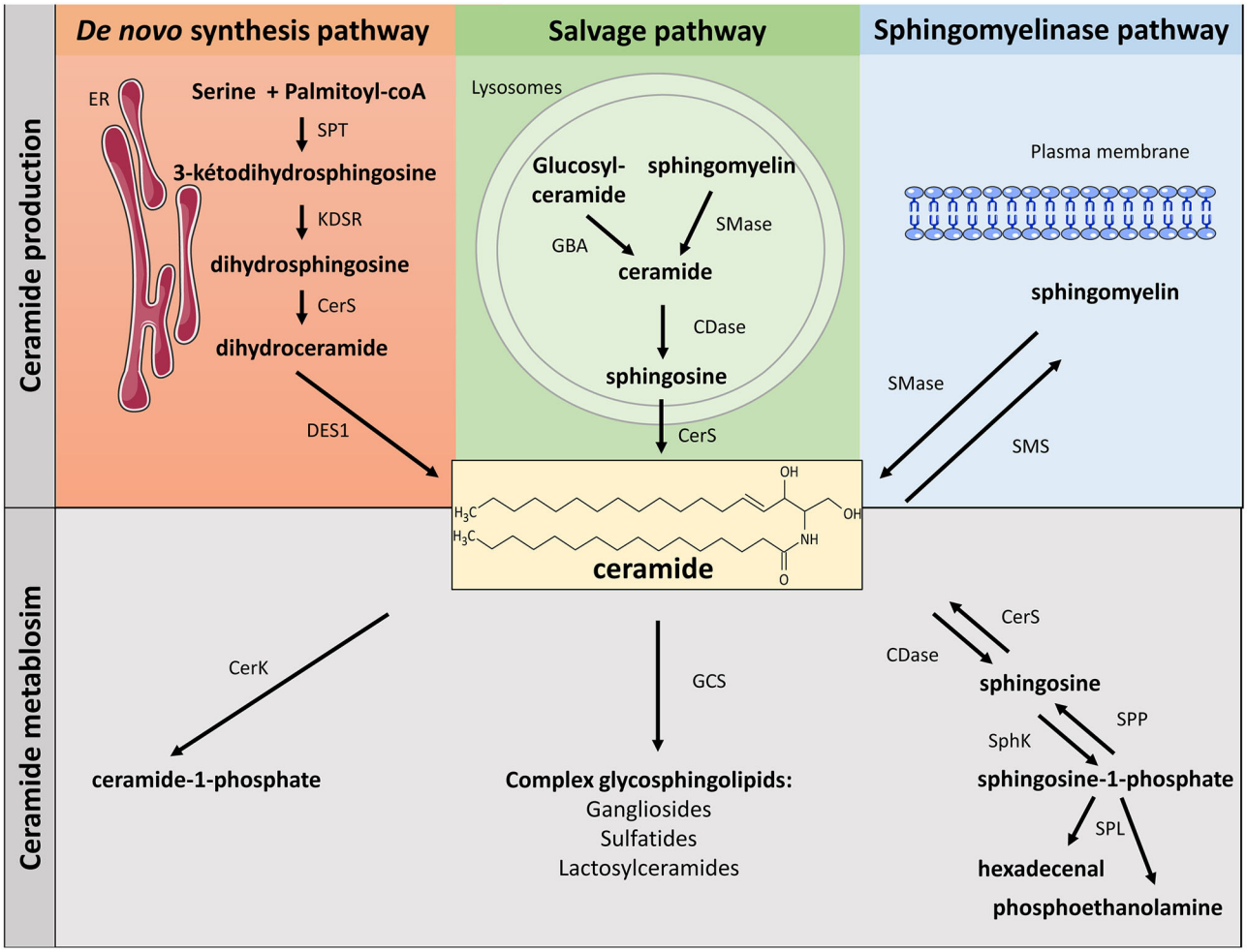

FIGURE 1 | Metabolism of sphingolipids. SLs can be synthetized through three major metabolic pathways: the *de-novo* pathway coming from the condensation of SFA (palmitate) with serine, the salvage pathway and the SMase pathway. ER, endoplasmic reticulum; SPT, serine palmitoyl transferase; KDSR, 3-keto-dihydrosphingosine; CerS, ceramide synthase; DES, dihydroceramide desaturase; GBA, acid β-glucosidase; SMase, sphingomyelinase; CDase, ceramidase; SMase, sphingomyelinase; SMS, sphingomyelin synthase; CerK, ceramide kinase; GCS, glucosyl ceramide synthase; SphK, sphingosine kinase; SPP, lipid sphingosine phosphatase; SPL, sphingosine-1-phosphate lyase.

group of SM to generate ceramide (**Figure 1**). Three kinds of SMases have been identified, based upon their optimum pH: acid sphingomyelinase (aSMase), alkaline SMase, and neutral sphingomyelinase (nSMase) (4). Even though they catalyze a similar reaction, these three enzymes are different and display different subcellular localizations. Alkaline SMase, exclusively expressed in the intestine and liver, plays a role in the absorption of dietary SM. On the other hand, both aSMase and nSMase are ubiquitously expressed and play major roles in SM catabolism in most tissues. aSMase is predominantly a lysosomal enzyme that metabolizes SM present on endosomal membranes, whereas three isoforms of nSMase (nSMase 1, nSMase 2, and nSMase 3) are present in the inner leaflet of the plasma membrane (23). nSMase 2 is the most studied isoform and has emerged as a key mediator in the generation of ceramide in response to many stresses to disturb diverse signaling pathways in the fields of cancer, growth and development, and in inflammatory responses (24).

SM degradation is a major pathway involved in producing ceramide and some of its lipid metabolites, and in particular sphingosine-1-phosphate (S1P), last SL before the final stage of SL degradation (**Figure 1**). Ceramide is first deacylated by ceramidase (CDase) to produce sphingosine (4). There are three isoforms of CDase classified, like SMases, according to their optimal pH of function and their subcellular location. Acid CDase (ASAH1) is localized in lysosomes and is more effective in deacylating medium-chain ceramides (C12- and C14- rather than C16- and C18-ceramide) (4, 25). Neutral CDase (ASAH2) is an important regulator for the production of sphingosine and S1P at the plasma membrane (4, 25). There are three alkaline CDases (ACER). ACER1 is mainly localized in the epidermis ER and it deacylates C24:0- and C24:1-ceramides. ACER2 is

localized in the Golgi and is very strongly expressed in the placenta. ACER2 deacylates C16-, C18-, C20-, C24:0, and C24:1-ceramides. ACER3 is a phytoceramidase that is localized in both the RE and the Golgi. It is expressed in many tissues, its most important expression being found in the placenta, like ACER2 (4, 25). Interestingly, sphingosine is considered to be exclusively generated from ceramide through the action of CDase. *De-novo* synthesis of sphingosine has been ruled out since DES1 specifically acts on Dcer, not on dihydrosphingosine (26).

Then, sphingosine is phosphorylated by sphingosine kinases (SphK) to generate S1P (**Figure 1**) (4). Two isoforms of SphK exist (SphK1 and SphK2). They catalyze the same reaction but with different subcellular localizations (27). SphK1 is mainly a cytosolic enzyme and contains residues that bind acidic phospholipids that contribute to SphK1 intracellular localization (28). SphK1 can be activated by growth factors, cytokines, and G protein-coupled receptors (GPCR) ligands. Extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylate SphK1 on its serine 225 residue, thus inducing its translocation to cell plasma membrane where sphingosine is localized (29). In contrast, SphK2 is localized in different cell compartments, such as the nucleus, endoplasmic reticulum, and mitochondria (27). The mechanism by which SphK2 is regulated remains obscure and, in opposite to SphK1-produced S1P that is rapidly excreted outside the cell, SphK2-produced S1P is rapidly degraded to be recycled into sphingosine to give ceramide (30).

S1P degradation is controlled either by specific lipid phosphate phosphohydrolases (LPP) that hydrolyze S1P to give sphingosine, or by the S1P-lyase (SPL), splitting S1P into hexadecenal and phosphoethanolamine (**Figure 1**) (31).

S1P is a strong signal mediator that affects several cellular functions essential for health and diseases (32). Many different S1P-mediated actions are explained by the fact that S1P modulates intracellular functions, but also, after secretion into the extracellular environment, S1P acts as a ligand of GPCR called S1P receptors that are present on most cell membranes (32). S1P possess five specific receptors (S1P1-5) on the cell surface (27). Binding of S1P to its receptors allows the transmission of various signals in cells. In addition to being able to relay its effects via its receptors, S1P has several direct intracellular targets involved in gene expression, inflammation, and mitochondrial function (27).

Interestingly, S1P has been shown to play a significant role in the control of cell survival and growth (effects opposite to those of ceramides), and SphK1 has emerged as a central signaling enzyme. As such, SphK1 is placed at a key point, controlling the balance between the prosurvival and proapoptotic SL metabolites, namely the "sphingolipid rheostat" (**Figure 2**) (32).

Salvage/Recycling Pathway

The recycling pathway for long-chain sphingoid bases leading to SL regeneration constitutes between 50 and 90% of SL biosynthesis (33, 34). This suggests a crucial role of this pathway in the biosynthesis/recycling of SL and an important implication in cell signaling.

The constitutive degradation of complex SL and glycosphingolipids (35, 36) occurs in acidic subcellular compartments such as endosomes and lysosomes. In the case of glycosphingolipids, exohydrolases (β-glucosidase acid) release monosaccharide units to generate ceramide. Likewise, SM present in the lysosomes are reconverted into ceramide via aSMase (37). The common metabolic product of these degradations (ceramide) is hydrolyzed by acid CDase to give sphingosine and free FAs capable (unlike ceramide) of leaving lysosomes (38, 39). Then, sphingosine and free FAs released from the lysosome enter the ceramide biosynthesis pathway (40, 41) to generate new ceramide molecules via CerS (42–44), or can be phosphorylated by sphingosine kinases into S1P (**Figure 1**) (43).

SPHINGOLIPIDS AND THE REGULATION OF MUSCLE BIOLOGY

In skeletal muscle, and in addition to their roles as structural membrane components, it has been shown that many metabolites derived from the metabolism of ceramide play different key roles in various biological effects, such as cell proliferation, differentiation, survival, and mobility. In addition, it is now clear that several SL play a major role in regulating muscle insulin response. Two SL derivatives have been mainly studied, namely ceramide and S1P, and it is interesting to note is that these two lipids often relay opposite biological actions. For example, ceramide is involved in apoptosis, cellular stress, and cell growth arrest, while S1P plays important roles in mitogenesis, cell differentiation, and migration. Similarly, S1P has been shown to prevent programmed cell death normally induced by ceramide. This homeostatic system is known as the ceramide/S1P rheostat (**Figure 2**) (32).

At the muscle level, ceramide and S1P have been also shown to exert opposite roles. Indeed, intracellular accumulation of ceramides displays largely negative actions, while an increase in S1P concentrations in muscle cells frequently potentiate opposite functions to those of ceramide.

Regulation of Growth, Differentiation, Regeneration, and Aging of Muscle Cells by SPHINGOLIPIDS

There is a mechanism that is regulated by the ceramide/S1P rheostat in muscle cells, which is growth and differentiation of skeletal muscle cells (**Figure 2**).

Most of the experiments studying *in-vitro* skeletal muscle growth and differentiation have been carried out in the C2C12 muscle cell line, derived from mouse muscle satellite cells (45). They showed that S1P plays a major role in the activation of satellite cells. More specifically, inhibition of S1P synthesis through the use of SphK inhibitors demonstrated the importance of S1P to induce the entry of satellite cells into the cell cycle by inhibiting cell proliferation and inducing their myogenic differentiation (46, 47). The importance of S1P receptors in the initiation of the C2C12 muscle cell differentiation phenotype has been highlighted in this mechanism (**Figure 2A**) (47), and it has been demonstrated that S1P stimulates positively cell migration through the activation of both S1P1 and S1P4, whereas S1P2 was found to negatively regulate cell migration (48). In addition,

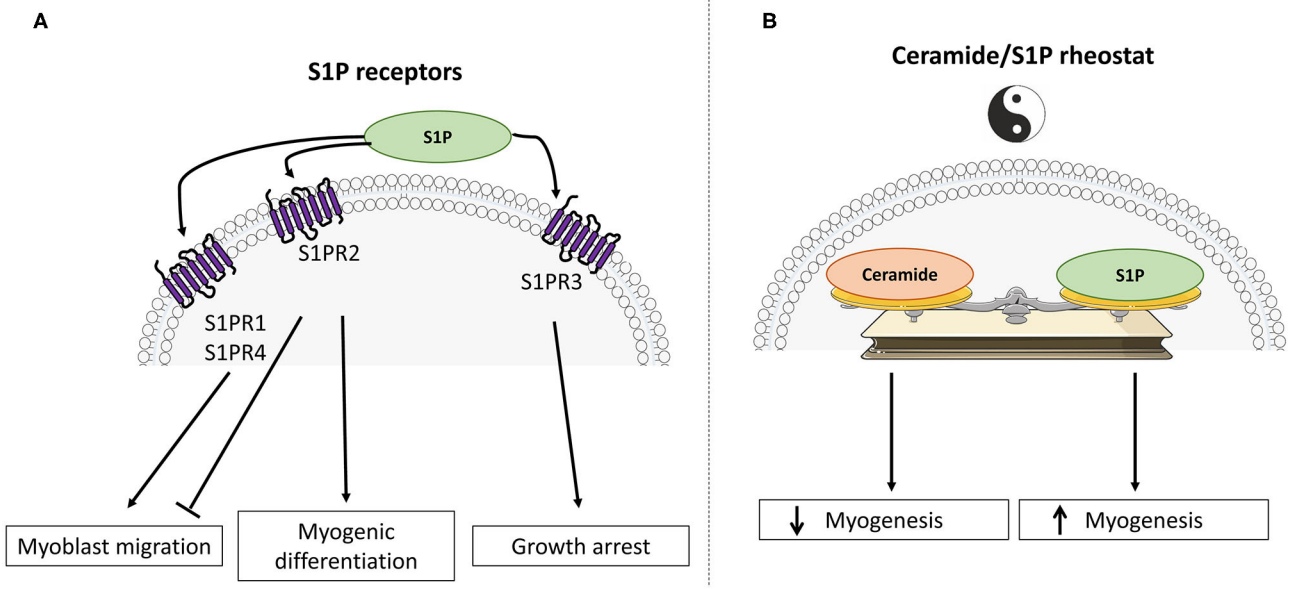

FIGURE 2 | Divergent roles of sphingosine-1-phosphate and ceramide in skeletal muscle cell fate. **(A)** Schematic representation of S1PR signaling activation in muscle differentiation. **(B)** The "ceramide / sphingosine-1-phosphate rheostat" model emphasizes antagonistic roles of ceramide and S1P in the regulation of myogenesis and glucose metabolism.

activation of the S1P3 suppresses cell cycle progression in muscle satellite cells (49).

Interestingly, S1P receptor expression changes occur during myoblast differentiation into myotubes. Indeed, it has been observed a downregulation of the S1P2, while expression of the S1PR3 was increased in differentiated cells (50). Using both pharmacological and genetic approaches, a study demonstrated that S1P, through its binding to S1P2, reduced serum-induced myoblast proliferation and potently stimulates myogenesis (47).

SphK1 also plays a role in this mechanism, because overexpression of this kinase in cells reduced myoblastic proliferation and increased the expression of myogenic differentiation markers (51). In contrast, inhibition of SphK1 expression increased cell proliferation and delayed the onset of myogenesis (51, 52). In addition, stimulation of myogenesis in cells overexpressing SphK1 is inhibited when the S1P2 receptor was suppressed, reinforcing the idea that the expression of the S1P2 receptor is critical for relaying the pro-myogenic action of S1P in muscle cells (53).

In addition to muscle differentiation, S1P plays an important role in skeletal muscle regeneration. Indeed, a notable property of skeletal muscle is its capacity to regenerate in response to injury. Satellite cells are activated, proliferate, migrate, differentiate, and fuse to form new myofibers (54), and several studies have shown that S1P signaling was involved in this process [reviewed in (55)]. S1P stimulates the proliferation of a muscle cell reserve through the activation of its S1P1 (56), and exogenous addition of S1P stimulates the growth of regenerating myofibers after a myotoxic injury induced by intramuscular injection of bupivacaine (57). Interestingly, S1P was shown to suppress muscle degeneration in Duchenne muscular dystrophy (58), and to exert a positive action for activating satellite cells and muscle regeneration in dystrophic muscles (59, 60). It was also reported that S1P could protect skeletal muscle tissue against eccentric contraction-induced damage, further emphasizing the relevance of S1P signaling in skeletal muscle protection and regeneration (61).

In contrast to S1P signaling, ceramide appears to negatively regulate myogenic differentiation. It has been shown in C2C12 muscle cells that a treatment with ceramide abrogated cell differentiation (62) and the myogenic marker myogenin (63). Inhibition of the *de-novo* ceramide synthesis pathway using potent inhibitors (fumonisin B1 and myriocin) decreased intracellular concentrations of ceramide and increased the appearance of a cell differentiation phenotype (63). Interestingly, ceramide has been shown to regulate negatively muscle differentiation through downregulation of phospholipase D expression and activity (63).

Similar results were obtained after treating myoblasts with the pro-inflammatory cytokine tumor necrosis factor α (TNFα), known to induce ceramide synthesis through the activation of the SMase pathway (64). Strle et al. (65) showed that TNFα-induced ceramide production inhibited the expression of critical muscle-specific transcription factors and myogenesis. In addition, inhibition of ceramide synthesis improved myocyte size under conditions inducing muscle atrophy (a mouse model of cancer-induced cachexia and in muscle cells treated with TNF-α) (66), thus reinforcing the negative action of the lipid on muscle-mass regulation.

All these results highlight the importance of the SL metabolism in regulating muscle differentiation mechanism. Ceramide and S1P act as a rheostat by regulating myogenic

differentiation of satellite cells rather than their survival, depending on cell conditions (**Figure 2B**).

Some data also demonstrated that SL metabolism could be involved in the development of sarcopenia with aging and muscle atrophy, often observed during obesity. Several studies showed that, during aging, there is a decrease in muscle protein synthesis (67) and increased tissue insulin resistance (68). Insulin is a powerful anabolic factor that promotes muscle protein synthesis, primarily by inhibiting protein breakdown (69, 70). A metabolic characteristic of aging is an increase of intramyocellular lipid content, and this lipid increase could play an important role in the development of insulin resistance of muscle cells with age. Studies have recently shown a significant accumulation (2- to 4-fold) of different SL, including ceramide, sphingosine, and their respective metabolites Dcer, sphingosine, and S1P in muscle cells from older mice compared to young congeners (71). In parallel, increased expression of inflammatory markers TLR2, TNF-α, and IL-1β, and a higher phosphorylation and ubiquitination of NF-κB key inhibitor IκBα were observed with aging (71). These data could demonstrate that an association exists between the accumulation of critical bioactive SL, such as ceramide, associated with inflammation, and a decrease in lean mass and muscular strength observed with age in mouse skeletal muscle. Unfortunately, no human studies corroborating these results obtained in rodents have been published so far.

Interestingly, an increase in ASMase expression has been shown to induce a decreased skeletal muscle force in mice (72). Thus, this modification ASMase expression could play in important role in changes of SL turnover observed with age (see above). Using various ASMase inhibitors, Babenko et al. (73) demonstrated that downregulation of ASMase reduced skeletal muscle ceramide content and ceramide/SM ratio and increased SM of old rats to the levels close to those observed in young animals. Unfortunately, S1P content had not been assessed in these conditions. Since S1P displays pro-differentiation activity (see above), S1P concentrations could vary inversely to those of ceramides. In addition, inhibition of ASMase activity improved skeletal muscle insulin response of old rats up to the level of younger animals (74). If confirmed in humans, these data suggest that age-dependent upregulation of ASMase could play an important role in the modulation of skeletal muscle ceramide content during age and could be a therapeutic target for treatment of age-dependent pathologies.

Sphingolipids and Insulin Resistance

According to several studies, skeletal muscle accounts for 30–70% of insulin-stimulated glucose disposal in the postprandial state (75, 76). Thus, most of the studies on the mechanisms of insulin resistance induced by lipotoxicity have been carried out mainly in muscle tissue.

In 1963, Randle and colleagues hypothesized that competition between glucose and FA for their oxidation and absorption was responsible for the development of insulin resistance in muscle and adipose tissue (77). *In-vivo* studies in rodents and humans have confirmed a relationship between lipids and insulin resistance, but they also showed that, unlike Randle's hypothesis, lipid-induced insulin resistance was not secondary to inhibition of glycolysis (78). They demonstrated that lipids act directly on insulin signaling, blocking the translocation of the insulin-sensitive glucose transporter GLUT4 to the plasma membrane in response to the hormone, and thus a decrease in glucose uptake in cells (78). In humans, data clearly showed a strong correlation between intramuscular lipid content and insulin cell resistance (79).

Thus, strong evidence exists between muscle lipid accumulation and insulin resistance. However, in some cases, like in the "athlete's paradox," no correlation between ectopic lipid accumulation and peripheral insulin resistance was found. Athletes display high insulin sensitivity but also present increased levels of intramuscular lipids (80), suggesting that it is not only an increased muscle lipid content that affects insulin sensitivity level, but rather the types of lipids stored in muscle. Indeed, FAs contribute to insulin resistance because they lead to the synthesis of many lipid derivative intermediates such as SL. Ceramide must have been the most studied SL for its critical role in the onset of muscle insulin resistance. However, other SL derivatives synthesized from ceramide (S1P, C1P, SM, and glucosylceramide) were also shown to modulate insulin signaling in muscle cells.

Ceramide and Insulin Resistance

One of the first studies to analyse intracellular ceramide concentrations in obese insulin-resistant Zucker fa/fa rats was that of Turinsky et al. (81, 82). They found that these rats had increased muscle ceramide concentrations (81, 82). Increased levels of ceramide have also been detected in skeletal muscle of several other insulin resistance models [insulin resistant rodents, such as ob/ob mice, high fat diet (HFD)-fed mice, and rats infused with intra-lipids] [reviewed in (83)]. Overall, these studies illustrate an inverse relationship between muscle ceramide concentrations and insulin sensitivity. Consistent with data obtained in rodents, human studies also confirm the inverse relationship between an accumulation of skeletal muscle ceramide and insulin sensitivity [reviewed in (84)] (85–87). In addition, the Goodpaster group showed that physical exercise reduced muscle ceramide concentrations in obese and insulin-resistant subjects, all correlated with improved insulin sensitivity (88).

Several studies highlighted the crucial role of muscle ceramide in the development of insulin resistance and type 2 diabetes (T2D) (89). This relationship has also been confirmed *in vitro* in mouse C2C12, rat L6, and human myotubes (90–92). Indeed, the exposure of cells to a saturated fatty acid (SFA) (palmitate) or directly to ceramide inhibited the activation by insulin of glycogen synthesis and glucose transport (89). Overall, these studies demonstrated a strong association between insulin resistance and an increase in ceramide concentrations in skeletal muscles.

In vivo, a direct implication of ceramide accumulation in the onset of insulin resistance and diabetes has been established in several studies. Mice fed a HFD and treated with myriocin (inhibitor of the SPT) displayed an inhibition of ceramide synthesis, improvement in muscle Akt phosphorylation, and a better glucose tolerance and insulin sensitivity (93). These

data were confirmed in DES1 KO mice. A decrease in ceramide concentrations in insulin-sensitive tissues was observed in dexamethasone-induced diabetes treated mice compared to ceramide concentrations observed in control mice (94). Increased insulin sensitivity was highlighted in the DES1 KO animals compared to their wild-type littermates (94). Finally, treatment with myriocin of HFD-fed rats induced a drop in soleus muscle ceramide concentrations, as well as a normalization of glucose tolerance and insulin sensitivity (95).

However, and despite the abundant literature linking ceramide and muscle insulin resistance, it is important to note that there are nevertheless several studies that do not fully report this causal link. Indeed, some studies did not show any modification of muscle ceramide concentrations in response to lipid overload in rats (96), healthy subjects (97), obese, and diabetic human subjects (98–100). Another study did not show any change in overall muscle ceramide content in T2D patients after endurance training despite a clear improvement in muscle insulin sensitivity (101). In addition, one study showed no difference in muscle ceramide content between T2D patients and healthy individuals (100). Finally, a study showed that a single prior bout of exercise could protect isolated muscle rat against palmitate-induced insulin resistance through a ceramide-independent mechanism (102).

Overall, while numerous studies showed that ceramide was an important player in the onset of muscle insulin resistance, the picture is not that clear-cut, and it cannot be ruled out that other contributors such as diacyl-glycerols [reviewed in (78)] must also play a significant contribution in the appearance of a defect in the action of insulin in muscle.

Importance of Ceramide Species in the Development of Muscle Insulin Resistance

Muscle plays a notable role in glucose homeostasis regulation. During obesity, when the buffering action of adipose tissue is saturated and cannot act as a protective metabolic sink, muscle faces an excess of circulating free FA, resulting in an ectopic storage of different lipid such as ceramide in muscle, which are known to induce insulin resistance. Because it remains partially unknown whether specific ceramide species from muscle contribute to insulin resistance and obesity, only recently, generation of different CerS knock-out (KO) mice allowed elucidating their specificities in metabolic disorders.

In-vitro studies suggested several ceramide species as key players in mediating deleterious effects of SFAs, such as palmitate on insulin signaling in muscle cells (84). Similarly to what was observed in both L6 and C2C12 myotubes, incubation of primary cultures of muscle cells derived from human biopsies with palmitate led to both an increase of endogenous C16- and C18-ceramide and an impaired insulin signaling (92). Interestingly, these observations were confirmed in primary culture of skeletal muscle cells obtained from diabetic patients (92, 103). Nevertheless, the identity of CerS isoforms implicated in ceramide biosynthesis responsible for muscle insulin resistance remains still elusive.

Each CerS displays characteristic substrate specificity and produces ceramides with specific acyl chain distributions, thereby regulating explicit cell functions. Among them, CerS1 total KO mice suffer from severe neurological modifications (104), while CerS2 KO mice develop hepatocarcinoma (105) and CerS3 KO is lethal (106). CerS4 regulates stem cell homeostasis and hair follicle cycling (107), CerS5 regulates lung phosphatidylcholine synthesis (108), and CerS6 plays a critical role in the development of autoimmune encephalomyelitis (109). These various biological functions regulated by all CerS suggest that ceramide exhibit chain length-specific functions.

Positive correlation between muscle C16- and C18-ceramide content and insulin resistance had been highlighted for some time (95, 110–112). However, molecular evidences of an intramuscular synthesis of these ceramide species related to their insulin resistant properties were still lacking. In the last few years, generation of CerS KO mice allowed to go further in the identification of which ceramide species is (are) involved in the development of muscle insulin resistance.

In 2016, Gosejacob et al. demonstrated that CerS5 played a central role to maintain C16-ceramide pool in insulin sensitive tissues (113). They showed that CerS5 expression was essential to maintain cellular C16-ceramide content in lung, spleen, muscle, white adipose tissue (WAT) and liver, independently of feeding conditions. Under HFD conditions, a massive increase in C16-ceramide content was mainly observed in WAT compared to control mice (113). CerS5 deficiency prevented WAT C16-ceramide increase and improved glucose intolerance, insulin resistance, WAT mass, adipocyte size, and triglycerides levels in mice challenged with HFD (113). Altogether, this study highlighted new insight about the implication of CerS5 in maintaining whole-body homeostasis, but independently from its expression in muscle, suggesting that C16-ceramide produced by CerS5 may not play a role in the onset of muscle insulin resistance. In agreement, Turpin-Nolan et al. (114) found that mice with a muscle-specific CerS5 deletion exhibited no alterations in adiposity, systemic insulin sensitivity, and glucose tolerance upon exposure to a HFD.

A study investigated a role for C16-ceramide produced by CerS6 (using CerS6 KO mice) in the progression of muscle insulin resistance. CerS6 is another CerS isoform known to generate ceramide with C16 acyl chain, and HFD-fed CerS6 KO were protected from HFD-induced obesity and glucose intolerance. Furthermore, mice exhibited reduced C16-ceramide content in liver, white and brown adipose tissues but not in muscle (115). Interestingly, mice with a muscle-specific CerS6 did no reverse the effect of HFD on insulin sensitivity (114). Altogether, these studies argue against a role of a direct role of a local production of C16-ceramide either by CerS5 or CerS6 into muscle insulin resistance.

Given the lack of molecular evidence for a role of intramuscular C16-ceramide production by these two CerS in muscle insulin resistance, other studies focused on C18-ceramide as another potential candidate. Gosejacob et al. (113) already showed that CerS5 KO mice challenged with a HFD were protected against C18-ceramide accumulation in muscle. These data, in conjunction with other studies showing that C18-ceramide content in muscle was increased with lipotoxicity (92, 103, 114), suggest that accumulation of this ceramide species may

play a role in muscle insulin resistance appearance (**Figure 3A**). Additional studies performed in rats (95, 116) and humans (117) also highlighted a direct role of C18-ceramide in the onset of muscle insulin resistance.

Synthetized by CerS1, C18-ceramide is one of the major ceramide species upregulated in skeletal muscle of mice fed with HFD (113, 114). Turpin-Nolan et al. (114) generated a global CerS1 KO mice that, under a HFD, displayed reduced C18-ceramide production in skeletal muscle, accompanied by an increase in C16-, C22:0, C22:1, C24:0, and C24:1 ceramide species. Particularly, CerS1 deficiency did not affect the SL content in heart, liver, and white adipose tissue (114). These changes in ceramide contents were accompanied with decreased body weight and adiposity and higher energy expenditure and a better glucose tolerance (114). In addition, CerS1 deficient mice improved their glucose tolerance and insulin sensitivity under HFD (114). Mice lacking CerS1 were also generated specifically in skeletal muscle (114). These mice under a HFD displayed a strong reduction in C18-ceramide in this tissue, but, and in opposite to global CerS1 KO mice, no difference in weight gain, adiposity, and food intake was observed (114). However, glucose metabolism was still improved in muscle CerS1 deficient mice under HFD in response due to an increase of Fibroblast Growth Factor 21 (Fgf21) release from skeletal muscle into the circulation (114). Fgf21 is a growth factor mainly synthetized by the liver and other organs, and it can be secreted as a compensatory mechanism to fight against metabolic disorders associated with obesogenic conditions, and is known to exert anti-inflammatory and insulin-sensitizing actions (118). In absence of CerS1 in muscle, muscle-derived Fgf21 seemed to improve the ability of insulin to suppress hepatic glucose production (114) (**Figure 3B**). Taken together, these studies highlighted CerS1 and its consequent reduction of C18-ceramide in muscle as key components in ameliorating whole-organism glucose metabolism. However, the latter study also reveals that intramuscular C18-ceramide produced by CerS1 may not play a direct role in muscle insulin resistance (114).

Beyond genetic models, a specific CerS1 pharmacological inhibitor, P053, has been generated (119). Its daily oral administration in mice fed an HFD decreased by 50% C18-ceramide content in skeletal muscle. This decrease was associated with reduced whole-body adiposity due to enhanced FA oxidation in this tissue. However, and unlike the CerS1 muscle deficient mice, this inhibitor did not improved glucose tolerance and insulin sensitivity in HFD-fed mice (119). This suggests that C18-ceramide may not be the main SL lipid actor for the development of insulin resistance, or possibly that the residual muscle C18-ceramide content (50%) was sufficient to inhibit insulin sensitivity (in opposite to 90% ablation observed in Turpin-Nolan's study). Interestingly, another study tended to suggest that other ceramide species could also be involved in muscle insulin resistance. Bandet et al. (120) showed that overexpression of CERT transporter in in tibialis anterior muscle from mice fed a HFD induced a decrease in concentrations of C16-, C22-, C24:1-, and C24-ceramide species associated with an improved insulin response. No change in both C18- and C20-ceramide species was observed (120). Thus, further studies will be necessary to exactly define whether other ceramide species than C18-ceramide produced by others CerS (i.e., CerS3 and 4) could also play a significant and deleterious role in the modulation of muscle insulin sensitivity.

Subcellular Localization and Mechanism of Action of Ceramide in Muscle Cells

Subcellular localization of SL has emerged to be crucial in relation to insulin resistance in skeletal muscle. Ceramide is generated in the ER through *de-novo* synthesis and is transported to the Golgi apparatus to be metabolized into other SL (**Figure 1**). This transport involves an ATP-dependent carrier called CERT (121). Ceramide can also be produced in the plasma membrane through conversion of SM into ceramide by SMase (**Figure 1**) (4). Interestingly, CerS isoforms were found in various intracellular organelles, such as the ER (122), the mitochondria (123), the nucleus (124), and the Golgi apparatus (123), suggesting a possible local ceramide production in all these compartments. Thus, contribution of ceramide to cellular dysfunctions could be related to these subcellular compartments.

Perreault et al. (125) found an accumulation of ceramide, sphingomyelin, and lactosylceramide in sarcolemma of insulin-resistant individuals. Similar data were observed by Chung et al. (112). Interestingly, association between raise in intramyocellular ceramide concentrations and hyperinsulinemia/hypertriglyceridemia was only limited to the sarcolemmal fraction (112), and only C16- and C18-ceramides were found to be positively correlated with insulin resistance markers (112). These data should be closely compared to the absence of role of CerS1, 5, and 6 isoforms in muscle insulin resistance found in some studies (113, 114, 119). In these latter studies, both C16 and C18-ceramides were quantified in whole muscle. Therefore, it would be important to determine whether these CerS could affect specifically subcellular localization of C16 and C18-ceramides.

In sarcolemma compartment, ceramides have been shown to inhibit insulin signaling through different mechanisms in muscle cells. The first step in activating the insulin signaling pathway is the hormone binding to its membrane receptor, leading to the autophosphorylation of its β subunits (126). Following this autophosphorylation, the insulin receptor catalyzes the activation of insulin receptor substrates (IRS). Six different isoforms of IRS are expressed, but only IRS1 and 2 control insulin signal transduction. IRS1 is predominantly expressed in skeletal muscle where it plays a crucial role in the transmission of the insulin signal (127). Activated IRS1 activates a protein called PI3K that generates specific membrane phospholipids essential for the activation of a protein kinase called Akt (also called Protein Kinase B) (126, 128). In response to insulin, Akt is recruited to the plasma membrane via phospholipids generated by PI3K where it is activated by phosphorylation at two critical sites: threonine 308 (T308) in the activation loop and serine 473 (S473) in the hydrophobic motif of the kinase (126). The phosphoinositide-dependent kinase 1 (PDK1) phosphorylates Akt at its T308 site and the S473 site is phosphorylated by the mTORC2 (mammalian target of rapamycin-2) complex (126). Akt relays the many metabolic actions of insulin in its different target tissues (128,

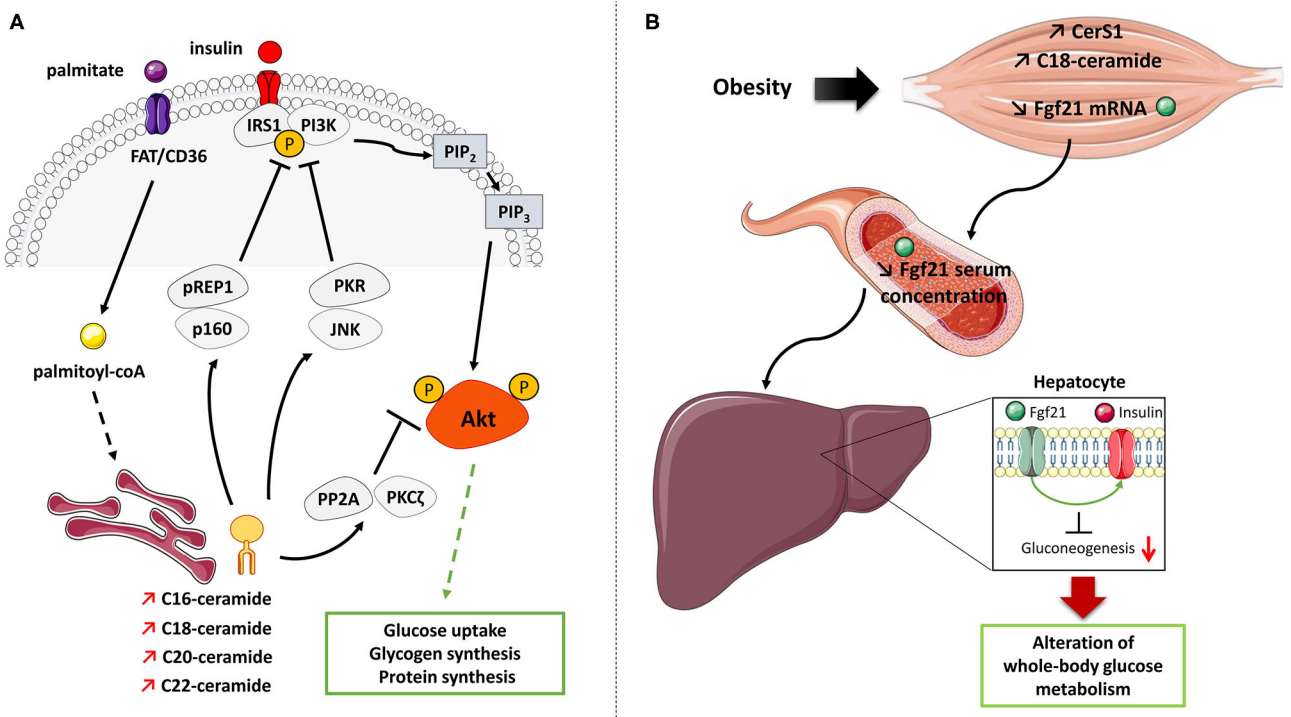

FIGURE 3 | Mechanisms of action of ceramide on insulin signaling and glucose metabolism in muscle cells. **(A)** Palmitate induces the *de-novo* production of several ceramide species in muscle cells. Ceramide inhibits the muscle insulin signaling pathway and muscle metabolism by targeting two important actors, Akt and IRS1. Ceramide rapidly activates either PP2A or PKCζ to inactivate Akt. Ceramides also induce PKR/JNK and/or Prep1/p160 axes to target IRS1. IRS1, Insulin receptor substrate 1; JNK, c-Jun NH2-terminal kinase; PI3K, Phosphoinositide-3-kinase; PKCζ, Protein kinase C ζ; PKR, Double stranded ARN-activated protein kinase; PP2A, Protein phosphatase 2A; Prep1, Pbx regulating protein 1; P, phosphorylation. **(B)** Proposed model of muscle ceramide action on regulating whole-body glucose metabolism. Obesity-induced lipid overflow in muscle induces an increase in CerS1 expression and C18-ceramide content which decrease in Fgf21 production and secretion from muscle cells. This lack of Fgf21 secretion could affect the ability of insulin to suppress hepatic glucose production and will induce hyperglycemia.

129). Insulin stimulates the uptake of glucose in skeletal muscles and adipocytes, induces the synthesis of glycogen and its storage in muscle and in the liver, induces protein synthesis in muscle, and inhibits adipocyte lipolysis and production hepatic glucose (89, 130).

The mechanisms by which ceramide acts on the insulin signaling pathway in insulin-sensitive tissues are now clear, at least in both rodent and human muscle cells. In these cells, ceramide targets the insulin signaling by specifically inhibiting in a time-dependent manner two specific actors in this pathway: Akt and IRS1. Regarding Akt, ceramide rapidly (within 2 h) activates the atypical protein kinase C ζ (PKCζ), which interacts and phosphorylates the pleckstrin homology (PH) domain of Akt on a Thr34/Ser34 residue, thus inducing its sequestration in specialized areas of the plasma membrane called Caveolin Enriched Microdomains (CEM) (131–136). Targeting Akt in CEM prevents the kinase from being recruited to the plasma membrane where it is normally activated in response to insulin (136, 137). In this time frame, the authors showed that the inhibition of the insulin signaling pathway was not mediated at the level of the insulin receptor, IRS1, PI3K, or PDK1, but was only due to a targeted action of ceramide on Akt (91, 138, 139). In CEM devoid cells, ceramide inhibits Akt by a mechanism involving the protein phosphatase 2A (PP2A) (90, 137). In the longer term, ceramide also target and inhibit IRS1 by mechanisms involving PKR/JNK (double-stranded RNA-dependent protein kinase/c-Jun kinase) and/or Prep1/p160 (**Figure 3A**) (140, 141).

De-novo synthetized ceramides are transported from the ER to the Golgi apparatus through a transporter called CERT, where they are metabolized into SM (4). Disrupted CERT-mediated transport of ceramide from the ER to the Golgi was observed in both mouse and human muscle cells under lipotoxic conditions, leading to a decrease in Golgi-based SM synthesis (120). Interestingly, in T2D patients, CERT expression was found to be downregulated in muscle (142), suggesting that, in addition to the increased *de-novo* ceramide biosynthesis observed in the ER, a reduced transport of ceramide due to decreased levels of CERT could play a role in muscle insulin resistance. However, how accumulation of ceramide in the ER through CERT inhibition plays a role in insulin resistance remains elusive.

The ER has been shown to be the major *de-novo* production site of ceramide during lipotoxicity, and ceramide accumulation in this compartment can induce changes in the ER homeostasis, and a durable unfolded protein response called ER stress (143). Long-term ER stress has been observed in obesity and insulin

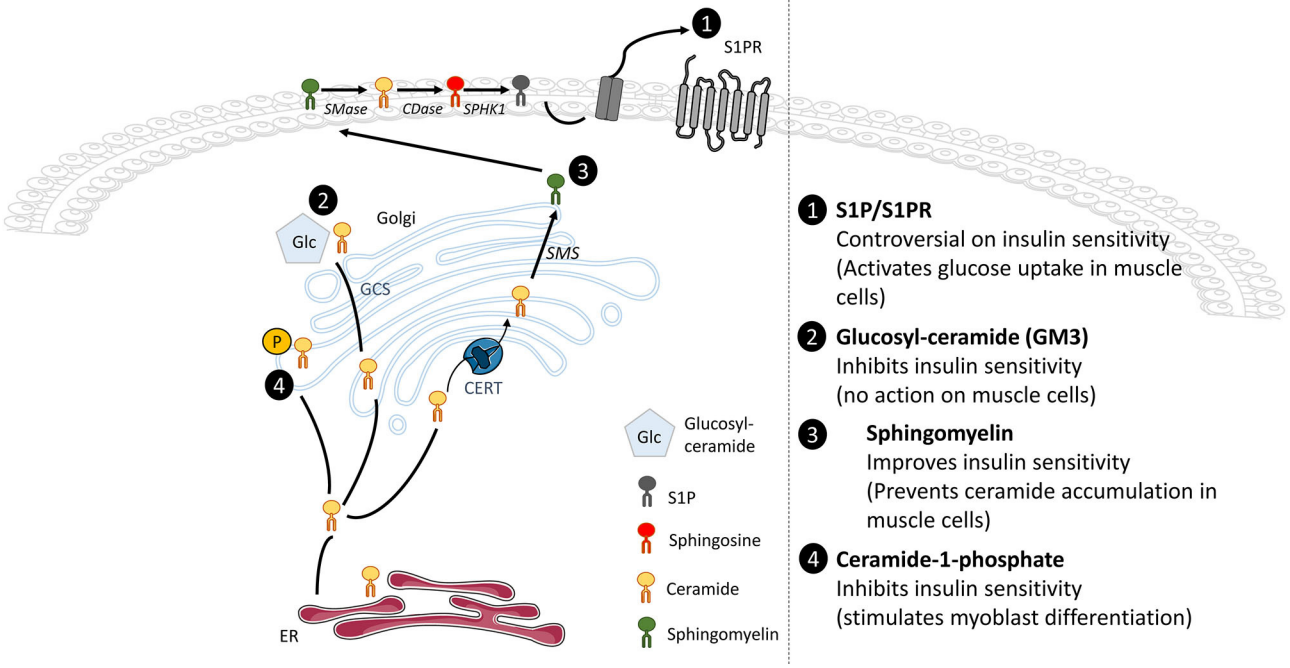

FIGURE 4 | Effects of ceramide metabolite accumulation on insulin signaling. Whole-tissue accumulation of ceramide-1-phosphate and glucosylceramide damages insulin sensitivity without significant action in muscle glucose metabolism. In opposite, accumulation of SM prevents ceramide buildup and improves insulin sensitivity in muscle cells. The SphK1/S1P/S1PR axis displays controversial actions on insulin sensitivity but activates glucose uptake in muscle cells.

resistance and is associated with cellular dysfunction in liver and adipose tissue (144, 145). In muscle cells, however, activation of ER stress in response to ceramide accumulation remains mild (146), and does not play a major role in the onset of insulin resistance (146, 147). These data could suggest that ER-produced ceramide must somehow transfer to the plasma membrane, maybe through specialized membrane contact sites between these two compartments, to exert their negative effects on insulin signaling.

Another possibility could be related to the accumulation of ceramide in the mitochondria. Indeed, mitochondria-based ceramide has also been observed (148) where they have been shown to inhibit mitochondrial fatty acid oxidation in liver and brain (115, 125, 149). Similar observations have been made in muscle mitochondria where addition of C2-ceramide to isolated mitochondria inhibited mitochondrial respiratory chain function (150). More recently, Perreault et al. showed that ceramide or dihydroceramide inhibited complex 1 and 3 of the electron transport chain (125), demonstrating a possible and important role for these lipids in the appearance of an oxidative stress, systemic inflammation and insulin resistance. Interestingly, disruption of mitochondria-associated ER membrane (MAM) integrity resulted in muscle insulin resistance in both mice and humans (151). Ceramide metabolism and especially CerS were located in MAM from liver (152), supporting the hypothesis that obesity could increase ceramide levels in MAM and could contribute to muscle insulin resistance.

Overall, these observations suggest that mechanisms directing the negative action of ceramide on muscle insulin sensitivity are rather complex, and may imply to consider SL subcellular localization in order to fight insulin resistance.

Ceramide Lipid Derivatives and Muscle Insulin Resistance

Several other SL derivatives synthesized from ceramide have also been shown to regulate insulin signaling in muscle cells (**Figure 4**).

Sphingosine-1-phosphate

Recent evidence suggests that obesity also increased other SL content such as S1P in muscle. Hu et al. determined that lipotoxicity positively increased S1P levels through upregulation of SphK1 activity in C2C12 myotubes, but also in isolated skeletal muscle from HFD-fed mice (153). These data suggested for the first time that both S1P and SphK1 could play a role in metabolic diseases such as obesity or T2D (153). Interestingly, Rapizzi et al. reported a crosstalk between S1P and insulin signaling in C2C12 myoblasts as they showed that S1P, through the transactivation of the insulin receptor, increased glucose uptake in muscle cells (154).

These data considered, successive studies wondered whether the muscle SphK/S1P axis could be implicated in modulating insulin signaling in a lipotoxicity context observed in obesity and T2D. Several studies highlighted a critical role of SphK1 in the regulation of the whole body glucose homeostasis (155,

156). HFD-fed SphK1 KO mice exhibited high fasting glucose levels and severe glucose intolerance compared to their WT HFD-fed littermates. However, this was not associated with an increase of the global insulin resistant state induced by obesity, but rather with a failure of compensatory insulin production by pancreatic β-cells in HFD-fed SphK1 KO mice (156). In fact, HFD-fed SphK1 KO mice displayed a significant reduction of β-cells mass, consequence of an increased β-cells apoptosis in comparison with HFD-fed WT mice. These data highlighted that SphK1 played a key role in regulation whole-body glucose homeostasis under lipotoxic conditions (156). However, whether muscle SphK1 expressed exerted a role in enhancing whole-body glucose homeostasis remained unclear. Bruce et al. (155) were pioneers to identify a role of muscle SphK1 in the etiology of obesity and insulin resistance. HFD-fed mice overexpressing SphK1 displayed increased SphK1 activity in skeletal muscle, were protected against ceramide accumulation and displayed an improved insulin-stimulated glucose uptake (155). In opposite, inhibition of SphK1 activity strengthened the deleterious effect of palmitate on insulin-stimulated glucose uptake and further reduced the activity of insulin signaling proteins in L6 myotubes (157). Furthermore, treatment of HFD-fed mice with FTY20, a S1P analog, improved whole-body glucose tolerance, reduced muscle ceramide content and increased muscle insulin-stimulated glucose uptake and Akt phosphorylation (158). Taken together, these results suggest that, under lipotoxic conditions, muscle SphK1 could play a protective role against the onset of insulin resistance.

However, genetic (SphK1 KO) or pharmacological (SphK1 inhibitor) approaches displayed improved glucose tolerance and insulin sensitivity, and lowered fasting glucose levels in obese mice (159). In addition, improved insulin-dependent phosphorylation of Akt was observed in adipose tissue, liver, and muscle of HFD-fed SphK1 KO mice. The discrepancy between these data and Bruce's could come from the diet they used throughout the studies (159, 160). The first study used a fructose-associated HFD to challenge pancreatic β-cell function in an obesity context, whereas the second study used a simple HFD to induce obesity. Altogether, these studies highlight a possible divergent role of the muscle SphK1/S1P axis on regulating whole-body glucose homeostasis in mice. Both studies used a global SphK1 KO approach, and further analyses of a muscle-targeted deletion of SphK1 would be more conclusive.

In opposite to SphK1, muscle SphK2 overexpression had no effect on muscle lipid content, suggesting that SphK2 did no regulate muscle ceramide levels (155). More recently, Ravichandran et al. (161) aimed to better understand the role of SphK2 in glucose and lipid metabolism. They characterized metabolic parameters of SphK2 KO old mice compared to control old mice, as aging is associated with weight gain and obesity (161). SphK2 KO mice seemed to be protected against age-related changes of adipose tissue as they displayed decreased weight and fat mass and increased lean mass (161). Moreover, old SphK2 KO mice saw their glucose tolerance and insulin sensitivity improved with aging compared with WT mice (161). These enhanced metabolic e?ects could be the result of increased adiponectin plasma levels and enhanced lipolysis (161). It still remains to know whether muscle insulin sensitivity was improved these conditions. Nevertheless, this study indicates that SphK2 may be a negative regulator of glucose homeostasis in an obesity context. Interestingly, a role of SphK2 in promoting β-cell lipotoxicity has also been demonstrated as SphK2 deficiency prevented β-cell mass loss in lipotoxic conditions (162). Overall, these data indicated that inhibition of SphK2 could be an attractive target to develop treatments to ameliorate insulin resistance but also insulin secretion associated with obesity and T2D.

A small number of studies have also suggested the involvement of additional SL derivatives as being able to modulate glucose homeostasis. However, their direct action in muscle cells is not always well defined and clear (**Figure 4**).

Ceramide-1-phosphate

A study has shown that the total silencing of CerK, ceramide kinase responsible for the formation of C1P from ceramide, protected animals from obesity and glucose intolerance induced by a HFD. In addition, CerK silencing also protected mouse adipose tissue from macrophage infiltration, thereby preventing adipose tissue inflammation of (163). At the muscle level, C1P stimulated myoblast proliferation via a lysophosphatidic acid signaling axis (164), in a PI3K, Akt, ERK 1/2, and mTOR dependent manner (165). This suggests that C1P could be involved in the regulation of muscle regeneration and repair.

Sphingomyelin

A metabolomic study demonstrated that reduced levels of plasma C16:1-SM species were predictive of T2D (166). In addition, inhibition of SMase in muscle cells induced an increase in ceramide content and impairment of insulin signaling (167). Obese individuals with glucose intolerance showed increased muscle ceramide content and lower muscle SM compared with obese individuals with normal glucose tolerance (168). Overall, these data suggest that SM biosynthesis from ceramide may be protective for maintaining insulin sensitivity, and that inhibition of SM synthesis could have serious consequences on cell insulin sensitivity. A study reinforced this hypothesis, as a pharmacological inhibition of SMS2 in C2C12 myotubes induced ceramide accumulation in cells and an alteration of insulin signaling (167). In addition, Bandet et al. showed in muscle cells that increased concentrations of ceramide in response to palmitate were associated with a malfunctioning transport of the SL from the ER to the Golgi apparatus, consequence of a defected expression of CERT, and a concomitant reduced SM biosynthesis (120). *In-vitro* overexpression of CERT in muscle cells under lipotoxic conditions, or *in vivo* in the anterior tibialis muscle of HFD-fed mice improved insulin sensitivity (120), demonstrating that transformation of ceramide into SM improved muscle insulin sensitivity.

Gangliosides

Inhibition of glycosphingolipid synthesis significantly improved insulin sensitivity and glucose homeostasis in Zucker diabetic fatty rat, *ob/ob* mice, and HFD-fed mice (169, 170). The involvement of a particular ganglioside, GM3 (ganglioside monosialo 3) was particularly highlighted in this process. TNFα-induced insulin resistance was shown to be associated with high concentrations of GM3 in adipocytes (171), and GM3 synthase

deficient mice fed a HFD displayed a better glucose tolerance than HFD-fed control mice (172). In humans, serum GM3 concentrations were increased in T2D patients (173), and they are associated with risk factors of metabolic syndrome (174). In skeletal muscle, GM3 content was significantly higher in T2D rats compared with control rats, but significantly lower in T1D rats compared with their controls (175). However, involvement of GM3 in inhibiting insulin action in muscle cells remains elusive since a direct addition of GM3 or GCS overexpression failed to inhibit insulin signaling in C2C12 myotubes (176). In addition, glucosylceramide concentration went down in muscle of mice fed a HFD, and GSL accumulated in adipocytes, where it exerted negative effects on insulin signaling (176).

Overall, these ceramide derivatives could therefore play an important role in modulating muscle insulin sensitivity, but crucial lack of human data on these SL as regulators of muscle metabolism prevents definitive conclusions.

SPHINGOLIPIDS AND PHYSICAL ACTIVITY

Several studies attempted to investigate whether a correlation existed between physical exercise, known to improve cell insulin sensitivity in diabetic patients (177), and intramyocyte levels of ceramide, acknowledged to be harmful to insulin sensitivity (see previous chapter). The rationale for this question was whether physical exercise, in addition to improving muscle uptake of glucose by an insulin-independent pathway (178), could prevent ceramide from accumulating in muscle cells, and thus resensitize them to the action of insulin.

A pioneering study carried out in 2004 in humans showed that, in opposite to what was expected, acute physical exercise (3 h bicycle exercise) induced an increase in muscle concentrations of ceramide (+ 39%) (179). Another study performed by the same group went more in detail and assessed SL content in rats exercised for 30 min, 90 min, and until exhaustion (180). The authors showed that exercise gradually increased muscle ceramide levels (+27%) in two different muscles types (soleus and red section of gastrocnemius). Surprisingly, in white section of gastrocnemius, ceramide content decreased progressively until the 90th minute of exercise and returned to control value when mice were exhausted (180). Dihydrosphingosine content was also assessed. It increased gradually over time in the three muscle types to reach maximum level (80 to 100%) at the point of exhaustion (180). Levels of sphingosine rapidly increased by 20–30% after 30 min of running and stayed at that level until exhaustion (180). S1P levels stayed stable until 90th minute of running and reached a twofold increase at the point of exhaustion in soleus. In red and white sections of the gastrocnemius, S1P levels went up and down throughout physical exercise (180). Circulating S1P levels were also increased in response to acute (60 min) physical exercise (181). Activities of key enzymes involved in ceramide metabolism (SPT, nSMase, aSMase, CDase) were also affected by exercise. Thus, SPT activity went up gradually with the duration of the exercise, while nSMase activity did not move, and both aSMase and CDase activities went down (180). These data indicate that the exercise strongly affected the activity of the key enzymes involved in ceramide metabolism and increased ceramide *de-novo* synthesis.

In an attempt to explain these results, the authors advanced the hypothesis that the increase in plasma concentrations of free FA (lipolysis), secondary to physical exercise (182), could be favorable to the increase of the *de-novo* synthesis of ceramide. It is also possible that acute exercise-induced ceramide accumulation could be the consequence of an increased inflammatory response to exercise. Furthermore, S1P accumulation could prolong exercise capacity and delay muscle fatigue (**Figure 5A**) (180). Indeed, studies also showed an increase in muscle S1P content with exercise (181), and that S1P improved muscle strength, mass, and metabolism (59, 183–185). These data were confirmed in a recent human study where they found that total muscle ceramide and other SL species concentrations were significantly increased by acute exercise (2 h), and then rapidly returned to basal after 2 h resting (117).

The role of this increase in muscle ceramide content during acute physical exertion remains currently unknown, but we can hypothesize that it would block insulin action and boost the use of lipids as an energy source for physical exercise. In opposite, increased S1P could exert beneficial effects on the promotion of fatigue resistance.

Endurance training studies gave different results. No modification or even a decrease in the concentrations of intramuscular ceramide was observed in rats trained for 6–8 weeks (wheel exercised) compared to sedentary rats (186, 187). Results of these studies were confirmed in humans where ceramide concentrations in skeletal muscles of non-obese sedentary volunteers and non-obese endurance-trained male athletes (aerobic exercise sessions) were measured, and no difference was observed (188, 189). Similar observations were obtained in lean women (**Figure 5B**) (190).

In contrast, ceramide concentrations measured in obese and T2D patients who were sedentary or had practiced moderate but prolonged physical activity were found to be decreased in response to exercise training (190–195). Interestingly, Bruce et al. showed that C16-, C16:1-, C18-, C18:1-, C18:2-, and C20-ceramide concentrations were decreased (191), and this study also showed that physical exercise increased the activity of CPT1, thus pushing FAs toward mitochondria oxidation and diverting them from ceramide synthesis (191). Even if this latter study suggests that physical exercise could inhibit ceramide to be synthetized *de novo* in obese and T2D patients, more work will be necessary to figure out whether CerS expression was modified by exercise, and to exclude an exercise-induced inhibition of either the SMase or the salvage/recycling pathway in this process (**Figure 5C**).

AMPK could play an important role in the inhibition of ceramide synthesis observed in skeletal muscle in response to exercise in T2D patients. Indeed, utilization of metformin, a ubiquitously prescribed anti-diabetic drug that was described to act through AMPK activation (196), improved insulin sensitivity at both systemic and muscle levels by augmenting

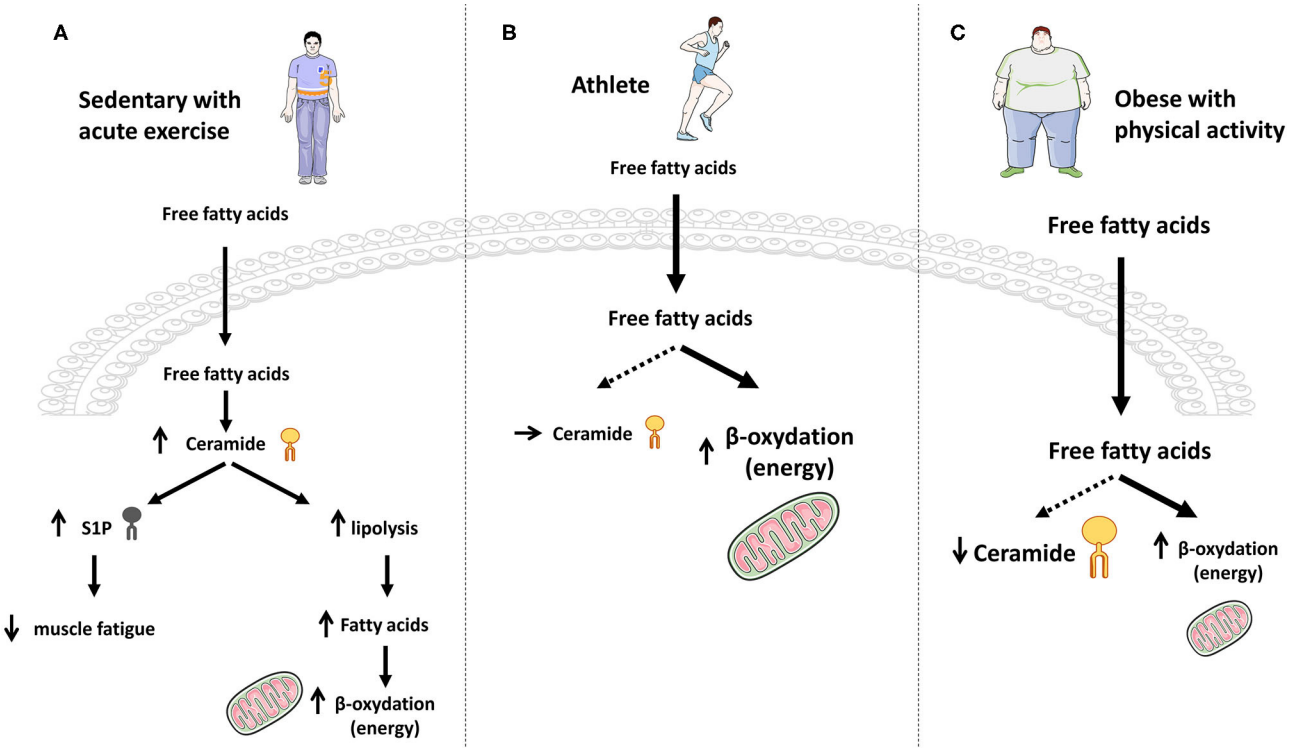

FIGURE 5 | Effect of exercise on ceramide accumulation and muscle energy requirements. **(A)** In sedentary lean people, acute exercise induces a transient increase in ceramide synthesis that momentarily blocks the anti-lipolytic action of insulin and therefore favors FA production and energy needs through β-oxidation. **(B)** Chronic exercise promotes increased entry of FA into muscle cells and their oxidation to fulfill the energy need, without any ceramide production. **(C)** In obese patients practicing physical activity, large intracellular FA concentrations are deflected toward β-oxidation for energy purposes, thereby reducing muscle ceramide synthesis flux and concentrations.

Akt phosphorylation and decreasing ceramide content (116). Direct involvement of AMPK in this process was further characterized after treatment of C2C12 muscle cells with 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR), a well-established AMPK activator. AICAR treatment prevented completely palmitate-induced ceramide content in cells (197), suggesting that AMPK activation could be an important exercise-associated mechanism to decrease muscle ceramide content and to improve metabolic functions in T2D patients.

In summary, physical exercise seems to exert opposite actions on ceramide metabolism, depending on the chronicity of the effort (**Figure 5**):

- an acute action inducing a transient increase of the *de-novo* ceramide synthesis, thus promoting muscle FA β-oxidation by temporarily blocking the anti-lipolytic action of insulin.
- a more long-term action in lean, obese and T2D individuals where lipids are deflected toward an increased β-oxidation for energy purposes, thereby reducing muscle ceramide biosynthesis flux (no need to block insulin action). Interestingly, chronic exercise-induced FA β-oxidation decreases FA conversion to ceramide, hence improving muscle sensitivity to insulin in obese/T2D patients.

CIRCULATING SPHINGOLIPIDS

Negative consequences of tissue-based ceramide (and some ceramide metabolites) accumulation have now been extensively highlighted over the years, but it looks now apparent that circulating SL also play a distant and additional role on insulin-sensitive organs such as skeletal muscle. Indeed, several studies have reported an increase in circulating ceramide content in the plasma of obese and T2D mice (94, 198, 199), as well as with insulin resistance in obese people with T2D (200, 201). Interestingly, weight loss induced by gastric bypass surgery or changes in lifestyle produced a decrease in plasma ceramide content in patients (199, 202–206). Importantly, strong correlations have been established between plasma ceramide concentrations and insulin resistance and inflammation (200, 201, 207, 208). It is interesting to note that one study has also shown that serum levels of the ceramide-derived ganglioside GM3 were also increased in T2D patients (173).

A possible and direct role of circulating ceramide in the development of skeletal muscle insulin resistance has been proposed by Boon et al. (199) a few years ago. They showed that ceramide concentrations were elevated specifically in low-density lipoproteins (LDL) of T2D patients compared with insulin-sensitive individuals, independently of obesity (199).

Interestingly, they demonstrated that LDL-ceramide caused whole-body insulin resistance in lean mice, an effect mediated by a decrease of insulin signal in skeletal muscle. *In vitro* incubation of L6 muscle cells with C24:0-ceramide-enriched LDL induced a loss in insulin response, demonstrating that LDL-ceramide were taken up by muscle cells and were directly affecting muscle insulin signaling (199). Secretion of ceramide into LDL lipoproteins by the liver into the circulation had already been shown long ago (209, 210), suggesting that, in addition to muscle-based *de-novo* synthetized ceramide, hepatocyte-produced ceramide can also affect remotely muscle insulin sensitivity, further complicating possible strategies envisaged to combat the deleterious peripheral action of ceramide on muscle insulin sensitivity.

Like ceramide, S1P can also be secreted into the bloodstream associated to high-density lipoproteins (HDL). Circulating S1P is mainly derived from erythrocytes and vascular endothelial cells in healthy subjects (211). However, plasma levels of S1P were found to be higher in different rodent models of insulin resistance (212), in obese patients compared to non-obese individuals (213), and correlated with HOMA-IR, HbA1c, and body mass index (212). In the diabetic state, S1P binds physically to apolipoprotein M (ApoM) and is excreted by the liver inside ApoM-containing HDL vesicles into the plasma (214–216). However, S1P is a key component in the antiatherogenic properties of HDL (217, 218), and two very recent studies just showed that apoM/S1P exerted a protective role against the development of insulin resistance. Indeed, Kurano et al. showed that deletion of apoM in mice was associated with a degradation of insulin sensitivity associated with a decreased Akt phosphorylation in skeletal muscle (219). In opposite, overexpression of apoM induced an improvement of insulin sensitivity in mice and enhanced muscle Akt phosphorylation (219). Identical results were observed in Goto-Kakizaki Rats (220). Altogether, these studies demonstrate that circulating S1P can exert protecting effects against the development of tissue insulin resistance.

Quantification of circulating SL could also be important in the context of monitoring the development of diabetes since it has recently been shown that certain SL could be used as biomarkers to identify individuals at risk of developing T2D. Indeed, it has been demonstrated that circulating concentrations of Dcer were significantly elevated in insulin-resistant individuals with T2D (221), and more importantly, in pre-diabetic individuals up to 9 years before the onset of the disease (222). Interestingly, circulating Dcer were better biomarkers of TD2 than circulating ceramides (223). Few data, at the cellular level or in animals, exist on the mechanistic role of Dcer on the pathophysiology of T2D. In addition, no data are available about the origin of these circulating Dcer, and about the carrier (lipoproteins, exosomes…) they are using in blood circulation.

Interestingly, 1-deoxysphinganine plasma levels were also found to be increased in non-human primates maintained on a HFD (224). 1-deoxysphinganine is produced through the condensation of alanine rather than serine with palmitoyl-CoA by SPT (7), suggesting that HFD feeding could increase the utilization frequency of alanine by SPT. Levels of 1-deoxysphinganine correlated with HOMA-IR, indicating that elevated plasma deoxysphingolipids could also be biomarkers of insulin resistance (224).

Thus, additional studies need to be carried out to clarify the interest of these ceramide-derived SL species as biomarkers or as therapeutic targets in T2D.

CONCLUSION

SL influence several important processes in skeletal muscle, from differentiation, regeneration, and fatigue to contraction and insulin response. SL metabolism is quite complex and depends on numerous enzymes and signaling pathways, and important roles of some SL metabolites, especially those of ceramide and S1P, in the regulation of physiological or pathophysiological skeletal muscle phenomena begin to be really highlighted. The discovery of the different pathways of ceramide intermediary metabolism has provided the field with a number of exciting targets in order to manipulate SL levels. As such, pharmacological and genetic strategies that could modulate levels of muscle SL should lead to beneficial effects in the progression and development of muscle disorders, thus supporting new approaches for potential treatments of muscle physiopathology.

AUTHOR CONTRIBUTIONS

EH: conceived the idea. ST-C, JG, OB, HL, and EH: wrote the manuscript and critically reviewed the manuscript and figures. ST-C: figure preparation. All authors: approved the final manuscript.

FUNDING

This study was supported by INSERM and the Fondation de France.

REFERENCES

1. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes.* (1981) 30:1000–7. doi: 10.2337/diab.30.12.1000
2. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med.* (1990) 322:223–8. doi: 10.1056/NEJM199001253220403
3. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol.* (2008) 9:139–50. doi: 10.1038/nrm2329
4. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol.* (2010) 688:1–23. doi: 10.1007/978-1-4419-6741-1_1
5. Futerman AH, Riezman H. The ins and outs of sphingolipid synthesis. *Trends Cell Biol.* (2005) 15:312–8. doi: 10.1016/j.tcb.2005.04.006

6. Futerman AH. Intracellular trafficking of sphingolipids: relationship to biosynthesis. *Biochim Biophys Acta.* (2006) 1758:1885–92. doi: 10.1016/j.bbamem.2006.08.004
7. Duan J, Merrill AH. 1-deoxysphingolipids encountered exogenously and made *de novo*: dangerous mysteries inside an enigma. *J Biol Chem.* (2015) 290:15380–9. doi: 10.1074/jbc.R115.658823
8. Dohrn MF, Othman A, Hirshman SK, Bode H, Alecu I, Fähndrich E, et al. Elevation of plasma 1-deoxy-sphingolipids in type 2 diabetes mellitus: a susceptibility to neuropathy? *Eur J Neurol.* (2015) 22:806–14.e55. doi: 10.1111/ene.12663
9. Gorden DL, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, et al. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res.* (2015) 56:722–36. doi: 10.1194/jlr.P056002
10. Zuellig RA, Hornemann T, Othman A, Hehl AB, Bode H, Güntert T, et al. Deoxysphingolipids, novel biomarkers for type 2 diabetes, are cytotoxic for insulin-producing cells. *Diabetes.* (2014) 63:1326–39. doi: 10.2337/db13-1042
11. Güntert T, Hänggi P, Othman A, Suriyanarayanan S, Sonda S, Zuellig RA, et al. 1-Deoxysphingolipid-induced neurotoxicity involves N-methyl-d-aspartate receptor signaling. *Neuropharmacology.* (2016) 110:211–22. doi: 10.1016/j.neuropharm.2016.03.033
12. Russo SB, Tidhar R, Futerman AH, Cowart LA. Myristate-derived d16:0 sphingolipids constitute a cardiac sphingolipid pool with distinct synthetic routes and functional properties. *J Biol Chem.* (2013) 288:13397–409. doi: 10.1074/jbc.M112.428185
13. Mizutani Y, Kihara A, Igarashi Y. Identification of the human sphingolipid C4-hydroxylase, hDES2, and its up-regulation during keratinocyte differentiation. *FEBS Lett.* (2004) 563:93–7. doi: 10.1016/S0014-5793(04)00274-1
14. Omae F, Miyazaki M, Enomoto A, Suzuki M, Suzuki Y, Suzuki A. DES2 protein is responsible for phytoceramide biosynthesis in the mouse small intestine. *Biochem J.* (2004) 379:687–95. doi: 10.1042/bj20031425
15. Fukasawa M, Nishijima M, Hanada K. Genetic evidence for ATP-dependent endoplasmic reticulum-to-Golgi apparatus trafficking of ceramide for sphingomyelin synthesis in Chinese hamster ovary cells. *J Cell Biol.* (1999) 144:673–85. doi: 10.1083/jcb.144.4.673
16. Huitema K, van den Dikkenberg J, Brouwers JF, Holthuis JC. Identification of a family of animal sphingomyelin synthases. *EMBO J.* (2004) 23:33–44. doi: 10.1038/sj.emboj.7600034
17. Ishibashi Y, Kohyama-Koganeya A, Hirabayashi Y. New insights on glucosylated lipids: metabolism and functions. *Biochim Biophys Acta.* (2013) 1831:1475–85. doi: 10.1016/j.bbalip.2013.06.001
18. Sprong H, Kruithof B, Leijendekker R, Slot JW, van Meer G, van der Sluijs P. UDP-galactose:ceramide galactosyltransferase is a class I integral membrane protein of the endoplasmic reticulum. *J Biol Chem.* (1998) 273:25880–8. doi: 10.1074/jbc.273.40.25880
19. Yamashita T, Wada R, Sasaki T, Deng C, Bierfreund U, Sandhoff K, et al. A vital role for glycosphingolipid synthesis during development and differentiation. *Proc Natl Acad Sci USA.* (1999) 96:9142–7. doi: 10.1073/pnas.96.16.9142
20. Zöller I, Büssow H, Gieselmann V, Eckhardt M. Oligodendrocyte-specific ceramide galactosyltransferase (CGT) expression phenotypically rescues CGT-deficient mice and demonstrates that CGT activity does not limit brain galactosylceramide level. *Glia.* (2005) 52:190–8. doi: 10.1002/glia.20230
21. Gangoiti P, Camacho L, Arana L, Ouro A, Granado MH, Brizuela L, et al. Control of metabolism and signaling of simple bioactive sphingolipids: implications in disease. *Prog Lipid Res.* (2010) 49:316–34. doi: 10.1016/j.plipres.2010.02.004
22. Gómez-Muñoz A, Gangoiti P, Granado MH, Arana L, Ouro A. Ceramide-1-phosphate in cell survival and inflammatory signaling. *Adv Exp Med Biol.* (2010) 688:118–30. doi: 10.1007/978-1-4419-6741-1_8
23. Clarke CJ, Snook CF, Tani M, Matmati N, Marchesini N, Hannun YA. The extended family of neutral sphingomyelinases. *Biochemistry.* (2006) 45:11247–56. doi: 10.1021/bi061307z
24. Clarke CJ, Hannun YA. Neutral sphingomyelinases and nSMase2: bridging the gaps. *Biochim Biophys Acta.* (2006) 1758:1893–901. doi: 10.1016/j.bbamem.2006.06.025
25. Parveen F, Bender D, Law SH, Mishra VK, Chen CC, Ke LY. Role of ceramidases in sphingolipid metabolism and human diseases. *Cells.* (2019) 8:1573. doi: 10.3390/cells8121573
26. Michel C, van Echten-Deckert G, Rother J, Sandhoff K, Wang E, Merrill AH. Characterization of ceramide synthesis. A dihydroceramide desaturase introduces the 4,5-trans-double bond of sphingosine at the level of dihydroceramide. *J Biol Chem.* (1997) 272:22432–7. doi: 10.1074/jbc.272.36.22432
27. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol.* (2012) 22:50–60. doi: 10.1016/j.tcb.2011.09.003
28. Stahelin RV, Hwang JH, Kim J-H, Park Z-Y, Johnson KR, Obeid LM, et al. The mechanism of membrane targeting of human sphingosine kinase 1. *J Biol Chem.* (2005) 280:43030–8. doi: 10.1074/jbc.M507574200
29. Pitson SM, Moretti PAB, Zebol JR, Lynn HE, Xia P, Vadas MA, et al. Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J.* (2003) 22:5491–500. doi: 10.1093/emboj/cdg540
30. Le Stunff H, Giussani P, Maceyka M, Lépine S, Milstien S, Spiegel S. Recycling of sphingosine is regulated by the concerted actions of sphingosine-1-phosphate phosphohydrolase 1 and sphingosine kinase 2. *J Biol Chem.* (2007) 282:34372–80. doi: 10.1074/jbc.M703329200
31. van Veldhoven PP. Sphingosine-1-phosphate lyase. *Methods Enzymol.* (2000) 311:244–54. doi: 10.1016/S0076-6879(00)11087-0
32. Fayyaz S, Japtok L, Kleuser B. Divergent role of sphingosine 1-phosphate on insulin resistance. *Cell Physiol Biochem.* (2014) 34:134–47. doi: 10.1159/000362990
33. Gillard BK, Clement RG, Marcus DM. Variations among cell lines in the synthesis of sphingolipids in *de novo* and recycling pathways. *Glycobiology.* (1998) 8:885–90. doi: 10.1093/glycob/8.9.885
34. Tettamanti G, Bassi R, Viani P, Riboni L. Salvage pathways in glycosphingolipid metabolism. *Biochimie.* (2003) 85:423–37. doi: 10.1016/S0300-9084(03)00047-6
35. Prinetti A, Bassi R, Riboni L, Tettamanti G. Involvement of a ceramide activated protein phosphatase in the differentiation of neuroblastoma Neuro2a cells. *FEBS Lett.* (1997) 414:475–9. doi: 10.1016/S0014-5793(97)00981-2
36. Kolter T, Sandhoff K. Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu Rev Cell Dev Biol.* (2005) 21:81–103. doi: 10.1146/annurev.cellbio.21.122303.120013
37. Riboni L, Prinetti A, Bassi R, Tettamanti G. Formation of bioactive sphingoid molecules from exogenous sphingomyelin in primary cultures of neurons and astrocytes. *FEBS Lett.* (1994) 352:323–6. doi: 10.1016/0014-5793(94)00984-8
38. Riboni L, Bassi R, Caminiti A, Prinetti A, Viani P, Tettamanti G. Metabolic fate of exogenous sphingosine in neuroblastoma neuro2A cells. Dose-dependence and biological effects. *Ann N Y Acad Sci.* (1998) 845:46–56. doi: 10.1111/j.1749-6632.1998.tb09661.x
39. Chatelut M, Leruth M, Harzer K, Dagan A, Marchesini S, Gatt S, et al. Natural ceramide is unable to escape the lysosome, in contrast to a fluorescent analogue. *FEBS Lett.* (1998) 426:102–6. doi: 10.1016/S0014-5793(98)00325-1
40. Olivera A, Spiegel S. Sphingosine kinase: a mediator of vital cellular functions. *Prostaglandins Other Lipid Mediat.* (2001) 64:123–34. doi: 10.1016/S0090-6980(01)00108-3
41. Baumruker T, Bornancin F, Billich A. The role of sphingosine and ceramide kinases in inflammatory responses. *Immunol Lett.* (2005) 96:175–85. doi: 10.1016/j.imlet.2004.09.001
42. Smith ER, Merrill AH. Differential roles of *de novo* sphingolipid biosynthesis and turnover in the "burst" of free sphingosine and sphinganine, and their 1-phosphates and N-acyl-derivatives, that occurs upon changing the medium of cells in culture. *J Biol Chem.* (1995) 270:18749–58. doi: 10.1074/jbc.270.32.18749
43. Kitatani K, Idkowiak-Baldys J, Hannun YA. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal.* (2008) 20:1010–8. doi: 10.1016/j.cellsig.2007.12.006
44. Mullen TD, Hannun YA, Obeid LM. Ceramide synthases at the centre of sphingolipid metabolism and biology. *Biochem J.* (2012) 441:789–802. doi: 10.1042/BJ20111626

45. Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature.* (1977) 270:725–7. doi: 10.1038/270725a0
46. Becciolini L, Meacci E, Donati C, Cencetti F, Rapizzi E, Bruni P. Sphingosine 1-phosphate inhibits cell migration in C2C12 myoblasts. *Biochim Biophys Acta.* (2006) 1761:43–51. doi: 10.1016/j.bbalip.2006.01.006
47. Donati C, Meacci E, Nuti F, Becciolini L, Farnararo M, Bruni P. Sphingosine 1-phosphate regulates myogenic differentiation: a major role for S1P2 receptor. *FASEB J.* (2005) 19:449–51. doi: 10.1096/fj.04-1780fje
48. Calise S, Blescia S, Cencetti F, Bernacchioni C, Donati C, Bruni P. Sphingosine 1-phosphate stimulates proliferation and migration of satellite cells: role of S1P receptors. *Biochim Biophys Acta.* (2012) 1823:439–50. doi: 10.1016/j.bbamcr.2011.11.016
49. Fortier M, Figeac N, White RB, Knopp P, Zammit PS. Sphingosine-1-phosphate receptor 3 influences cell cycle progression in muscle satellite cells. *Dev Biol.* (2013) 382:504–16. doi: 10.1016/j.ydbio.2013.07.006
50. Meacci E, Cencetti F, Donati C, Nuti F, Farnararo M, Kohno T, et al. Down-regulation of EDG5/S1P2 during myogenic differentiation results in the specific uncoupling of sphingosine 1-phosphate signalling to phospholipase D. *Biochim Biophys Acta.* (2003) 1633:133–42. doi: 10.1016/S1388-1981(03)00106-9
51. Meacci E, Nuti F, Donati C, Cencetti F, Farnararo M, Bruni P. Sphingosine kinase activity is required for myogenic differentiation of C2C12 myoblasts. *J Cell Physiol.* (2008) 214:210–20. doi: 10.1002/jcp.21187
52. Olivera A, Kohama T, Edsall L, Nava V, Cuvillier O, Poulton S, et al. Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. *J Cell Biol.* (1999) 147:545–58. doi: 10.1083/jcb.147.3.545
53. Donati C, Nincheri P, Cencetti F, Rapizzi E, Farnararo M, Bruni P. Tumor necrosis factor-alpha exerts pro-myogenic action in C2C12 myoblasts via sphingosine kinase/S1P2 signaling. *FEBS Lett.* (2007) 581:4384–8. doi: 10.1016/j.febslet.2007.08.007
54. Zammit PS, Partridge TA, Yablonka-Reuveni Z. The skeletal muscle satellite cell: the stem cell that came in from the cold. *J Histochem Cytochem.* (2006) 54:1177–91. doi: 10.1369/jhc.6R6995.2006
55. Sassoli C, Pierucci F, Zecchi-Orlandini S, Meacci E. Sphingosine 1-phosphate (S1P)/ S1P receptor signaling and mechanotransduction: implications for intrinsic tissue repair/regeneration. *Int J Mol Sci.* (2019) 20:5545. doi: 10.3390/ijms20225545
56. Rapizzi E, Donati C, Cencetti F, Nincheri P, Bruni P. Sphingosine 1-phosphate differentially regulates proliferation of C2C12 reserve cells and myoblasts. *Mol Cell Biochem.* (2008) 314:193–9. doi: 10.1007/s11010-008-9780-y
57. Danieli-Betto D, Peron S, Germinario E, Zanin M, Sorci G, Franzoso S, et al. Sphingosine 1-phosphate signaling is involved in skeletal muscle regeneration. *Am J Physiol Cell Physiol.* (2010) 298:C550–8. doi: 10.1152/ajpcell.00072.2009
58. Pantoja M, Fischer KA, Ieronimakis N, Reyes M, Ruohola-Baker H. Genetic elevation of sphingosine 1-phosphate suppresses dystrophic muscle phenotypes in Drosophila. *Development.* (2013) 140:136–46. doi: 10.1242/dev.087791
59. Ieronimakis N, Pantoja M, Hays AL, Dosey TL, Qi J, Fischer KA, et al. Increased sphingosine-1-phosphate improves muscle regeneration in acutely injured mdx mice. *Skelet Muscle.* (2013) 3:20. doi: 10.1186/2044-5040-3-20
60. Loh KC, Leong W-I, Carlson ME, Oskouian B, Kumar A, Fyrst H, et al. Sphingosine-1-phosphate enhances satellite cell activation in dystrophic muscles through a S1PR2/STAT3 signaling pathway. *PLoS ONE.* (2012) 7:e37218. doi: 10.1371/journal.pone.0037218
61. Sassoli C, Formigli L, Bini F, Tani A, Squecco R, Battistini C, et al. Effects of S1P on skeletal muscle repair/regeneration during eccentric contraction. *J Cell Mol Med.* (2011) 15:2498–511. doi: 10.1111/j.1582-4934.2010.01250.x
62. Meadows KA, Holly JM, Stewart CE. Tumor necrosis factor-alpha-induced apoptosis is associated with suppression of insulin-like growth factor binding protein-5 secretion in differentiating murine skeletal myoblasts. *J Cell Physiol.* (2000) 183:330–7. doi: 10.1002/(SICI)1097-4652(200006)183:3<330::AID-JCP5>3.0.CO;2-N
63. Mebarek S, Komati H, Naro F, Zeiller C, Alvisi M, Lagarde M, et al. Inhibition of de novo ceramide synthesis upregulates phospholipase D and enhances myogenic differentiation. *J Cell Sci.* (2007) 120:407–16. doi: 10.1242/jcs.03331
64. Dbaibo GS, El-Assaad W, Krikorian A, Liu B, Diab K, Idriss NZ, et al. Ceramide generation by two distinct pathways in tumor necrosis factor alpha-induced cell death. *FEBS Lett.* (2001) 503:7–12. doi: 10.1016/S0014-5793(01)02625-4
65. Strle K, Broussard SR, McCusker RH, Shen WH, Johnson RW, Freund GG, et al. Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramide. *Endocrinology.* (2004) 145:4592–602. doi: 10.1210/en.2003-1749
66. De Larichaudy J, Zufferli A, Serra F, Isidori AM, Naro F, Dessalle K, et al. TNF-α- and tumor-induced skeletal muscle atrophy involves sphingolipid metabolism. *Skelet Muscle.* (2012) 2:2. doi: 10.1186/2044-5040-2-2
67. Haran PH, Rivas DA, Fielding RA. Role and potential mechanisms of anabolic resistance in sarcopenia. *J Cachexia Sarcopenia Muscle.* (2012) 3:157–62. doi: 10.1007/s13539-012-0068-4
68. Anton SD, Karabetian C, Naugle K, Buford TW. Obesity and diabetes as accelerators of functional decline: can lifestyle interventions maintain functional status in high risk older adults? *Exp Gerontol.* (2013) 48:888–97. doi: 10.1016/j.exger.2013.06.007
69. Proud CG. Regulation of protein synthesis by insulin. *Biochem Soc Trans.* (2006) 34:213–6. doi: 10.1042/BST0340213
70. Gelfand RA, Barrett EJ. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest.* (1987) 80:1–6. doi: 10.1172/JCI113033
71. Rivas DA, McDonald DJ, Rice NP, Haran PH, Dolnikowski GG, Fielding RA. Diminished anabolic signaling response to insulin induced by intramuscular lipid accumulation is associated with inflammation in aging but not obesity. *Am J Physiol Regul Integr Comp Physiol.* (2016) 310:R561–9. doi: 10.1152/ajpregu.00198.2015
72. Loehr JA, Abo-Zahrah R, Pal R, Rodney GG. Sphingomyelinase promotes oxidant production and skeletal muscle contractile dysfunction through activation of NADPH oxidase. *Front Physiol.* (2014) 5:530. doi: 10.3389/fphys.2014.00530
73. Babenko NA, Garkavenko VV, Storozhenko GV, Timofiychuk OA. Role of acid sphingomyelinase in the age-dependent dysregulation of sphingolipids turnover in the tissues of rats. *Gen Physiol Biophys.* (2016) 35:195–205. doi: 10.4149/gpb_2015046
74. Hassouneh LK, Timofiychuk OA, Babenko NA. Acid sphingomyelinase inhibitors, imipramine and zoledronic acid, increase skeletal muscle tissue sensitivity to insulin action at old age. *Gen Physiol Biophys.* (2018) 37:163–74. doi: 10.4149/gpb_2017020
75. Taylor R, Price TB, Katz LD, Shulman RG, Shulman GI. Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. *Am J Physiol.* (1993) 265:E224–9. doi: 10.1152/ajpendo.1993.265.2.E224
76. Katz LD, Glickman MG, Rapoport S, Ferrannini E, DeFronzo RA. Splanchnic and peripheral disposal of oral glucose in man. *Diabetes.* (1983) 32:675–9. doi: 10.2337/diabetes.32.7.675
77. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* (1963) 1:785–9. doi: 10.1016/S0140-6736(63)91500-9
78. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* (2018) 98:2133–223. doi: 10.1152/physrev.00063.2017
79. McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes.* (2002) 51:7–18. doi: 10.2337/diabetes.51.1.7
80. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab.* (2001) 86:5755–61. doi: 10.1210/jcem.86.12.8075
81. Turinsky J, Bayly BP, O'Sullivan DM. 1, 2-Diacylglycerol and ceramide levels in rat skeletal muscle and liver *in vivo*. Studies with insulin, exercise, muscle denervation, and vasopressin. *J Biol Chem.* (1990) 265:7933–8.
82. Turinsky J, O'Sullivan DM, Bayly BP. 1, 2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat *in vivo*. *J Biol Chem.* (1990) 265:16880–5.

83. Holland WL, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from *in vivo* manipulation of sphingolipid metabolism. *Endocr Rev.* (2008) 29:381–402. doi: 10.1210/er.2007-0025
84. Bandet CL, Tan-Chen S, Bourron O, Stunff HL, Hajduch E. Sphingolipid metabolism: new insight into ceramide-induced lipotoxicity in muscle cells. *Int J Mol Sci.* (2019) 20:E479. doi: 10.3390/ijms20030479
85. Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, et al. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes.* (2004) 53:25–31. doi: 10.2337/diabetes.53.1.25
86. Coen PM, Dube JJ, Amati F, Stefanovic-Racic M, Ferrell RE, Toledo FG, et al. Insulin resistance is associated with higher intramyocellular triglycerides in type I but not type II myocytes concomitant with higher ceramide content. *Diabetes.* (2010) 59:80–8. doi: 10.2337/db09-0988
87. Moro C, Galgani JE, Luu L, Pasarica M, Mairal A, Bajpeyi S, et al. Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. *J Clin Endocrinol Metab.* (2009) 94:3440–47. doi: 10.1210/jc.2009-0053
88. Dube JJ, Amati F, Toledo FG, Stefanovic-Racic M, Rossi A, Coen P, et al. Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia.* (2011) 54:1147–56. doi: 10.1007/s00125-011-2065-0
89. Hage Hassan R, Bourron O, Hajduch E. Defect of insulin signal in peripheral tissues: important role of ceramide. *World J Diabetes.* (2014) 5:244–57. doi: 10.4239/wjd.v5.i3.244
90. Cazzolli R, Carpenter L, Biden TJ, Schmitz-Peiffer C. A role for protein phosphatase 2A-like activity, but not atypical protein kinase czeta, in the inhibition of protein kinase B/Akt and glycogen synthesis by palmitate. *Diabetes.* (2001) 50:2210–8. doi: 10.2337/diabetes.50.10.2210
91. Hajduch E, Balendran A, Batty I, Litherland GJ, Blair AS, Downes CP, et al. Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia.* (2001) 44:173–83. doi: 10.1007/s001250051596
92. Mahfouz R, Khoury R, Blachnio-Zabielska A, Turban S, Loiseau N, Lipina C, et al. Characterising the inhibitory actions of ceramide upon insulin signaling in different skeletal muscle cell models: a mechanistic insight. *PLoS ONE.* (2014) 9:e101865. doi: 10.1371/journal.pone.0101865
93. Ussher JR, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, et al. Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. *Diabetes.* (2010) 59:2453–64. doi: 10.2337/db09-1293
94. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* (2007) 5:167–79. doi: 10.1016/j.cmet.2007.01.002
95. Blachnio-Zabielska AU, Chacinska M, Vendelbo MH, Zabielski P. The crucial role of C18-cer in fat-induced skeletal muscle insulin resistance. *Cell Physiol Biochem.* (2016) 40:1207–20. doi: 10.1159/000453174
96. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem.* (2002) 277:50230–6. doi: 10.1074/jbc.M200958200
97. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes.* (2002) 51:2005–11. doi: 10.2337/diabetes.51.7.2005
98. Vistisen B, Hellgren LI, Vadset T, Scheede-Bergdahl C, Helge JW, Dela F, et al. Effect of gender on lipid-induced insulin resistance in obese subjects. *Eur J Endocrinol.* (2008) 158:61–8. doi: 10.1530/EJE-07-0493
99. Serlie MJ, Meijer AJ, Groener JE, Duran M, Endert E, Fliers E, et al. Short-term manipulation of plasma free fatty acids does not change skeletal muscle concentrations of ceramide and glucosylceramide in lean and overweight subjects. *J Clin Endocrinol Metab.* (2007) 92:1524–9. doi: 10.1210/jc.2006-2347
100. Skovbro M, Baranowski M, Skov-Jensen C, Flint A, Dela F, Gorski J, et al. Human skeletal muscle ceramide content is not a major factor in muscle insulin sensitivity. *Diabetologia.* (2008) 51:1253–60. doi: 10.1007/s00125-008-1014-z
101. Søgaard D, Østergård T, Blachnio-Zabielska AU, Baranowski M, Vigelsø AH, Andersen JL, Dela F, et al. Training does not alter muscle ceramide and diacylglycerol in offsprings of type 2 diabetic patients despite improved insulin sensitivity. *J Diabetes Res.* (2016) 2016:2372741. doi: 10.1155/2016/2372741
102. Thrush AB, Harasim E, Chabowski A, Gulli R, Stefanyk L, Dyck DJ. A single prior bout of exercise protects against palmitate-induced insulin resistance despite an increase in total ceramide content. *Am J Physiol Regul Integr Comp Physiol.* (2011) 300:R1200–8. doi: 10.1152/ajpregu.00091.2010
103. Bajpeyi S, Myrland CK, Covington JD, Obanda D, Cefalu WT, Smith SR, et al. Lipid in skeletal muscle myotubes is associated to the donors' insulin sensitivity and physical activity phenotypes. *Obesity.* (2013) 22:426–34. doi: 10.1002/oby.20556
104. Ginkel C, Hartmann D, vom Dorp K, Zlomuzica A, Farwanah H, Eckhardt M, et al. Ablation of neuronal ceramide synthase 1 in mice decreases ganglioside levels and expression of myelin-associated glycoprotein in oligodendrocytes. *J Biol Chem.* (2012) 287:41888–902. doi: 10.1074/jbc.M112.413500
105. Pewzner-Jung Y, Brenner O, Braun S, Laviad EL, Ben-Dor S, Feldmesser E, et al. A critical role for ceramide synthase 2 in liver homeostasis: II. Insights into molecular changes leading to hepatopathy. *J Biol Chem.* (2010) 285:10911–23. doi: 10.1074/jbc.M109.077610
106. Jennemann R, Rabionet M, Gorgas K, Epstein S, Dalpke A, Rothermel U, et al. Loss of ceramide synthase 3 causes lethal skin barrier disruption. *Hum Mol Genet.* (2012) 21:586–608. doi: 10.1093/hmg/ddr494
107. Peters F, Vorhagen S, Brodesser S, Jakobshagen K, Brüning JC, Niessen CM, et al. Ceramide synthase 4 regulates stem cell homeostasis and hair follicle cycling. *J Invest Dermatol.* (2015) 135:1501–9. doi: 10.1038/jid.2015.60
108. Xu Z, Zhou J, McCoy DM, Mallampalli RK. LASS5 is the predominant ceramide synthase isoform involved in *de novo* sphingolipid synthesis in lung epithelia. *J Lipid Res.* (2005) 46:1229–38. doi: 10.1194/jlr.M500001-JLR200
109. Schiffmann S, Ferreiros N, Birod K, Eberle M, Schreiber Y, Pfeilschifter W, et al. Ceramide synthase 6 plays a critical role in the development of experimental autoimmune encephalomyelitis. *J Immunol.* (2012) 188:5723–33. doi: 10.4049/jimmunol.1103109
110. Turner N, Kowalski GM, Leslie SJ, Risis S, Yang C, Lee-Young RS, et al. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia.* (2013) 56:1638–48. doi: 10.1007/s00125-013-2913-1
111. Hla T, Kolesnick R. C16:0-ceramide signals insulin resistance. *Cell Metab.* (2014) 20:703–5. doi: 10.1016/j.cmet.2014.10.017
112. Chung JO, Koutsari C, Blachnio-Zabielska AU, Hames KC, Jensen MD. Intramyocellular ceramides: subcellular concentrations and fractional *de novo* synthesis in postabsorptive humans. *Diabetes.* (2017) 66:2082–91. doi: 10.2337/db17-0082
113. Gosejacob D, Jäger PS, Vom Dorp K, Frejno M, Carstensen AC, Köhnke M, et al. Ceramide synthase 5 is essential to maintain C16:0-ceramide pools and contributes to the development of diet-induced obesity. *J Biol Chem.* (2016) 291:6989–7003. doi: 10.1074/jbc.M115.691212
114. Turpin-Nolan SM, Hammerschmidt P, Chen W, Jais A, Timper k, Awazawa M, et al. CerS1-derived C18:0 ceramide in skeletal muscle promotes obesity-induced insulin resistance. (2019) 26:1–10.e7. doi: 10.1016/j.celrep.2018.12.031
115. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* (2014) 20:678–86. doi: 10.1016/j.cmet.2014.08.002
116. Zabielski P, Chacinska M, Charkiewicz K, Baranowski M, Gorski J, Blachnio-Zabielska AU. Effect of metformin on bioactive lipid metabolism in insulin-resistant muscle. *J Endocrinol.* (2017) 233:329–40. doi: 10.1530/JOE-16-0381
117. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, et al. Muscle sphingolipids during rest and exercise: a C18:0 signature for insulin resistance in humans. *Diabetologia.* (2016) 59:785–98. doi: 10.1007/s00125-015-3850-y
118. Fisher FM, Maratos-Flier E. Understanding the physiology of FGF21. *Annu Rev Physiol.* (2016) 78:223–41. doi: 10.1146/annurev-physiol-021115-105339

119. Turner N, Lim XY, Toop HD, Osborne B, Brandon AE, Taylor EN, et al. A selective inhibitor of ceramide synthase 1 reveals a novel role in fat metabolism. *Nat Commun.* (2018) 9:3165. doi: 10.1038/s41467-018-05613-7
120. Bandet CL, Mahfouz R, Veret J, Sotiropoulos A, Poirier M, Giussani P, et al. Ceramide Transporter CERT is involved in muscle insulin signaling defects under lipotoxic conditions. *Diabetes.* (2018) 67:1258–71. doi: 10.2337/db17-0901
121. Yamaji T, Hanada K. Sphingolipid metabolism and interorganellar transport: localization of sphingolipid enzymes and lipid transfer proteins. *Traffic.* (2015) 16:101–22. doi: 10.1111/tra.12239
122. Riebeling C, Allegood JC, Wang E, Merrill AH, Futerman AH. Two mammalian longevity assurance gene (LAG1) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. *J Biol Chem.* (2003) 278:43452–9. doi: 10.1074/jbc.M307104200
123. Sridevi P, Alexander H, Laviad EL, Min J, Mesika A, Hannink M, et al. Stress-induced ER to golgi translocation of ceramide synthase 1 is dependent on proteasomal processing. *Exp Cell Res.* (2010) 316:78–91. doi: 10.1016/j.yexcr.2009.09.027
124. White-Gilbertson S, Mullen T, Senkal C, Lu P, Ogretmen B, Obeid L, et al. Ceramide synthase 6 modulates TRAIL sensitivity and nuclear translocation of active caspase-3 in colon cancer cells. *Oncogene.* (2009) 28:1132–41. doi: 10.1038/onc.2008.468
125. Perreault L, Newsom SA, Strauss A, Kerege A, Kahn DE, Harrison KA, et al. Intracellular localization of diacylglycerols and sphingolipids influences insulin sensitivity and mitochondrial function in human skeletal muscle. *JCI Insight.* (2018) 3:e96805. doi: 10.1172/jci.insight.96805
126. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol.* (2014) 6:a009191. doi: 10.1101/cshperspect.a009191
127. Lavin DP, White MF, Brazil DP. IRS proteins and diabetic complications. *Diabetologia.* (2016) 59:2280–91. doi: 10.1007/s00125-016-4072-7
128. Hajduch E, Litherland G, Hundal H. Protein kinase B (PKB/Akt) - a key regulator of glucose transport? *Febs Lett.* (2001) 492:199–203. doi: 10.1016/S0014-5793(01)02242-6
129. Litherland GJ, Hajduch E, Hundal HS. Intracellular signalling mechanisms regulating glucose transport in insulin-sensitive tissues (review). *Mol Membr Biol.* (2001) 18:195–204. doi: 10.1080/09687680110076407
130. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci.* (2018) 14:1483–96. doi: 10.7150/ijbs.27173
131. Hajduch E, Turban S, Le L, Le Lay S, Lipina C, Dimopoulos N, et al. Targeting of PKCzeta and PKB to caveolin-enriched microdomains represents a crucial step underpinning the disruption in PKB-directed signalling by ceramide. *Biochem J.* (2008) 410:369–79. doi: 10.1042/BJ20070936
132. Fox TE, Houck KL, O'Neill SM, Nagarajan M, Stover TC, Pomianowski PT, et al. Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. *J Biol Chem.* (2007) 282:12450–7. doi: 10.1074/jbc.M700082200
133. Hajduch E, Alessi D, Hemmings B, Hundal HS. Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. *Diabetes.* (1998) 47:1006–13. doi: 10.2337/diabetes.47.7.1006
134. Bourbon NA, Sandirasegarane L, Kester M. Ceramide-induced inhibition of Akt is mediated through protein kinase Czeta: implications for growth arrest. *J Biol Chem.* (2002) 277:3286–92. doi: 10.1074/jbc.M110541200
135. Powell D, Turban S, Gray A, Hajduch E, Hundal HS. Intracellular ceramide synthesis and protein kinase C zeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J.* (2004) 382:619–29.
136. Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol.* (2003) 23:7794–808. doi: 10.1128/MCB.23.21.7794-7808.2003
137. Blouin CM, Prado C, Takane KK, Lasnier F, Garcia-Ocana A, Ferre P, et al. Plasma membrane subdomain compartmentalization contributes to distinct mechanisms of ceramide action on insulin signaling. *Diabetes.* (2010) 59:600–10. doi: 10.2337/db09-0897
138. Powell DJ, Turban S, Gray A, Hajduch E, Hundal HS. Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J.* (2004) 382:619–29. doi: 10.1042/BJ20040139
139. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol.* (1998) 18:5457–64. doi: 10.1128/MCB.18.9.5457
140. Hage Hassan R, Pacheco de Sousa AC, Mahfouz R, Hainault I, Blachnio-Zabielska A, Bourron O, et al. Sustained action of ceramide on the insulin signaling pathway in muscle cells: implication of the double-stranded rna-activated protein kinase. *J Biol Chem.* (2016) 291:3019–29. doi: 10.1074/jbc.M115.686949
141. Cimmino I, Lorenzo V, Fiory F, Doti N, Ricci S, Cabaro S, et al. A peptide antagonist of Prep1-p160 interaction improves ceramide-induced insulin resistance in skeletal muscle cells. *Oncotarget.* (2017) 8:71845–58. doi: 10.18632/oncotarget.18286
142. Pralhada Rao R, Vaidyanathan N, Rengasamy M, Mammen Oommen A, Somaiya N, Jagannath MR. Sphingolipid metabolic pathway: an overview of major roles played in human diseases. *J Lipids.* (2013) 2013:178910. doi: 10.1155/2013/178910
143. Flamment M, Hajduch E, Ferre P, Foufelle F. New insights into ER stress-induced insulin resistance. *Trends Endocrinol Metab.* (2012) 23:381–90. doi: 10.1016/j.tem.2012.06.003
144. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol.* (2020) 21:421–38. doi: 10.1038/s41580-020-0250-z
145. Hotamisligil GS. Inflammation and endoplasmic reticulum stress in obesity and diabetes. *Int J Obes (Lond).* (2008) 32(Suppl. 7):S52–4. doi: 10.1038/ijo.2008.238
146. Hage Hassan R, Hainault I, Vilquin JT, Samama C, Lasnier F, Ferre P, et al. Endoplasmic reticulum stress does not mediate palmitate-induced insulin resistance in mouse and human muscle cells. *Diabetologia.* (2012) 55:204–14. doi: 10.1007/s00125-011-2328-9
147. Rieusset J, Chauvin MA, Durand A, Bravard A, Laugerette F, Michalski MC, et al. Reduction of endoplasmic reticulum stress using chemical chaperones or Grp78 overexpression does not protect muscle cells from palmitate-induced insulin resistance. *Biochem Biophys Res Commun.* (2012) 417:439–45. doi: 10.1016/j.bbrc.2011.11.135
148. Novgorodov SA, Chudakova DA, Wheeler BW, Bielawski J, Kindy MS, Obeid LM, et al. Developmentally regulated ceramide synthase 6 increases mitochondrial Ca^{2+} loading capacity and promotes apoptosis. *J Biol Chem.* (2011) 286:4644–58. doi: 10.1074/jbc.M110.164392
149. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab.* (2014) 20:687–95. doi: 10.1016/j.cmet.2014.09.015
150. Gudz TI, Tserng KY, Hoppel CL. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. *J Biol Chem.* (1997) 272:24154–8. doi: 10.1074/jbc.272.39.24154
151. Tubbs E, Chanon S, Robert M, Bendridi N, Bidaux G, Chauvin M-A, et al. Disruption of mitochondria-associated endoplasmic reticulum membrane (MAM) integrity contributes to muscle insulin resistance in mice and humans. *Diabetes.* (2018) 67:636–50. doi: 10.2337/db17-0316
152. Bionda C, Portoukalian J, Schmitt D, Rodriguez-Lafrasse C, Ardail D. Subcellular compartmentalization of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? *Biochem J.* (2004) 382:527–33. doi: 10.1042/BJ20031819
153. Hu W, Bielawski J, Samad F, Merrill AH, Cowart LA. Palmitate increases sphingosine-1-phosphate in C2C12 myotubes via upregulation of sphingosine kinase message and activity. *J Lipid Res.* (2009) 50:1852–62. doi: 10.1194/jlr.M800635-JLR200
154. Rapizzi E, Taddei ML, Fiaschi T, Donati C, Bruni P, Chiarugi P. Sphingosine 1-phosphate increases glucose uptake through trans-activation of insulin receptor. *Cell Mol Life Sci.* (2009) 66:3207–18. doi: 10.1007/s00018-009-0106-3
155. Bruce CR, Risis S, Babb JR, Yang C, Kowalski GM, Selathurai A, et al. Overexpression of sphingosine kinase 1 prevents ceramide accumulation and

ameliorates muscle insulin resistance in high-fat diet–fed Mice. *Diabetes.* (2012) 61:3148–55. doi: 10.2337/db12-0029
156. Qi Y, Chen J, Lay A, Don A, Vadas M, Xia P. Loss of sphingosine kinase 1 predisposes to the onset of diabetes via promoting pancreatic beta-cell death in diet-induced obese mice. *FASEB J.* (2013) 27:4294–304.
157. Mikłosz A, Łukaszuk B, Baranowski M, Górski J, Chabowski A. Effects of inhibition of serine palmitoyltransferase (SPT) and sphingosine kinase 1 (SphK1) on palmitate induced insulin resistance in L6 myotubes. *PLoS ONE.* (2013) 8:e85547. doi: 10.1371/journal.pone.0085547
158. Bruce CR, Risis S, Babb JR, Yang C, Lee-Young RS, Henstridge DC, et al. The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. *Endocrinology.* (2013) 154:65–76. doi: 10.1210/en.2012-1847
159. Wang J, Badeanlou L, Bielawski J, Ciaraldi TP, Samad F. Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance. *Am J Physiol Endocrinol Metab.* (2014) 306:E756–68. doi: 10.1152/ajpendo.00549.2013
160. Qi Y, Chen J, Lay A, Don A, Vadas M, Xia P. Loss of sphingosine kinase 1 predisposes to the onset of diabetes via promoting pancreatic β-cell death in diet-induced obese mice. *FASEB J.* (2013) 27:4294–304. doi: 10.1096/fj.13-230052
161. Ravichandran S, Finlin BS, Kern PA, Özcan S. Sphk2-/- mice are protected from obesity and insulin resistance. *Biochim Biophys Acta Mol Basis Dis.* (2019) 1865:570–6. doi: 10.1016/j.bbadis.2018.12.012
162. Song Z, Wang W, Li N, Yan S, Rong K, Lan T, et al. Sphingosine kinase 2 promotes lipotoxicity in pancreatic β-cells and the progression of diabetes. *FASEB J.* (2019) 33:3636–46. doi: 10.1096/fj.201801496R
163. Mitsutake S, Date T, Yokota H, Sugiura M, Kohama T, Igarashi Y. Ceramide kinase deficiency improves diet-induced obesity and insulin resistance. *FEBS Lett.* (2012) 586:1300–5. doi: 10.1016/j.febslet.2012.03.032
164. Bernacchioni C, Cencetti F, Ouro A, Bruno M, Gomez-Muñoz A, Donati C, et al. Lysophosphatidic acid signaling axis mediates ceramide 1-phosphate-induced proliferation of C2C12 myoblasts. *Int J Mol Sci.* (2018) 19:139. doi: 10.3390/ijms19010139
165. Gangoiti P, Bernacchioni C, Donati C, Cencetti F, Ouro A, Gómez-Muñoz A, et al. Ceramide 1-phosphate stimulates proliferation of C2C12 myoblasts. *Biochimie.* (2012) 94:597–607. doi: 10.1016/j.biochi.2011.09.009
166. Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost HG, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes.* (2013) 62:639–48. doi: 10.2337/db12-0495
167. Park M, Kaddai V, Ching J, Fridianto KT, Sieli RJ, Sugii S, et al. A role for ceramides, but not sphingomyelins, as antagonists of insulin signaling and mitochondrial metabolism in C2C12 myotubes. *J Biol Chem.* (2016) 291:23978–88. doi: 10.1074/jbc.M116.737684
168. Straczkowski M, Kowalska I, Baranowski M, Nikolajuk A, Otziomek E, Zabielski P, et al. Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes. *Diabetologia.* (2007) 50:2366–73. doi: 10.1007/s00125-007-0781-2
169. Zhao H, Przybylska M, Wu IH, Zhang J, Siegel C, Komarnitsky S, et al. Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. *Diabetes.* (2007) 56:1210–8. doi: 10.2337/db06-0719
170. Aerts JM, Ottenhoff R, Powlson AS, Grefhorst A, van EM, Dubbelhuis PF, et al. Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. *Diabetes.* (2007) 56:1341–9. doi: 10.2337/db06-1619
171. Tagami S, Inokuchi JJ, Kabayama K, Yoshimura H, Kitamura F, Uemura S, et al. Ganglioside GM3 participates in the pathological conditions of insulin resistance. *J Biol Chem.* (2002) 277:3085–92. doi: 10.1074/jbc.M103705200
172. Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci USA.* (2003) 100:3445–9. doi: 10.1073/pnas.0635898100
173. Sato T, Nihei Y, Nagafuku M, Tagami S, Chin R, Kawamura M, et al. Circulating levels of ganglioside GM3 in metabolic syndrome: a pilot study. *Obes Res Clin Pract.* (2008) 2:I–II. doi: 10.1016/j.orcp.2008.06.001
174. Veillon L, Go S, Matsuyama W, Suzuki A, Nagasaki M, Yatomi Y, et al. Identification of ganglioside GM3 molecular species in human serum associated with risk factors of metabolic syndrome. *PLoS ONE.* (2015) 10:e0129645. doi: 10.1371/journal.pone.0129645
175. Bozic J, Markotic A, Cikes-Culic V, Novak A, Borovac JA, Vucemilovic H, et al. Ganglioside GM3 content in skeletal muscles is increased in type 2 but decreased in type 1 diabetes rat models: implications of glycosphingolipid metabolism in pathophysiology of diabetes. *J Diabetes.* (2018) 10:130–139. doi: 10.1111/1753-0407.12569
176. Chavez JA, Siddique MM, Wang ST, Ching J, Shayman JA, Summers SA. Ceramides and glucosylceramides are independent antagonists of insulin signaling. *J Biol Chem.* (2014) 289:723–34. doi: 10.1074/jbc.M113.522847
177. Richter EA, Derave W, Wojtaszewski JF. Glucose, exercise and insulin: emerging concepts. *J Physiol.* (2001) 535:313–22. doi: 10.1111/j.1469-7793.2001.t01-2-00313.x
178. Evans PL, McMillin SL, Weyrauch LA, Witczak CA. Regulation of skeletal muscle glucose transport and glucose metabolism by exercise training. *Nutrients.* (2019) 11:2432. doi: 10.3390/nu11102432
179. Helge JW, Dobrzyn A, Saltin B, Gorski J. Exercise and training effects on ceramide metabolism in human skeletal muscle. *Exp Physiol.* (2004) 89:119–27. doi: 10.1113/expphysiol.2003.002605
180. Błachnio-Zabielska A, Baranowski M, Zabielski P, Górski J. Effect of exercise duration on the key pathways of ceramide metabolism in rat skeletal muscles. *J Cell Biochem.* (2008) 105:776–84. doi: 10.1002/jcb.21877
181. Baranowski M, Błachnio-Zabielska AU, Charmas M, Helge JW, Dela F, Ksiazek M, et al. Exercise increases sphingoid base-1-phosphate levels in human blood and skeletal muscle in a time- and intensity-dependent manner. *Eur J Appl Physiol.* (2015) 115:993–1003. doi: 10.1007/s00421-014-3080-x
182. Newsom SA, Schenk S, Li M, Everett AC, Horowitz JF. High fatty acid availability after exercise alters the regulation of muscle lipid metabolism. *Metab Clin Exp.* (2011) 60:852–9. doi: 10.1016/j.metabol.2010.08.004
183. Weske S, Vaidya M, Reese A, von Wnuck Lipinski K, Keul P, Bayer JK, et al. Targeting sphingosine-1-phosphate lyase as an anabolic therapy for bone loss. *Nat Med.* (2018) 24:667–78. doi: 10.1038/s41591-018-0005-y
184. Cordeiro AV, Silva VRR, Pauli JR, da Silva ASR, Cintra DE, Moura LP, et al. The role of sphingosine-1-phosphate in skeletal muscle: physiology, mechanisms, and clinical perspectives. *J Cell Physiol.* (2019) 234:10047–59. doi: 10.1002/jcp.27870
185. Danieli-Betto D, Germinario E, Esposito A, Megighian A, Midrio M, Ravara B, et al. Sphingosine 1-phosphate protects mouse extensor digitorum longus skeletal muscle during fatigue. *Am J Physiol, Cell Physiol.* (2005) 288:C1367–73. doi: 10.1152/ajpcell.00246.2004
186. Tsalouhidou S, Petridou A, Mougios V. Effect of chronic exercise on DNA fragmentation and on lipid profiles in rat skeletal muscle. *Exp Physiol.* (2009) 94:362–70. doi: 10.1113/expphysiol.2008.045732
187. Dobrzyn A, Zendzian-Piotrowska M, Górski J. Effect of endurance training on the sphingomyelin-signalling pathway activity in the skeletal muscles of the rat. *J Physiol Pharmacol.* (2004) 55:305–13.
188. Amati F, Dubé JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, et al. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes.* (2011) 60:2588–97. doi: 10.2337/db10-1221
189. Samjoo IA, Safdar A, Hamadeh MJ, Glover AW, Mocellin NJ, Santana J, et al. Markers of skeletal muscle mitochondrial function and lipid accumulation are moderately associated with the homeostasis model assessment index of insulin resistance in obese men. *PLoS ONE.* (2013) 8:e66322. doi: 10.1371/journal.pone.0066322
190. Devries MC, Samjoo IA, Hamadeh MJ, McCready C, Raha S, Watt MJ, et al. Endurance training modulates intramyocellular lipid compartmentalization and morphology in skeletal muscle of lean and obese women. *J Clin Endocrinol Metab.* (2013) 98:4852–62. doi: 10.1210/jc.2013-2044
191. Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJ, et al. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab.* (2006) 291:E99–107. doi: 10.1152/ajpendo.00587.2005
192. Coen PM, Menshikova EV, Distefano G, Zheng D, Tanner CJ, Standley RA, et al. Exercise and weight loss improve muscle mitochondrial respiration, lipid partitioning, and insulin sensitivity after gastric

bypass surgery. *Diabetes.* (2015) 64:3737–50. doi: 10.2337/db15-0809
193. Dubé JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. *Am J Physiol Endocrinol Metab.* (2008) 294:E882–8. doi: 10.1152/ajpendo.00769.2007
194. Kasumov T, Solomon TPJ, Hwang C, Huang H, Haus JM, Zhang R, et al. Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes. *Obesity.* (2015) 23:1414–21. doi: 10.1002/oby.21117
195. Shepherd SO, Cocks M, Meikle PJ, Mellett NA, Ranasinghe AM, Barker TA, et al. Lipid droplet remodelling and reduced muscle ceramides following sprint interval and moderate-intensity continuous exercise training in obese males. *Int J Obes.* (2017) 41:1745–54. doi: 10.1038/ijo.2017.170
196. An H, He L. Current understanding of metformin effect on the control of hyperglycemia in diabetes. *J Endocrinol.* (2016) 228:R97–106. doi: 10.1530/JOE-15-0447
197. Erickson KA, Smith ME, Anthonymuthu TS, Evanson MJ, Brassfield ES, Hodson AE, et al. AICAR inhibits ceramide biosynthesis in skeletal muscle. *Diabetol Metab Syndr.* (2012) 4:45. doi: 10.1186/1758-5996-4-45
198. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes.* (2006) 55:2579–87. doi: 10.2337/db06-0330
199. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, et al. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes.* (2013) 62:401–10. doi: 10.2337/db12-0686
200. Lemaitre RN, Yu C, Hoofnagle A, Hari N, Jensen PN, Fretts AM, et al. Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: the strong heart family study. *Diabetes.* (2018) 67:1663–72. doi: 10.2337/db17-1449
201. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, DeFronzo RA, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes.* (2009) 58:337–43. doi: 10.2337/db08-1228
202. Huang H, Kasumov T, Gatmaitan P, Heneghan HM, Kashyap SR, Schauer PR, et al. Gastric bypass surgery reduces plasma ceramide subspecies and improves insulin sensitivity in severely obese patients. *Obesity.* (2011) 19:2235–40. doi: 10.1038/oby.2011.107
203. Promrat K, Longato L, Wands JR, de la Monte SM. Weight loss amelioration of non-alcoholic steatohepatitis linked to shifts in hepatic ceramide expression and serum ceramide levels. *Hepatol Res.* (2011) 41:754–62. doi: 10.1111/j.1872-034X.2011.00815.x
204. Kayser BD, Lhomme M, Dao MC, Ichou F, Bouillot J-L, Prifti E, et al. Serum lipidomics reveals early differential effects of gastric bypass compared with banding on phospholipids and sphingolipids independent of differences in weight loss. *Int J Obes.* (2017) 41:917–25. doi: 10.1038/ijo.2017.63
205. Mathews AT, Famodu OA, Olfert MD, Murray PJ, Cuff CF, Downes MT, et al. Efficacy of nutritional interventions to lower circulating ceramides in young adults: FRUVEDomic pilot study. *Physiol Rep.* (2017) 5:e13329. doi: 10.14814/phy2.13329
206. Leal-Witt MJ, Ramon-Krauel M, Samino S, Llobet M, Cuadras D, Jimenez-Chillaron JC, et al. Untargeted metabolomics identifies a plasma sphingolipid-related signature associated with lifestyle intervention in prepubertal children with obesity. *Int J Obes.* (2018) 42:72–8. doi: 10.1038/ijo.2017.201
207. Majumdar I, Mastrandrea LD. Serum sphingolipids and inflammatory mediators in adolescents at risk for metabolic syndrome. *Endocrine.* (2012) 41:442–9. doi: 10.1007/s12020-011-9589-4
208. de Mello VDF, Lankinen M, Schwab U, Kolehmainen M, Lehto S, Seppänen-Laakso T, et al. Link between plasma ceramides, inflammation and insulin resistance: association with serum IL-6 concentration in patients with coronary heart disease. *Diabetologia.* (2009) 52:2612–5. doi: 10.1007/s00125-009-1482-9
209. Merrill AH, Lingrell S, Wang E, Nikolova-Karakashian M, Vales TR, Vance DE. Sphingolipid biosynthesis de novo by rat hepatocytes in culture. Ceramide and sphingomyelin are associated with, but not required for, very low density lipoprotein secretion. *J Biol Chem.* (1995) 270:13834–41. doi: 10.1074/jbc.270.23.13834
210. Lightle S, Tosheva R, Lee A, Queen-Baker J, Boyanovsky B, Shedlofsky S, et al. Elevation of ceramide in serum lipoproteins during acute phase response in humans and mice: role of serine-palmitoyl transferase. *Arch Biochem Biophys.* (2003) 419:120–8. doi: 10.1016/j.abb.2003.08.031
211. Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL, et al. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res.* (2008) 102:669–76. doi: 10.1161/CIRCRESAHA.107.165845
212. Kowalski GM, Carey AL, Selathurai A, Kingwell BA, Bruce CR. Plasma sphingosine-1-phosphate is elevated in obesity. *PLoS ONE.* (2013) 8:e72449. doi: 10.1371/journal.pone.0072449
213. Ito S, Iwaki S, Koike K, Yuda Y, Nagasaki A, Ohkawa R, et al. Increased plasma sphingosine-1-phosphate in obese individuals and its capacity to increase the expression of plasminogen activator inhibitor-1 in adipocytes. *Coron Artery Dis.* (2013) 24:642–50. doi: 10.1097/MCA.0000000000000033
214. Tong X, Peng H, Liu D, Ji L, Niu C, Ren J, et al. High-density lipoprotein of patients with type 2 diabetes mellitus upregulates cyclooxgenase-2 expression and prostacyclin I-2 release in endothelial cells: relationship with HDL-associated sphingosine-1-phosphate. *Cardiovasc Diabetol.* (2013) 12:27. doi: 10.1186/1475-2840-12-27
215. Liu M, Seo J, Allegood J, Bi X, Zhu X, Boudyguina E, et al. Hepatic apolipoprotein M (apoM) overexpression stimulates formation of larger apoM/sphingosine 1-phosphate-enriched plasma high density lipoprotein. *J Biol Chem.* (2014) 289:2801–14. doi: 10.1074/jbc.M113.499913
216. Nojiri T, Kurano M, Tokuhara Y, Ohkubo S, Hara M, Ikeda H, et al. Modulation of sphingosine-1-phosphate and apolipoprotein M levels in the plasma, liver and kidneys in streptozotocin-induced diabetic mice. *J Diabetes Investig.* (2014) 5:639–48. doi: 10.1111/jdi.12232
217. Poti F, Simoni M, Nofer JR. Atheroprotective role of high-density lipoprotein (HDL)-associated sphingosine-1-phosphate (S1P). *Cardiovasc Res.* (2014) 103:395–404. doi: 10.1093/cvr/cvu136
218. Mihanfar A, Nejabati HR, Fattahi A, Latifi Z, Pezeshkian M, Afrasiabi A, et al. The role of sphingosine 1 phosphate in coronary artery disease and ischemia reperfusion injury. *J Cell Physiol.* (2019) 234:2083–94. doi: 10.1002/jcp.27353
219. Kurano M, Tsukamoto K, Shimizu T, Kassai H, Nakao K, Aiba A, et al. Protection against insulin resistance by apolipoprotein M/sphingosine-1-phosphate. *Diabetes.* (2020) 69:867–81. doi: 10.2337/db19-0811
220. Yu Y, Zhang J, Yao S, Pan L, Luo G, Xu N. Apolipoprotein M overexpression through adeno-associated virus gene transfer improves insulin secretion and insulin sensitivity in Goto-Kakizaki rats. *J Diabetes Investig.* (2020). doi: 10.1111/jdi.13261
221. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, et al. Serum sphingolipids: relationships to insulin sensitivity and changes with exercise in humans. *Am J Physiol Endocrinol Metab.* (2015) 309:E398–408. doi: 10.1152/ajpendo.00134.2015
222. Wigger L, Cruciani-Guglielmacci C, Nicolas A, Denom J, Fernandez N, Fumeron F, et al. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep.* (2017) 18:2269–79. doi: 10.1016/j.celrep.2017.02.019
223. Szpigel A, Hainault I, Carlier A, Venteclef N, Batto AF, Hajduch E, et al. Lipid environment induces ER stress, TXNIP expression and inflammation in immune cells of individuals with type 2 diabetes. *Diabetologia.* (2018) 61:399–412. doi: 10.1007/s00125-017-4462-5
224. Brozinick JT, Hawkins E, Hoang BH, Kuo MS, Tan B, Kievit P, et al. Plasma sphingolipids are biomarkers of metabolic syndrome in non-human primates maintained on a Western-style diet. *Int J Obes.* (2013) 37:1064–70. doi: 10.1038/ijo.2012.191

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 15 September 2020
doi: 10.3389/fendo.2020.00652

Sphingolipids in the Heart: From Cradle to Grave

Anna Kovilakath[1†], *Maryam Jamil*[1†] *and Lauren Ashley Cowart*[2,3*]

[1] Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA, United States, [2] Department of Biochemistry and Molecular Biology and the Massey Cancer Center, Virginia Commonwealth University, Richmond, VA, United States, [3] Hunter Holmes McGuire Veteran's Affairs Medical Center, Richmond, VA, United States

Cardiovascular diseases are the leading cause of mortality worldwide and this has largely been driven by the increase in metabolic disease in recent decades. Metabolic disease alters metabolism, distribution, and profiles of sphingolipids in multiple organs and tissues; as such, sphingolipid metabolism and signaling have been vigorously studied as contributors to metabolic pathophysiology in various pathological outcomes of obesity, including cardiovascular disease. Much experimental evidence suggests that targeting sphingolipid metabolism may be advantageous in the context of cardiometabolic disease. The heart, however, is a structurally and functionally complex organ where bioactive sphingolipids have been shown not only to mediate pathological processes, but also to contribute to essential functions in cardiogenesis and cardiac function. Additionally, some sphingolipids are protective in the context of ischemia/reperfusion injury. In addition to mechanistic contributions, untargeted lipidomics approaches used in recent years have identified some specific circulating sphingolipids as novel biomarkers in the context of cardiovascular disease. In this review, we summarize recent literature on both deleterious and beneficial contributions of sphingolipids to cardiogenesis and myocardial function as well as recent identification of novel sphingolipid biomarkers for cardiovascular disease risk prediction and diagnosis.

Keywords: sphingolipids, ceramide, sphingosine-1-phosphate, heart development, cardiovascular disease

OPEN ACCESS

Edited by:
Scott A. Summers,
The University of Utah, United States

Reviewed by:
Taesik Park,
Gachon University, South Korea
Annarita Di Lorenzo,
Cornell University, United States

***Correspondence:**
Lauren Ashley Cowart
lauren.cowart@vcuhealth.org

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *22 June 2020*
Accepted: *11 August 2020*
Published: *15 September 2020*

Citation:
Kovilakath A, Jamil M and Cowart LA (2020) Sphingolipids in the Heart: From Cradle to Grave. Front. Endocrinol. 11:652. doi: 10.3389/fendo.2020.00652

INTRODUCTION

Sphingolipids, which constitute a large and diverse lipid class, were originally recognized over a century ago as structural components of cell membranes. More recently they are recognized as crucial bioactive lipids that regulate many cell processes (1). Sphingolipid biosynthesis commences with condensation of an amino acid with acyl-CoA to yield an amino alcohol, or sphingoid base, which is the defining structural component of the sphingolipid class. The sphingoid base can subsequently be modified by acylation, phosphorylation, glycosylation, and/or addition of multiple headgroups or other functional groups (2, 3). These structural modifications generate hundreds of sphingolipid subspecies involved in most if not all major aspects of cell regulation including cell division and senescence, migration, differentiation, apoptosis, autophagy, nutrient uptake, metabolism, and protein synthesis (1). Commensurate with their multiple regulatory roles, disruption of sphingolipid metabolism has emerged as a component of many diseases including cardiometabolic disease. As such, sphingolipid metabolism may be a suitable therapeutic target in the context of cardiovascular disease (CVD). However, perhaps less appreciated are the constitutive and protective roles of sphingolipids in some contexts including heart development

and ischemic injury, and these desirable and homeostatic roles should be considered for both *in vivo* experimental design and, more importantly, developing pharmacologic strategies for clinical use. A comprehensive awareness of both deleterious and beneficial roles of sphingolipids will inform successful therapeutic approaches based on targeting sphingolipid metabolism.

SPHINGOLIPID BIOSYNTHESIS: A BRIEF OVERVIEW

De novo synthesis of sphingolipids starts in the endoplasmic reticulum (ER) where the enzyme serine palmitoyltransferase (SPT) catalyzes the condensation of an amino acid with acyl-CoA into 3-ketodihydrophingosine (KDHSph). The second step occurs through 3-Ketodihydrosphingosine reductase, which rapidly converts KDHSph to dihydrosphingosine (DHS). DHS is the first easily detected sphingolipid metabolite and serves as the sphingoid base for synthesis of ceramides and downstream complex sphingolipids. DHS can be phosphorylated (forming DHS-1-phosphate) but more often undergoes N-acylation. This is accomplished by a family of (dihydro)ceramide synthase enzymes (CerS) consisting of 6 isoforms with various enzymological distinctions including partially distinct substrate preferences for the incorporation of fatty acids with different chain lengths (4–6). In mammals the length of the ceramide acyl chain length ranges from medium (12-14C), long (16-20C), very long (22-26C), and ultra-long chain fatty acids (>26C). Dihydroceramide (DHC) is converted into ceramide by DHC desaturase (DES), which introduces a double bond into the sphingoid base. Once formed, ceramide can be hydrolyzed by ceramidase enzymes, yielding sphingosine, which can be reincorporated into ceramides by CerS or phosphorylated by sphingosine kinases (SphK1 and SphK2) to produce sphingosine-1-phosphate. Ceramide can also undergo phosphorylation, yielding ceramide-1-phosphate, or O-acylation, yielding a structure similar to a triacylglycerol (TAG), and similarly, is stored in lipid droplets (7). Most ceramide, however, is shuttled to the Golgi apparatus via vesicular transport or ceramide transport protein (CERT) for further metabolism to complex sphingolipids including glycosphingolipids (GSLs) and sphingomyelins (SM) through the addition of sugars or phosphocholine, respectively. These complex sphingolipids can be catabolized to yield ceramide, which plays an essential role in regulating cell ceramide profiles. A less well-studied pathway of sphingomyelin catabolism generates sphingosylphosphorylcholine (SPC). SPC is composed of a long-chain sphingosine and phosphorylcholine and is essentially lyso-sphingomyelin, thus sharing similar structure with S1P and other lysophospholipids. Phosphorylated sphingoid bases are the only known sphingolipids that can exit the cell sphingolipid pool. This occurs via S1P lyase (SPL) which catabolizes S1P into non-sphingolipid components; fatty aldehyde and ethanolamine phosphate. Because SPL is the only exit from the sphingolipid metabolic pathway, it has been proposed as a major regulator of total cell sphingolipid levels (8).

The diversity of sphingolipid species arises not only from the length of the N-acyl chain of ceramide and its derivatives or the functional groups added to the sphingoid base, but also from the length of the sphingoid base. Synthesis of the sphingoid base occurs through SPT, a multimeric enzyme comprised of catalytic subunits and various regulatory proteins. SPTLC1 and SPTLC2 form the canonical catalytic complex, but SPTLC3 can also be included and/or substitute for SPTLC2. The composition of the SPT complex determines substrate and product specificity. The SPTLC1/SPTLC2 complex condenses serine with palmitoyl-CoA giving rise to canonical sphingolipids with an 18-carbon sphingoid backbone (d18:0 DHS) (9). In contrast, inclusion of SPTLC3 in the SPT complex renders a more promiscuous enzyme, using 14-carbon myristoyl-CoA, 18-carbon stearoyl-CoA, and potentially others. This seems to be regulated at least in part by inclusion of small SPT subunits (ssSPTa and b) in the SPT complex (10). These bases are also incorporated into downstream sphingolipids, though not evenly across all sphingolipid species (11). Importantly, variations in both sphingoid base and N-acyl chain determine biological functions (3, 12). Several proteins, namely, neurite outgrowth inhibitor (Nogo-A/B) and orosomucoid-like proteins (ORMDL) negatively regulate SPT. Upon their respective ablation or inhibition, the resulting heart phenotype drastically varies, suggesting sphingolipid levels from *de novo* synthesis must be within a narrow range to maintain normal heart homeostasis (13–15). In the cardiovascular system, many sphingolipid subclasses and even specific molecules have distinct functions, some of which are desirable and others deleterious; for example, increased ceramide and SM oftentimes with concomitant decrease of S1P have been implicated in dilated cardiomyopathy, diabetic cardiomyopathy (DbCM), ischemic heart disease (IHD), and myocarditis (16–20). Therefore, the alterations that occur in sphingolipid content and profiles in disease contexts have emerged as a central focus in cardiovascular biology.

SPHINGOLIPIDS IN HEART DEVELOPMENT AND THE CARDIAC CONDUCTION SYSTEM

Multiple processes are involved in forming the heart, which is the first functional organ in vertebrate embryos. Cardiogenesis begins with formation and positioning of the primitive heart tube followed by heart looping, and finally chamber and septal formation (Summarized in **Figure 1**). In the context of cardiovascular biology, sphingolipids, especially ceramides, are most often considered deleterious; however, data show that the sphingosine kinase/sphingosine-1-phosphate signaling pathway is essential for heart development.

Studies in both zebrafish and mice support a regulatory function for sphingosine-1-phosphate (S1P) in formation and positioning of the primitive heart tube. Sphingosine produced by hydrolytic de-acylation of ceramides can be phosphorylated by sphingosine kinases (SphKs) to produce S1P, which signals

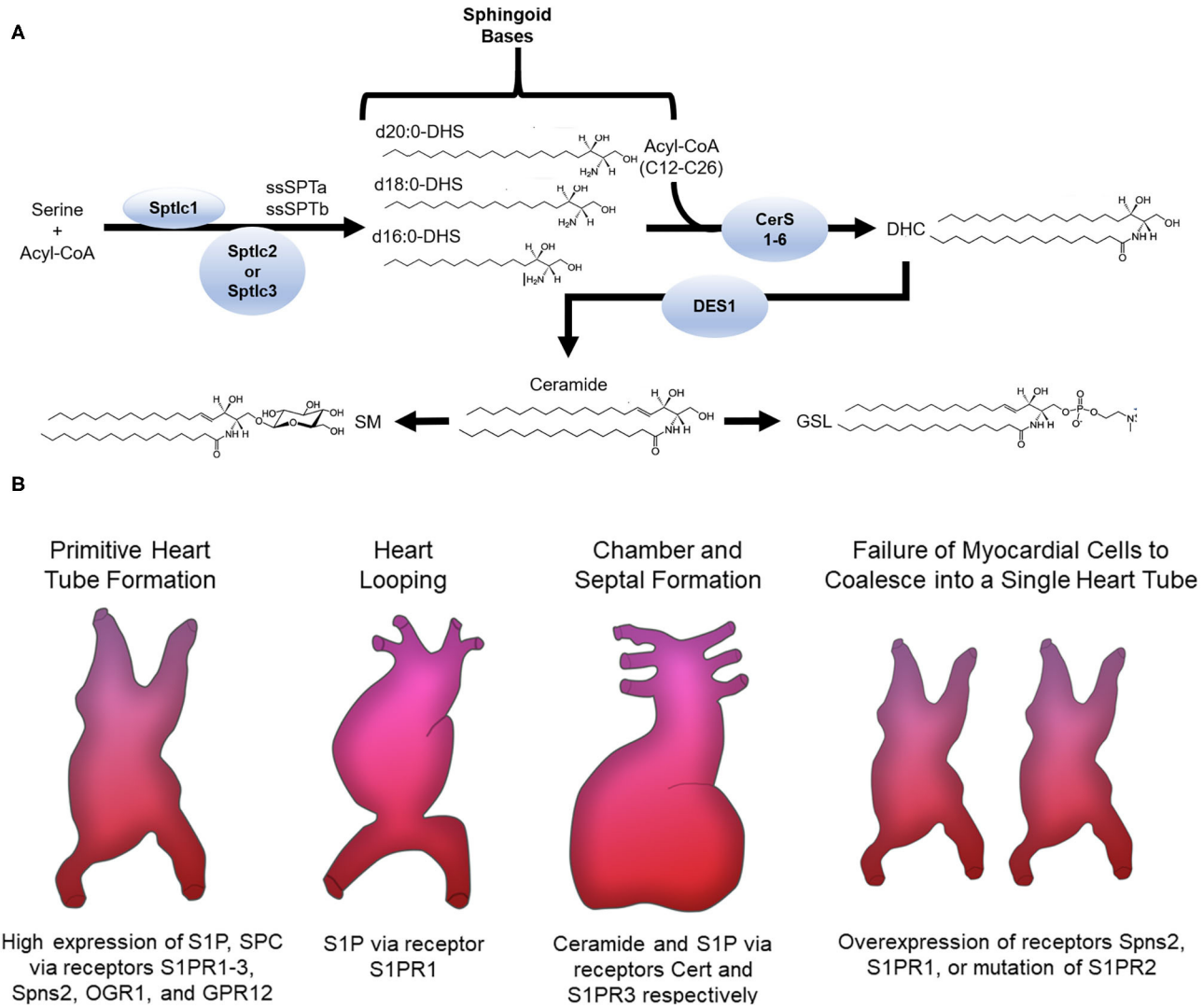

FIGURE 1 | (A) Sphingolipid *de novo* synthesis. Overview of sphingolipid structure and metabolism. ssSPTa, small SPT subunit a; ssSPTb, small SPT subunit b; DHS, dihydrosphingosine; CerS, ceramide synthase; DHC, dihydroceramide; DES, DHC desaturase; SM, sphingomyelin; GSL, glycosphingolipid. **(B)** Sphingolipids involved in cardiogenesis. The receptors S1PR1-3, Spns2, OGR1, GPR12 and the sphingolipids S1P and SPC are highly expressed in stem cells that migrate to form the primitive heart tube. Normal expression of S1P via the S1PR1 receptor is needed for normal heart looping. Ceramide and S1P via the Cert and S1PR3 receptors, respectively, are required for chamber and septal formation. Cardia bifida is observed after failure of the myocardial cells to coalesce into one single primitive heart tube. Overexpression of Spns2 or S1PR1 or mutation of S1PR2 causes cardia bifida.

through endocrine, paracrine, and autocrine mechanisms depending on context (21). For endocrine and paracrine functions S1P is transported into the extracellular milieu through plasma membrane ATP binding cassette family members (ABC) or spinster two (Spns2) transporters where it then signals through one of five different G protein-coupled receptors (S1PR1-5) (21). In Zebrafish, mutations in *s1pr2*, but not any of the other S1P receptors, led to cardia bifida or formation of two laterally positioned hearts (22) (Summarized in **Figure 1**). This phenotype was also observed in multiple studies with a *spns2* mutant or overexpression of *s1pr1* (22–29). In mice, conditional knockout (KO) of S1PR1 caused embryonic lethality due to ventricular non-compaction, ventricular septal defects, absence of normal increase in the number of cardiomyocytes and decreased myofibril organization (28). These studies suggest S1P signaling components S1PR1, S1PR2, and Spns2 are necessary for cardiomyocyte expansion and myocardial precursor migration to the ventral midline of the embryo where they develop into the primitive heart tube. At later developmental stages, knockdown of the zebrafish gene homolog to *s1pr1* caused an improperly looped heart (30, 31). Another study showed that an *s1pr1* morpholino in zebrafish affected heart valve orientation, an indicator of incorrect looping (28). During the looping process, precursors of cardiac valves—endocardial

cushioning (EC) and atrioventricular canal (AV)—are also formed. In mice, S1PR1$^{-/-}$, but not S1PR2 or S1PR3 KO, resulted in embryonic lethality due to severe heart hemorrhaging (32, 33). This may arise from the well-known role of this receptor in maintaining cell-cell contacts in the vascular endothelium (34). In mouse whole embryo cultures, inhibition of sphingosine kinase led to cell death, but elevating S1P levels prevented differentiation of cells into distinct cardiac cell types (35). These studies show that appropriate concentrations of S1P must be maintained for normal development of cardiac valve precursors. It should be noted, however, that affinities of S1P receptors for S1P are in the nanomolar range (e.g., 50–250 nM) and therefore, effects observed with higher concentrations *in vitro* may arise from non-specific activity, for example cross-reactivity with other lysophospholipid receptors, or even gross membrane perturbations due to the detergent properties of S1P. Therefore, care must be taken in interpreting data from experiments using supraphysiological S1P concentrations.

In addition to development, cardiac function also suffers from perturbed sphingolipid synthesis. For example, mouse embryos treated with exogenous S1P exhibited sinus bradycardia (decreased heart rate) (35, 36). In addition, S1PR3 KO mice treated with FTY720, an S1P receptor agonist known to cause sinus bradycardia in humans, did not show altered heart rates, whereas arrhythmias were observed in S1PR3 knock-in (KI) mice (36). S1PR3 is expressed on neural crest-derived atrioventricular nodes (AVN), His bundles, cardiac Purkinje fibers and vascular smooth muscles of the coronary arteries in mice. Extensive studies have suggested S1P plays a multifaceted role as a primary and secondary messenger in regulating both calcium and potassium ion channels (37, 38). This suggests S1P binds its receptor, S1PR3 within the AVN conduction block to regulate intracellular calcium and potassium levels which in turn alter the heart rate (39–41). Taken together these studies show broad roles for S1P receptors in the cardiac conduction system.

SPC displays cross-reactivity to S1P receptors due to its structural similarity to S1P. In addition, SPC also signals via OGR1 and GPR12 receptors (42). SPC, like S1P, is important for heart development, playing a pivotal role for end stage differentiation of committed multipotent cardiovascular progenitors to cardiomyocytes, vascular smooth muscle and endothelial cells (43). Importantly, SPC also induced differentiation of resident cardiac stem cells to cardiomyocytes, a finding which may hold tremendous therapeutic potential (44), as there is currently great interest in therapeutic strategies that leverage the potential for stem cell differentiation to cardiomyocytes in treating cardiac injury. In sum, several lines of evidence point to essential functions of S1P, SPC and their respective receptors in normal heart development and function (summarized in **Table 1**). Therefore, while sphingolipids have largely been implicated in cardiac pathology, they make essential contributions to cardiogenesis and therefore a broader cognizance of sphingolipids in the heart may benefit efforts to develop sphingolipid-based therapeutic approaches.

SPHINGOLIPIDS IN CARDIOVASCULAR DISEASE

Cardiovascular diseases (CVDs) are the leading cause of death in USA and worldwide, and it is estimated that by 2030 upward of 40% of the American population will be afflicted with some form of CVD (64). CVD occurs largely in the context of metabolic disease, such as diabetes, and obesity which are known to reconfigure sphingolipid profiles in multiple organs and tissues. It is unsurprising, then that a wide spectrum of sphingolipid species have been implicated in the pathophysiology of numerous CVDs (65, 66).

Myocardial Lipotoxicity

It is now well-established that ceramide metabolism is altered in the context of type 2 diabetes mellitus (T2DM) and obesity which are both linked to CVD (67–69). In fact, higher plasma ceramide levels have been associated with visceral obesity, non-alcoholic fatty liver disease, and T2DM, which are also predictors of CVDs (70–76). Genetically modified mouse models of lipotoxicity have greatly facilitated understanding of sphingolipid contributions to lipotoxic cardiomyopathy (The outcomes of many of these lipotoxic animal models are summarized in **Table 1**). In fact, the first recognition of a potential link between sphingolipids and cardiac lipotoxicity arose from mice with cardiomyocyte-specific overexpression of long-chain acyl-CoA synthetase (77). This increased lipid uptake generated a lipotoxic cardiomyopathy phenotype. Hearts from these mice showed increased TAG concomitant with increased lipid droplets (77, 78). Because increased cell death was observed in these mice the investigators measured total ceramide, a known apoptotic mediator, and found a 50% increase in total ventricular ceramide content. Metabolic disease increases uptake, utilization, and storage of fatty acids in lipid droplets, but the potential toxicity of lipid droplets/TAG, or other lipids remained in question. To address this, a follow-up study crossed these transgenic mice with another strain overexpressing diacylglycerol acyltransferase 1 (DGAT1), which increased intracellular TAG and reduced ceramide (79). Because DGAT1 catalyzes the final step in TAG synthesis, it diverts Acyl-CoA into neutral sphingolipid pools, thereby reducing toxic lipids in myocardial lipotoxicity such as ceramides (80, 81). Long-chain acyl-CoA synthetase 1 (ACSL1) catalyzes the conversion of long-chain fatty acids to fatty acyl-CoAs, which are then used as substrates by SPT in *de novo* sphingolipid biosynthesis (82). Thus, crossing the DGAT1 mice with the acyl coenzyme A synthetase-1 (ACSL1) transgenic mice increased TAG and lipid droplets, but lowered ceramides and improved the cardiac phenotype of ACSL1 mice, indicating that triacylglycerols (TAGs) are not lipotoxic *per se* but serve as an indicator of lipid oversupply (83). These studies and others gave rise to the concept that routing of lipids into TAGs could decrease their incorporation into bioactive lipids and therefore improve cardiac outcomes of lipotoxicity; however, whether sphingolipids *per se* were the underlying toxic lipid species remained to be addressed.

The first studies to effectively identify a mechanistic link between ceramide and lipotoxic cardiomyopathy employed mice with transgenic overexpression of a GPI-anchored lipoprotein

TABLE 1 | Sphingolipid knockout models and their cardiac tissue phenotypes.

Animal model	Cardiac tissue phenotype	References
Constitutive heterozygous *Sptlc1* knockout & glycosylphosphatidylinositol (GPI)-anchored human lipoprotein lipase transgenic	- Decreased cardiac ceramides comparable to WT mice - Prevention of lipotoxic cardiomyopathy induced by glycosylphosphatidylinositol (GPI)-anchored human lipoprotein lipase knockout	(16)
Cardiomyocyte-specific *Sptlc2* knockout	- Decreased C18:0 and very long chain ceramides - Increased ER stress markers - Increased apoptosis - Upregulation of heart failure markers - Decreased fractional shortening - Thinner cardiac walls	(45, 46)
Constitutive *α-galactosidase A* knockout (Fabry disease)	- Progressive accumulation of globotrioasylceramide in aged mice - Decreased glucosylceramides - No alteration in total ceramide	(47, 48)
Constitutive *Smpd1* knockout	- Accumulation of aSMase in aged mice	(49)
Heterozygous smooth muscle-specific deletion of *Asah1* (acid ceramidase)	- Severe arterial medial calcification in aorta and coronary arteries	(50)
Constitutive heterozygous *SPL* knockout	- Smaller infarct size after ischemic/reperfusion (I/R) injury - Increased S1P - Increased functional recovery after I/R	(51)
Constitutive mutant *Spns2* allele (zebrafish)	- Cardia bifida - Shortened anterior–posterior distance in the ventral pharyngeal arch - Embryonic lethality	(30)
Constitutive *SphK1* knockout	- Decreased S1P levels - Poor animal resuscitation after cardiac arrest - Impaired survival post-resuscitation after cardiac arrest - Increased infarct sizes after I/R	(52, 53)
Constitutive maternal and zygotic *SphK2* knockout (zebrafish)	- Cardia bifida - Failure of cardiac progenitor migration to form primitive heart tube - Decreased S1P levels	(54)
Cardiomyocyte-specific *S1pr1* knockout	- Ventricular non-compaction - Ventricular septal defects - Perinatal lethality - Decreased cardiomyocyte proliferation - Decreased myofibril organization - No alteration in coronary I/R injury	(55)
Cardiomyocyte, endocardial & epicardial-specific *S1pr1* knockout	- Ventricular non-compaction - Ventricular septal defects Perinatal lethality	(55)
Cardiomyocyte-specific *S1pr2* knockout	- No alteration in coronary I/R injury	(56)
Constitutive mutant *s1pr2* allele (zebrafish, *mil*)	- Cardia bifida - Failure of cardiac progenitor migration to form primitive heart tube - Embryonic lethality	(22)
Cardiomyocyte-specific *S1pr3* knockout	- No alteration in coronary I/R injury	(57)
Constitutive *S1pr2* & *S1pr3* double knockout	- Increased infarct size after I/R injury - Perinatal lethality	(58, 59)
Constitutive *S1pr2* & *ApoE* double knockout	- Decreased atherosclerotic lesions - Decreased number of macrophages in lesions	(60)
Constitutive *S1pr3* & *ApoE* double knockout	- No change in atherosclerotic lesions - Decreased number of macrophages in lesions	(61, 62)
Constitutive *Cert* knockout	- Severely compromised cardiac function - Accumulation of ceramide - Embryonic lethality	(63)

lipase on the cardiomyocyte surface (LpL^{GPI}) (78). Lipoprotein lipase (LpL) degrades circulating TAGs into free fatty acids, thus increasing fatty acids to cardiomyocytes. Similar to the ACSL1 mice, these mice showed a robust lipotoxic cardiomyopathy phenotype. These mice were treated with an inhibitor of *de novo* sphingolipid biosynthesis, myriocin, which lowered ceramide and ameliorated the phenotype. Additionally, crossing the LpL^{GPI} mice to mice haploinsufficient in SPTLC1, which mediates *de novo* sphingolipid synthesis, showed a similar effect (16). These studies suggest that in the lipotoxic context,

sphingolipid synthesis is deleterious, and increased incorporation of fatty acids into TAG is cardioprotective. This would imply that myocardial TAG levels are not indicative of cardiac dysfunction but rather reflect lipid metabolic dysfunction within the heart.

These studies were highly informative and led our lab to investigate specific sphingolipid species that may play roles in lipotoxic cardiomyopathy and the mechanism(s) by which they contributed. Based on our previous studies on the effects of saturated vs. unsaturated fatty acids on sphingolipid metabolism, we developed a high saturated fat diet (84–86). Mice on this high fat diet exhibited elevations in total myocardial ceramides and also DbCM-like cardiac hypertrophy and dysfunction (87). Further examination of specific ceramide chain lengths revealed that C14:0 ceramide as well as very long-chain (VLC) ceramides increased specifically in the high saturated fat-fed mice compared to mice on control and lard diets. Reducing sphingolipid synthesis by inhibition of SPT with Myriocin treatment inhibited sphingolipid synthesis including C14:0 ceramide production and also restored cardiac function. These animals also showed increased autophagosomes in cardiomyocytes; indeed, treatment of cultured primary cardiomyocytes with myristate (C14:0) increased autophagy. As noted above, ceramide synthase enzymes have partially distinct substrate preferences, and C14:0 ceramide is a product of CerS5, while VLC ceramides are products of CerS2. Indeed, overexpression of CerS5 in cardiomyocytes increased autophagic flux, and treating cardiomyocytes lacking CerS5 with myristate did not increase autophagy. These studies were the first to identify a specific ceramide species and ceramide synthase isoform in cardiac lipotoxicity. Upon overexpression of CerS2, VLC ceramides were elevated inducing insulin resistance, oxidative stress, mitochondrial dysfunction and mitophagy. As gain and loss-of function studies targeting CerS5 had no effect on these same functions, a distinct role was established for CerS2 (87, 88). These studies established specific roles for subsets of ceramide species and/or CerS enzymes in lipotoxic outcomes in the context of high fat feeding and subsequent diabetes (87, 88). Though these studies were conducted in mice and various primary and cultured cardiomyocyte models, the findings may nonetheless bear some relevance to humans (45). Importantly, many of these studies addressed lipotoxicity in the context of metabolic disease; however, other cardiac insults also cause lipotoxicity, and this may proceed by alternative mechanisms (69–76, 89). For example, metabolic tracing studies in mice subjected to pressure overload via transverse aortic constriction (TAC) showed that transgenic overexpression of acyl coenzyme A synthetase-1 (ACSL1) mitigated heart dysfunction relative to WT mice (83). In this context, ACSL1 overexpression prevented *de novo* synthesis of C16-, C24-, and C24:1-ceramides, which are synthesized by CerS5 and CerS2, respectively, but increased C20- and C22-ceramides. These subspecies can be generated by CerS4 and therefore, CerS4-derived ceramides may have a distinct, protective function in the context of HF, though this remains to be tested.

In addition to the n-acyl chain length, dictated by CerS, we showed that the sphingoid base chain length also distinguishes activities of sphingolipids. While the canonical SPT complex includes SPTLC1 and 2, SPTLC3 is an alternate subunit that can substitute for SPTLC2 and alter sphingoid base chain length. Therefore, the SPTLC1/2 complex generates an 18-carbon sphingoid base, but inclusion of SPTLC3 alters substrate preference to generate sphingoid bases of alternate chain lengths. We showed that high saturated fat feeding in mice induced SPTLC3 and altered cell sphingolipid profiles to include a high proportion of sphingolipids containing a 16-carbon sphingoid base within the myocardium (11). This shortened base did not cause autophagy but rather led to apoptotic cell death in cardiomyocytes, further exemplifying how distinct sphingolipid molecules can have divergent effects. Emerging literature continues to build a case for a role for SPTLC3 in human CVDs. Interestingly, a study of three German populations linked single nucleotide polymorphisms (SNPs) in the SPTLC3 locus to MI (90). Another study identified 28 SNPs close to the SPTLC3 locus which were significantly associated with reduced C22:0 and C24:0 ceramide concentrations, which are thought to correlate with disease risk (91).

Initial studies including our own showed that inhibition of overall sphingolipid biosynthesis prevented cardiac lipotoxicity, suggesting that merely reducing ceramide in the lipotoxic heart may be a "magic bullet." However, rather than improving cardiac function, a cardiomyocyte-specific SPTLC2 null mouse showed an exacerbated cardiac phenotype (46). While the mechanism for this was not revealed in that study, a speculative hypothesis is that SPTLC3 may show compensatory upregulation in the context of SPTLC2 depletion. If so, this could explain the phenotype observed in the SPTLC2 depletion mouse model. These studies coupled with observations of cardioprotection in TAC correlating to increased C20:0 and C22:0 ceramides clearly demonstrate that roles of ceramides in cardiac pathology are complex. In addition to ceramides, however, alterations in dihydroceramides, ceramide-1-phopshates, sphingomyelins, and glycosphingolipids likely play disparate roles in cardiac pathology, and genetic manipulations or use of myriocin *in vivo* does not necessarily enable identification of specific lipid classes involved in pathological processes. Further research is required to fully understand the links between specific sphingolipid pools and molecular structures and deleterious outcomes through modulation of cell signaling.

Coronary Artery Disease

Coronary Artery Disease (CAD) or IHD is the most common type of CVD worldwide and has been the leading cause of death for the past 16 years. CAD is caused by narrowing of arteries and subsequent reduction of blood flow to the heart due to build-up of plaque (atherosclerosis) within the arteries of the heart, ultimately leading to heart failure (HF). More often than not, coronary atherosclerosis observed in CAD occurs in the context of metabolic disease. As a result of chronic CAD, myocardial infarction (MI) and HF often occur. In contrast to much literature implicating ceramides as inducers of CVDs, pronounced cardiogenic and cardioprotective properties have been attributed to S1P (92–101).

Current therapeutics already undergoing clinical trials for CAD, ischemia/reperfusion injury, MI and HF target the

S1P/SphK axis, specifically drugs targeting S1P receptors (102–104). Mice with combined deletion of S1PR2 and S1PR3 subject to ischemia/reperfusion injury showed increased infarct size, however, infarct size was not altered when either S1PR2 or S1PR3 was deleted (58, 105). However, another study showed that intravenous SPC treatment of S1PR3 null mice subject to IR injury reduced infarct size (106). Agonists specific to S1PR1 protected mouse cardiomyocytes from hypoxia, while the opposite effect was observed with S1PR1 antagonists (107–111). Another study showed that S1PR1 attenuated cardiac fibrosis and hypertrophy in mice with HF induced by TAC (112). Nogo-A/B deficient mice were protected from HF for up to 3 months after TAC, while also seeing a significant induction of S1P (14).

In mouse, rabbit and rat models of ischemia/reperfusion injury (IRI) it was noted that ceramide, membrane neutral sphingomyelinase (nSMase) and acidic sphingomyelinase (aSMase) increased in the infarct site and blood with concomitant decrease of S1P (113–120). Ischemic preconditioning with S1P is a tried and proven method to induce significant recovery of cardiac function and infarct reduction in IRI (57, 121–123). The absence of improved cardiac function in SphK1 and SphK2 ablated mice subject to IR levels suggest the significance of S1P in ischemic conditioning (121, 124). These studies suggest targeting the S1P-SphK axis satisfies the criterion as an effective therapeutic agent to overcome the damages elicited by IRI. Though further investigation into the crosstalk between S1P receptors and analogs signaling behavior in comorbidities would better optimize the therapeutic potential of S1P in IRI.

Multiple *in vitro* studies observed upregulation of aSMase and nSMase along with increased SM in animal models of HF (125, 126). The nSMase and aSMase hydrolyze sphingomyelin to release ceramide, and thus the accumulation of ceramide in post-ischemic heart may arise from SM catabolism and not *de novo* sphingolipid biosynthesis (127, 128). Another study in both mice and humans with HF noted increased levels of SPTLC2, which participates in *de novo* sphingolipid synthesis and likely contributes to the significant increase of total ceramides in the aforementioned studies (45). However, this same study did not note any changes in aSMase or nSMase in HF, the primary catabolic enzymes for ceramide production (45). Therefore, it may be that chronic conditions leading to HF increase *de novo* synthesis, while acute insults such as MI activate sphingolipid catabolism, though there are clear exceptions to this concept, including nSMase activity was increased 2–3 months post-MI and SPTLC2 was observed to increase 2 weeks post-MI (45, 127).

While the roles of ceramides and S1P are well-established and antagonistic in CVDs, some literature suggests sphingosine plays a dichotomous role as a cardio protectant and CVD inducer. Induction of sphingosine in patients with and animal models of IRI points toward the maladaptive role of sphingosine in CVDs (129, 130). However, the same group preconditioned animals with sphingosine prior to ischemia or perfusion resulting in massive reduction of infarct size. This contradictory evidence points toward sphingosine as a cardio protectant in CVDs (131). Importantly, sphingosine can be used as a substrate for generation of either ceramides or S1P; therefore, whether it is protective or deleterious may arise from its metabolic fate. However, sphingosine does have its own signaling functions and thus may make these contributions directly and without further metabolism (132–134).

SPHINGOLIPIDS AS EMERGING BIOMARKERS IN ASSESSING CARDIOVASCULAR DISEASE

Over the past several decades various heart studies including the Framingham Heart Study, Busselton Family Heart Study, Strong Heart Family Study, Utah CAD and others have sought to determine CVD development, risks and therapeutics all aimed at combating CVD. As a result, conventional risk factors such as age, sex, ethnicity, blood pressure, total triglyceride levels and total cholesterol levels emerged as biomarkers for risk of major adverse cardiac events. However, given the substantial and rising burden of CVDs, ongoing efforts to shed light on novel, more specific biomarkers for CVD are needed. In response to this need, more sensitive lipidomics analysis have been developed focusing on sphingoid base and acyl chain length composition of total sphingolipids. Using these advanced techniques, risk assessment scores utilizing sphingolipid species levels have been recently developed in detecting CVDs and they continuously outperform conventional cardiovascular risk markers (135). For example, A Busselton Family Heart Study identified many classes of the sphingolipid species ceramides, DHC, mono-, di- and tri-hexosylceramides, SM, GM1, GM3 and sulfatides associated with heritable cardiac events, most of which were positively genetically correlated with LDL, HDL, total cholesterol and negatively correlated with triglyceride levels. In another study, the serum of patients with a clinical diagnosis of HF with preserved ejection fraction (HFpEF), the most common form of HF and also strongly associated with diabetes, in The Alberta Heart Failure Etiology and Analysis Research Team (HEART) project showed reduced SM compared to non-HF controls (136). These sphingolipids seemed to have no relationship to conventional risk factors such as diastolic blood pressure, systolic blood pressure, BMI and waist-hip ratio, suggesting they may be used as more specific markers to identify high-risk patients especially likely to have CVD (137–140).

A growing body of literature suggests long-chain ceramides and very long-chain ceramides and SM are associated with adverse cardiac outcomes. Ceramide analysis on an aggregate of 29,818 individuals from 7 cohort studies and determined plasma Ceramide (d18:1/16:0), (d18:1/18:0), and (d18:1/24:1) levels were associated with major adverse CVD events. Whereas, Ceramide (d18:1/22:0) were not associated (141). Javaheri et al., observed significant association of increased circulating concentrations of C16:0 and C18:0 ceramides in participants with HFpEF (142). This study was especially informative, as HFpEF is difficult to diagnose and controversy still exists as to diagnostic algorithms (143). Similarly, another study showed that levels of ceramide and SM with 16-carbon acyl chain length were directly associated with higher risk of mortality deaths from CVD (144). Analysis of myocardial tissue and serum from patients with HF showed increased ceramide, the significance of which was mainly driven

by the very long chain ceramides (45). When the HFpEF patients underwent left ventricular assist devices (LVAD) these changes were reversible. In contrast, another study observed that levels of Ceramide with a 22-, or 24-carbon acyl chain length and SM with a 20-, 22-, or 24- carbon acyl chain were directly associated with lower risks of CVD mortality (142, 144).

While these biomarkers and diagnostic indicators represent advances in identifying and diagnosing CVD, the distinction between long chain (i.e., C16–C18) and very long chain (i.e., C20–C24) ceramides, which are generated by distinct ceramide synthase isoforms (CerS1/5/6 vs. CerS2/4, respectively) hints at potential mechanistic involvement. However, though much circulating lipid in general arises from liver, a detailed study demonstrated that circulating ceramides but not SM are derived from the endothelium of blood vessels (145). Interestingly the endothelium is functionally impaired in CVDs and could thus contribute to the plasma sphingolipid profile reported in HF. Therefore, further determining the points of origin of these lipids may enable further study on potential mechanistic functions of these lipids in CVD.

DISCUSSION

In this review we highlighted recent studies implicating sphingolipids in heart development, ischemic injury, and CVDs. In general, the SphK/S1P/S1P receptor signaling axis appears beneficial both in development and in ischemic injury. On the other hand, elevation of myocardial ceramides appears mostly deleterious. Therapeutic potentials of targeting the SphK/S1P/S1P and ceramide pathways are beneficial and have been approved for treatment in patients in other systems, for example the FDA approved FTY-720, an S1P antagonist for treating relapsing multiple sclerosis (146). Since these have been tried in other systems they can be applied to CVDs, as targeting this axis in animal models of CVDs suggested prevention or reversal (147). Targeting CerS1-6 have a great therapeutic potential but there have been multiple developmental roadblocks, however a recent study observed that a CerS1 selective inhibitor endogenously inhibited mitochondrial fatty acid oxidation in muscle and regulated whole-body adiposity, which could potentially benefit in treating patients with some forms of CVDs (148). Further, relationships among circulating sphingolipid species are novel biomarkers of CVD. Therefore, sphingolipid metabolism and signaling is a constant thread from heart development to CVD, with distinct pathways playing beneficial or deleterious roles.

Lipidomic analyses based on mass spectrometry has enabled identification of different sphingoid bases and acyl chain lengths allowing for novel biomarkers in diagnosing and assessing risk in the context of CVDs. These novel risk scores and biomarkers that utilize sphingolipids show the full complexity of the altered lipid metabolism and outperform traditional lipid measurements. Sphingolipid risk scores are better predictors of major adverse cardiac events than conventional risk factors, including total and LDL cholesterol. As such, ceramide testing is now routinely performed as a diagnostic tool in CVD (149–152).

Many studies highlighted in this review contradict one another with respect to the significant sphingolipid species associated with a particular heart development stage or in association with a CVD. It is important to note these controversial observations can arise from the type of lipidomics technology utilized, the size of the population studied, the type of sample collected, sex distribution, and race/ethnicity. For example, an African American healthy control population had significantly higher plasma levels of most SM species and lower levels of lactosylceramide species compared to Caucasian control subjects in the same study (153). These differences were noted in healthy individuals; therefore, stratification of a study population based on race/ethnicity is essential to provide clear conclusions and, moreover, to identify health disparities when considering disease patients between different ethnicities.

Implications of the association of SPTLC3 SNPs with MI and other cardiovascular events is an emergent finding deserving further mechanistic study (90, 91). Synthesis of d16-, and d20-sphingoid based sphingolipids are entirely dependent while d18-sphingoid based sphingolipids are partially dependent on SPTLC3. Our lab previously showed that 1/3rd of the mouse myocardial sphingolipid pool is comprised of d16-sphingoid based sphingolipids presumably derived from SPTLC3 (11). Moreover, HF patients showed reduced SPTLC3 upon placement of left-ventricular assist devices (LVAD) (45). In addition to recognizing the function of SPTLC3 to generate non-canonical sphingoid bases, our understanding of the complexity of sphingolipid metabolism and the diversity of sphingolipid species has exploded in recent years. Thus, when it comes to matters of the heart, untargeted sphingolipidomics has the unique potential for revealing non-canonical sphingolipid species that indicate and/or play mechanistic roles in CVD.

Importantly, while most *in vivo* studies in the heart that address sphingolipid function have manipulated SPT, either genetically or pharmacologically (e.g., with myriocin treatment), these approaches inhibit biosynthesis of all sphingolipids, both desirable and deleterious, and, therefore, would be much too broad for clinical application. However, identification of specific enzymes that participate in distinct branches and pathways of sphingolipid metabolism would provide much greater specificity for therapeutic intervention in the CVD context. However, the dearth of isoform-specific CerS inhibitors has been an impediment to therapeutic targeting of CerS, though efforts to develop specific agents are beginning to yield results (148).

Over the last decade our lab has focused on studying heart sphingolipids showing that ceramides with not only distinct N-acyl-chains but particular sphingoid base backbone lead to apoptosis, mitochondrial damage and lipotoxicity in cardiomyocytes. It should be noted that identification of specific CerS isoforms that mediate CVD does not necessarily implicate ceramides, *per se*, but could implicate dihydroceramides as well as downstream products of ceramides including ceramide-1-phosphate, O-acylceramides, sphingomyelin, and glycosphingolipids. This understanding is crucial both for enabling further specificity of therapeutic targeting and facilitating the

potential identification of additional therapeutic targets. Ultimately, more research is needed to elucidate the regulatory pathways by which sphingolipids regulate cardiogenesis and cardiovascular function in both health and disease.

AUTHOR CONTRIBUTIONS

LC and AK: writing—review and editing, visualization, and conceptualization. LC, AK, and MJ: writing—original draft and data curation. LC: supervision and project administration. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by a National Heart, Lung, and Blood Institute Grant 1R01HL151243, National Institutes of Health Grant 1R01HL117233, and Veterans Affairs Merit Award 2I0BX000200.

REFERENCES

1. Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. *Nat Rev Mol Cell Biol.* (2018) 19:175–91. doi: 10.1038/nrm.2017.107
2. Menaldino DS, Bushnev A, Sun A, Liotta DC, Symolon H, Desai K, et al. Sphingoid bases and *de novo* ceramide synthesis: enzymes involved, pharmacology and mechanisms of action. *Pharmacol Res.* (2003) 47:373–81. doi: 10.1016/S1043-6618(03)00054-9
3. Bushnev A, Hagedorn K, Adiga M, Haynes C, Sullards M, Liotta D, et al. Thematic review series: sphingolipids. Biodiversity of sphingoid bases ("sphingosines") and related amino alcohols. *J Lipid Res.* (2008) 49:1621–39. doi: 10.1194/jlr.R800012-JLR200
4. Venkataraman K, Riebeling C, Bodennec J, Riezman H, Allegood JC, Sullards MC, et al. Upstream of growth and differentiation factor 1 (uog1), a mammalian homolog of the yeast longevity assurance gene 1 (LAG1), regulatesN-Stearoyl-sphinganine (C18-(Dihydro) ceramide) synthesis in a fumonisin B1-independent manner in mammalian cells. *J Biol Chem.* (2002) 277:35642–9. doi: 10.1074/jbc.M205211200
5. Riebeling C, Allegood JC, Wang E, Merrill AH, Futerman AH. Two mammalian longevity assurance gene (LAG1) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. *J Biol Chem.* (2003) 278:43452–9. doi: 10.1074/jbc.M307104200
6. Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH, et al. Characterization of ceramide synthase 2 tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. *J Biol Chem.* (2008) 283:5677–84. doi: 10.1074/jbc.M707386200
7. Senkal CE, Salama MF, Snider AJ, Allopenna JJ, Rana NA, Koller A, et al. Ceramide is metabolized to acylceramide and stored in lipid droplets. *Cell Metab.* (2017) 25:686–97. doi: 10.1016/j.cmet.2017.02.010
8. Aguilar A, Saba JD. Truth and consequences of sphingosine-1-phosphate lyase. *Adv Biol Regul.* (2012) 52:17–30. doi: 10.1016/j.advenzreg.2011.09.015
9. Nagiec MM, Baltisberger JA, Wells GB, Lester RL, Dickson RC. The LCB2 gene of saccharomyces and the related LCB1 gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid synthesis. *Proc Natl Acad Sci USA.* (1994) 91:7899–902. doi: 10.1073/pnas.91.17.7899
10. Hornemann T, Richard S, Rütti MF, Wei Y, von Eckardstein A. Cloning and initial characterization of a new subunit for mammalian serine-palmitoyltransferase. *J Biol Chem.* (2006) 281:37275–81. doi: 10.1074/jbc.M608066200
11. Russo SB, Tidhar R, Futerman AH, Cowart LA. Myristate-derived d16:0 sphingolipids constitute a cardiac sphingolipid pool with distinct synthetic routes and functional properties. *J Biol Chem.* (2013) 288:13397–409. doi: 10.1074/jbc.M112.428185
12. Zheng W, Kollmeyer J, Symolon H, Momin A, Munter E, Wang E, et al. Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. *Biochim Biophys Acta.* (2006) 1758:1864–84. doi: 10.1016/j.bbamem.2006.08.009
13. Sasset L, Zhang Y, Dunn TM, Di Lorenzo A. Sphingolipid *de novo* biosynthesis: a rheostat of cardiovascular homeostasis. *Trends Endocrinol Metab.* (2016) 27:807–19. doi: 10.1016/j.tem.2016.07.005
14. Zhang Y, Huang Y, Cantalupo A, Azevedo PS, Siragusa M, Bielawski J, et al. Endothelial Nogo-B regulates sphingolipid biosynthesis to promote pathological cardiac hypertrophy during chronic pressure overload. *JCI Insight.* (2016) 1:e85484. doi: 10.1172/jci.insight.85484
15. Davis D, Kannan M, Wattenberg B. Orm/ORMDL proteins: gate guardians and master regulators. *Adv Biol Regul.* (2018) 70:3–18. doi: 10.1016/j.jbior.2018.08.002
16. Park T-S, Hu Y, Noh H-L, Drosatos K, Okajima K, Buchanan J, et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res.* (2008) 49:2101–12. doi: 10.1194/jlr.M800147-JLR200
17. Maekawa K, Hirayama A, Iwata Y, Tajima Y, Nishimaki-Mogami T, Sugawara S, et al. Global metabolomic analysis of heart tissue in a hamster model for dilated cardiomyopathy. *J Mol Cell Cardiol.* (2013) 59:76–85. doi: 10.1016/j.yjmcc.2013.02.008
18. Knapp M, Zendzian-Piotrowska M, Błachnio-Zabielska A, Zabielski P, Kurek K, Górski J. Myocardial infarction differentially alters sphingolipid levels in plasma, erythrocytes and platelets of the rat. *Basic Res Cardiol.* (2012) 107:294. doi: 10.1007/s00395-012-0294-0
19. Tan G, Zhou Q, Liu K, Dong X, Li L, Liao W, et al. Cross-platform metabolic profiling deciphering the potential targets of shenfu injection against acute viral myocarditis in mice. *J Pharm Biomed Anal.* (2018) 160:1–11. doi: 10.1016/j.jpba.2018.07.042
20. Alonso N, Moliner P, Mauricio D. Pathogenesis, clinical features and treatment of diabetic cardiomyopathy. In: *Heart Failure: From Research to Clinical Practice.* Badalona; Madrid; Lleida; Barcelona: Springer (2017). p. 197–217. doi: 10.1007/5584_2017_105
21. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol.* (2012) 22:50–60. doi: 10.1016/j.tcb.2011.09.003
22. Kupperman E, An S, Osborne N, Waldron S, Stainier DY. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature.* (2000) 406:192–5. doi: 10.1038/35018092
23. Matsui T, Raya Á, Callol-Massot C, Kawakami Y, Oishi I, Rodriguez-Esteban C, et al. miles-apart-mediated regulation of cell–fibronectin interaction and myocardial migration in zebrafish. *Nat Clin Pract Cardiovasc Med.* (2007) 4:S77–82. doi: 10.1038/ncpcardio0764
24. Kawahara A, Nishi T, Hisano Y, Fukui H, Yamaguchi A, Mochizuki N. The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. *Science.* (2009) 323:524–7. doi: 10.1126/science.1167449
25. de Faria Poloni J, Chapola H, Feltes BC, Bonatto D. The importance of sphingolipids and reactive oxygen species in cardiovascular development. *Biol Cell.* (2014) 106:167–81. doi: 10.1111/boc.201400008
26. Kraut R. Roles of sphingolipids in drosophila development and disease. *J Neurochem.* (2011) 116:764–78. doi: 10.1111/j.1471-4159.2010.07022.x
27. Osborne N, Brand-Arzamendi K, Ober EA, Jin S-W, Verkade H, Holtzman NG, et al. The spinster homolog, two of hearts, is required for sphingosine 1-phosphate signaling in zebrafish. *Curr Biol.* (2008) 18:1882–8. doi: 10.1016/j.cub.2008.10.061
28. Guzzolino E, Chiavacci E, Ahuja N, Mariani L, Evangelista M, Ippolito C, et al. Post-transcriptional modulation of sphingosine-1-phosphate receptor 1 by miR-19a affects cardiovascular development in zebrafish. *Front Cell Dev Biol.* (2018) 6:58. doi: 10.3389/fcell.2018.00058

29. Hisano Y, Inoue A, Taimatsu K, Ota S, Ohga R, Kotani H, et al. Comprehensive analysis of sphingosine-1-phosphate receptor mutants during zebrafish embryogenesis. *Genes Cells.* (2015) 20:647–58. doi: 10.1111/gtc.12259
30. Hisano Y, Ota S, Takada S, Kawahara A. Functional cooperation of spns2 and fibronectin in cardiac and lower jaw development. *Biol Open.* (2013) 2:789–94. doi: 10.1242/bio.20134994
31. Mendelson K, Zygmunt T, Torres-Vázquez J, Evans T, Hla T. Sphingosine 1-phosphate receptor signaling regulates proper embryonic vascular patterning. *J Biol Chem.* (2013) 288:2143–56. doi: 10.1074/jbc.M112.427344
32. Kono M, Mi Y, Liu Y, Sasaki T, Allende ML, Wu Y-P, et al. The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. *J Biol Chem.* (2004) 279:29367–73. doi: 10.1074/jbc.M403937200
33. Allende ML, Proia RL. Sphingosine-1-phosphate receptors and the development of the vascular system. *Biochim Biophys Acta.* (2002) 1582:222–7. doi: 10.1016/S1388-1981(02)00175-0
34. Wilkerson BA, Grass GD, Wing SB, Argraves WS, Argraves KM. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier high density lipoprotein (HDL)-S1P prolongs endothelial barrier enhancement as compared with albumin-S1P via effects on levels, trafficking, and signaling of S1P1. *J Biol Chem.* (2012) 287:44645–53. doi: 10.1074/jbc.M112.423426
35. Wendler CC, Rivkees SA. Sphingosine-1-phosphate inhibits cell migration and endothelial to mesenchymal cell transformation during cardiac development. *Dev Biol.* (2006) 291:264–77. doi: 10.1016/j.ydbio.2005.12.013
36. Sanna MG, Vincent KP, Repetto E, Nguyen N, Brown SJ, Abgaryan L, et al. Bitopic sphingosine 1-phosphate receptor 3 (S1P3) antagonist rescue from complete heart block: pharmacological and genetic evidence for direct S1P3 regulation of mouse cardiac conduction. *Mol Pharmacol.* (2016) 89:176–86. doi: 10.1124/mol.115.100222
37. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol.* (2000) 1:11–21. doi: 10.1038/35036035
38. zu Heringdorf DM, Lass H, Alemany R, Laser KT, Neumann E, Zhang C, et al. Sphingosine kinase-mediated Ca2+ signalling by G-protein-coupled receptors. *EMBO J.* (1998) 17:2830–7. doi: 10.1093/emboj/17.10.2830
39. Mangoni ME, Couette B, Bourinet E, Platzer J, Reimer D, Striessnig J, et al. Functional role of L-type Cav1. 3 Ca2+ channels in cardiac pacemaker activity. *Proc Natl Acad Sci USA*. (2003) 100:5543–8. doi: 10.1073/pnas.0935295100
40. Zhang Q, Timofeyev V, Lu L, Li N, Singapuri A, Long MK, et al. Functional roles of a Ca2+-activated K+ channel in atrioventricular nodes. *Circulat Res.* (2008) 102:465–71. doi: 10.1161/CIRCRESAHA.107.161778
41. Mesirca P, Torrente AG, Mangoni ME. Functional role of voltage gated Ca2+ channels in heart automaticity. *Front Physiol.* (2015) 6:19. doi: 10.3389/fphys.2015.00019
42. Ge D, Yue H-w, Liu H-h, Zhao J. Emerging roles of sphingosylphosphorylcholine in modulating cardiovascular functions and diseases. *Acta Pharmacol Sinica.* (2018) 39:1830–6. doi: 10.1038/s41401-018-0036-4
43. Kattman SJ, Huber TL, Keller GM. Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. *Dev Cell.* (2006) 11:723–32. doi: 10.1016/j.devcel.2006.10.002
44. Li W, Liu H, Liu P, Yin D, Zhang S, Zhao J. Sphingosylphosphorylcholine promotes the differentiation of resident Sca-1 positive cardiac stem cells to cardiomyocytes through lipid raft/JNK/STAT3 and β-catenin signaling pathways. *Biochim Biophys Acta.* (2016) 1863:1579–88. doi: 10.1016/j.bbamcr.2016.04.006
45. Ji R, Akashi H, Drosatos K, Liao X, Jiang H, Kennel PJ, et al. Increased *de novo* ceramide synthesis and accumulation in failing myocardium. *JCI Insight.* (2017) 2:e82922. doi: 10.1172/jci.insight.82922
46. Lee S-Y, Kim JR, Hu Y, Khan R, Kim S-J, Bharadwaj KG, et al. Cardiomyocyte specific deficiency of serine palmitoyltransferase subunit 2 reduces ceramide but leads to cardiac dysfunction. *J Biol Chem.* (2012) 287:18429–39. doi: 10.1074/jbc.M111.296947
47. Bangari DS, Ashe KM, Desnick RJ, Maloney C, Lydon J, Piepenhagen P, et al. α-Galactosidase a knockout mice: progressive organ pathology resembles the type 2 later-onset phenotype of fabry disease. *Am J Pathol.* (2015) 185:651–65. doi: 10.1016/j.ajpath.2014.11.004
48. Quinta R, Rodrigues D, Assunção M, Macedo MF, Azevedo O, Cunha D, et al. Reduced glucosylceramide in the mouse model of fabry disease: correction by successful enzyme replacement therapy. *Gene.* (2014) 536:97–104. doi: 10.1016/j.gene.2013.11.073
49. Marathe S, Miranda SR, Devlin C, Johns A, Kuriakose G, Williams KJ, et al. Creation of a mouse model for non-neurological (type B) niemann–pick disease by stable, low level expression of lysosomal sphingomyelinase in the absence of secretory sphingomyelinase: relationship between brain intra-lysosomal enzyme activity and central nervous system function. *Hum Mol Genet.* (2000) 9:1967–76. doi: 10.1093/hmg/9.13.1967
50. Bhat OM, Li G, Yuan X, Huang D, Gulbins E, Kukreja RC, et al. Arterial medial calcification through enhanced small extracellular vesicle release in smooth muscle-specific asah1 gene knockout mice. *Sci Rep.* (2020) 10:1645. doi: 10.1038/s41598-020-58568-5
51. Bandhuvula P, Honbo N, Wang G-Y, Jin Z-Q, Fyrst H, Zhang M, et al. S1P lyase: a novel therapeutic target for ischemia-reperfusion injury of the heart. *Am J Physiol Heart Circul Physiol.* (2011) 300:H1753–61. doi: 10.1152/ajpheart.00946.2010
52. Gorshkova IA, Wang H, Orbelyan GA, Goya J, Natarajan V, Beiser DG, et al. Inhibition of sphingosine-1-phosphate lyase rescues sphingosine kinase-1-knockout phenotype following murine cardiac arrest. *Life Sci.* (2013) 93:359–66. doi: 10.1016/j.lfs.2013.07.017
53. Karliner JS. Sphingosine kinase and sphingosine 1-phosphate in cardioprotection. *J Cardiovasc Pharmacol.* (2009) 53:189. doi: 10.1097/FJC.0b013e3181926706
54. Hisano Y, Inoue A, Okudaira M, Taimatsu K, Matsumoto H, Kotani H, et al. Maternal and zygotic sphingosine kinase 2 are indispensable for cardiac development in zebrafish. *J Biol Chem.* (2015) 290:14841–51. doi: 10.1074/jbc.M114.634717
55. Clay H, Wilsbacher LD, Wilson SJ, Duong DN, McDonald M, Lam I, et al. Sphingosine 1-phosphate receptor-1 in cardiomyocytes is required for normal cardiac development. *Dev Biol.* (2016) 418:157–65. doi: 10.1016/j.ydbio.2016.06.024
56. Means CK, Miyamoto S, Chun J, Brown JH. S1P1 receptor localization confers selectivity for Gi-mediated cAMP and contractile responses. *J Biol Chem.* (2008) 283:11954–63. doi: 10.1074/jbc.M707422200
57. Theilmeier G, Schmidt C, Herrmann Jr, Keul P, Schäfers M, Herrgott I, et al. Clinical perspective. *Circulation.* (2006) 114:1403–9. doi: 10.1161/CIRCULATIONAHA.105.607135
58. Means CK, Xiao C-Y, Li Z, Zhang T, Omens JH, Ishii I, et al. Sphingosine 1-phosphate S1P2 and S1P3 receptor-mediated Akt activation protects against *in vivo* myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circul Physiol.* (2007) 292:H2944–H51. doi: 10.1152/ajpheart.01331.2006
59. Ishii I, Ye X, Friedman B, Kawamura S, Contos JJ, Kingsbury MA, et al. Marked perinatal lethality and cellular signaling deficits in mice null for the two sphingosine 1-phosphate (S1P) receptors, S1P2/LPB2/EDG-5 and S1P3/LPB3/EDG-3. *J Biol Chem.* (2002) 277:25152–9. doi: 10.1074/jbc.M200137200
60. Skoura A, Michaud J, Im D-S, Thangada S, Xiong Y, Smith JD, et al. Sphingosine-1-phosphate receptor-2 function in myeloid cells regulates vascular inflammation and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2011) 31:81–5. doi: 10.1161/ATVBAHA.110.213496
61. Schuchardt M, Tölle M, Huang T, Zidek W, Van Der Giet M. HDL DIMINISHES MMP-9 PRODUCTION IN A S1P3/TGF-β DEPENDENT MANNER. *J Hypertension.* (2011) 29:e186. doi: 10.1097/00004872-201106001-00487
62. Keul P, Lucke S, von Wnuck Lipinski K, Bode C, Gräler M, Heusch G, et al. Sphingosine-1-phosphate receptor 3 promotes recruitment of monocyte/macrophages in inflammation and atherosclerosis. *Circulation Res.* (2011) 108:314–23. doi: 10.1161/CIRCRESAHA.110.235028
63. Wang X, Rao RP, Kosakowska-Cholody T, Masood MA, Southon E, Zhang H, et al. Mitochondrial degeneration and not apoptosis is the primary cause of embryonic lethality in ceramide transfer protein mutant mice. *J Cell Biol.* (2009) 184:143–58. doi: 10.1083/jcb.200807176
64. American Heart Association. *Cardiovascular Disease: A Costly Burden for America Projections Through*. American Heart Association (2017).

65. Kovilakath A, Cowart LA. Sphingolipid mediators of myocardial pathology. *J Lipid Atherosc.* (2020) 9:23–49. doi: 10.12997/jla.2020.9.1.23
66. Kovilakath A, Cowart AL. A novel class of sphingolipids mediate autophagy and apoptosis in models of ischemia. *Circul Res.* (2019) 125(Suppl. 1):A735–A. doi: 10.1161/res.125.suppl_1.735
67. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* (2014) 20:678–86. doi: 10.1016/j.cmet.2014.08.002
68. Kuzmenko D, Klimentyeva T. Role of ceramide in apoptosis and development of insulin resistance. *Biochemistry.* (2016) 81:913–27. doi: 10.1134/S0006297916090017
69. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, DeFronzo RA, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes.* (2009) 58:337–43. doi: 10.2337/db08-1228
70. Lopez X, Goldfine AB, Holland WL, Gordillo R, Scherer PE. Plasma ceramides are elevated in female children and adolescents with type 2 diabetes. *J Pediatr Endocrinol Metab.* (2013) 26:995–8. doi: 10.1515/jpem-2012-0407
71. Kasumov T, Li L, Li M, Gulshan K, Kirwan JP, Liu X, et al. Ceramide as a mediator of non-alcoholic fatty liver disease and associated atherosclerosis. *PLoS ONE.* (2015) 10:e0126910. doi: 10.1371/journal.pone.0126910
72. Raichur S, Brunner B, Bielohuby M, Hansen G, Pfenninger A, Wang B, et al. The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Mol Metab.* (2019) 21:36–50. doi: 10.1016/j.molmet.2018.12.008
73. Choromańska B, Myśliwiec P, Razak Hady H, Dadan J, Myśliwiec H, Chabowski A, et al. Metabolic syndrome is associated with ceramide accumulation in visceral adipose tissue of women with morbid obesity. *Obesity.* (2019) 27:444–53. doi: 10.1002/oby.22405
74. Torretta E, Barbacini P, Al-Daghri NM, Gelfi C. Sphingolipids in obesity and correlated co-morbidities: the contribution of gender, age and environment. *Int J Mol Sci.* (2019) 20:5901. doi: 10.3390/ijms20235901
75. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, et al. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes.* (2013) 62:401–10. doi: 10.2337/db12-0686
76. Boini KM, Koka S, Xia M, Ritter JK, Gehr TW, Li P-L. Sphingolipids in obesity and related complications. *Front Biosci.* (2017) 22:4474. doi: 10.2741/4474
77. Chiu H-C, Kovacs A, Ford DA, Hsu F-F, Garcia R, Herrero P, et al. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest.* (2001) 107:813–22. doi: 10.1172/JCI10947
78. Yagyu H, Chen G, Yokoyama M, Hirata K, Augustus A, Kako Y, et al. Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. *J Clin Invest.* (2003) 111:419–26. doi: 10.1172/JCI16751
79. Liu L, Shi X, Bharadwaj KG, Ikeda S, Yamashita H, Yagyu H, et al. DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. *J Biol Chem.* (2009) 284:36312–23. doi: 10.1074/jbc.M109.049817
80. Liu L, Yu S, Khan RS, Homma S, Schulze PC, Blaner WS, et al. Diacylglycerol acyl transferase 1 overexpression detoxifies cardiac lipids in PPARγ transgenic mice. *J Lipid Res.* (2012) 53:1482–92. doi: 10.1194/jlr.M024208
81. Liu L, Trent CM, Fang X, Son N-H, Jiang H, Blaner WS, et al. Cardiomyocyte-specific loss of diacylglycerol acyltransferase 1 (DGAT1) reproduces the abnormalities in lipids found in severe heart failure. *J Biol Chem.* (2014) 289:29881–91. doi: 10.1074/jbc.M114.601864
82. Ellis JM, Mentock SM, DePetrillo MA, Koves TR, Sen S, Watkins SM, et al. Mouse cardiac acyl coenzyme a synthetase 1 deficiency impairs fatty acid oxidation and induces cardiac hypertrophy. *Mol Cell Biol.* (2011) 31:1252–62. doi: 10.1128/MCB.01085-10
83. Goldenberg JR, Carley AN, Ji R, Zhang X, Fasano M, Schulze PC, et al. Preservation of acyl coenzyme a attenuates pathological and metabolic cardiac remodeling through selective lipid trafficking. *Circulation.* (2019) 139:2765–77. doi: 10.1161/CIRCULATIONAHA.119.039610
84. Hu W, Bielawski J, Samad F, Merrill AH, Cowart LA. Palmitate increases sphingosine-1-phosphate in C2C12 myotubes via upregulation of sphingosine kinase message and activity. *J Lipid Res.* (2009) 50:1852–62. doi: 10.1194/jlr.M800635-JLR200
85. Hu W, Ross J, Geng T, Brice SE, Cowart LA. Differential regulation of dihydroceramide desaturase by palmitate versus monounsaturated fatty acids Implications for insulin resistance. *J Biolo Chem.* (2011) 286:16596–605. doi: 10.1074/jbc.M110.186916
86. Ross JS, Hu W, Rosen B, Snider AJ, Obeid LM, Cowart LA. Sphingosine kinase 1 is regulated by peroxisome proliferator-activated receptor α in response to free fatty acids and is essential for skeletal muscle interleukin-6 production and signaling in diet-induced obesity. *J Biol Chem.* (2013) 288:22193–206. doi: 10.1074/jbc.M113.477786
87. Russo SB, Baicu CF, Van Laer A, Geng T, Kasiganesan H, Zile MR, et al. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. *J Clin Invest.* (2012) 122:3919–30. doi: 10.1172/JCI63888
88. Law BA, Liao X, Moore KS, Southard A, Roddy P, Ji R, et al. Lipotoxic very-long-chain ceramides cause mitochondrial dysfunction, oxidative stress, and cell death in cardiomyocytes. *FASEB J.* (2018) 32:1403–16. doi: 10.1096/fj.201700300R
89. Chaurasia B, Summers SA. Ceramides–lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab.* (2015) 26:538–50. doi: 10.1016/j.tem.2015.07.006
90. Hicks AA, Pramstaller PP, Johansson Å, Vitart V, Rudan I, Ugocsai P, et al. Genetic determinants of circulating sphingolipid concentrations in European populations. *PLoS Genet.* (2009) 5:e1000672. doi: 10.1371/journal.pgen.1000672
91. Cresci S, Zhang R, Yang Q, Duncan MS, Xanthakis V, Jiang X, et al. Genetic architecture of circulating very-long-chain (C24:0 and C22:0) ceramide concentrations. *J Lipid Atherosc.* (2020) 9:172–83. doi: 10.12997/jla.2020.9.1.172
92. Alessenko A, Lebedev A, Kurochkin I. The role of sphingolipids in cardiovascular pathologies. *Biochem.* (2019) 13:122–31. doi: 10.1134/S1990750819020021
93. Kurano M, Yatomi Y. Sphingosine 1-phosphate and atherosclerosis. *J Atheroscler Thromb*. (2017) 25:16–26. doi: 10.5551/jat.RV17010
94. Okajima F. Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? *Biochim Biophys Acta.* (2002) 1582:132–7. doi: 10.1016/S1388-1981(02)00147-6
95. Peters SL, Alewijnse AE. Sphingosine-1-phosphate signaling in the cardiovascular system. *Curr Opin Pharmacol.* (2007) 7:186–92. doi: 10.1016/j.coph.2006.09.008
96. Soltau I, Mudersbach E, Geissen M, Schwedhelm E, Winkler MS, Geffken M, et al. Serum-sphingosine-1-phosphate concentrations are inversely associated with atherosclerotic diseases in humans. *PLoS ONE.* (2016) 11:e0168302. doi: 10.1371/journal.pone.0168302
97. Baranowski M, Górski J. Heart sphingolipids in health and disease. *Sphing Metab Disease.* Białystok: Springer. (2011). p. 41–56. doi: 10.1007/978-1-4614-0650-1_3
98. Borodzicz S, Czarzasta K, Kuch M, Cudnoch-Jedrzejewska A. Sphingolipids in cardiovascular diseases and metabolic disorders. *Lipids Health Dis.* (2015) 14:55. doi: 10.1186/s12944-015-0053-y
99. Park T-S, Goldberg IJ. Sphingolipids, lipotoxic cardiomyopathy, and cardiac failure. *Heart Failure Clin.* (2012) 8:633–41. doi: 10.1016/j.hfc.2012.06.003
100. Keul P, van Borren MM, Ghanem A, Müller FU, Baartscheer A, Verkerk AO, et al. Sphingosine-1-phosphate receptor 1 regulates cardiac function by modulating Ca2+ sensitivity and Na+/H+ exchange and mediates protection by ischemic preconditioning. *J Am Heart Assoc.* (2016) 5:e003393. doi: 10.1161/JAHA.116.003393
101. Levkau B. Cardiovascular effects of sphingosine-1-phosphate (S1P). *Sphingolipids in Disease:* Essen: Springer. (2013). p. 147–70. doi: 10.1007/978-3-7091-1511-4_8
102. Yung BS, Brand CS, Xiang SY, Gray CB, Means CK, Rosen H, et al. Selective coupling of the S1P3 receptor subtype to S1P-mediated RhoA activation and cardioprotection. *J Mol Cell Cardiol.* (2017) 103:1–10. doi: 10.1016/j.yjmcc.2016.12.008
103. Brait VH, Tarrasón G, Gavaldà A, Godessart N, Planas AM. Selective sphingosine 1-phosphate receptor 1 agonist is

protective against ischemia/reperfusion in mice. *Stroke.* (2016) 47:3053–6. doi: 10.1161/STROKEAHA.116.015371
104. Sugahara K, Maeda Y, Shimano K, Mogami A, Kataoka H, Ogawa K, et al. Amiselimod, a novel sphingosine 1-phosphate receptor-1 modulator, has potent therapeutic efficacy for autoimmune diseases, with low bradycardia risk. *Br J Pharmacol.* (2017) 174:15–27. doi: 10.1111/bph.13641
105. Cannavo A, Liccardo D, Komici K, Corbi G, de Lucia C, Femminella GD, et al. Sphingosine kinases and sphingosine 1-phosphate receptors: signaling and actions in the cardiovascular system. *Front Pharmacol.* (2017) 8:556. doi: 10.3389/fphar.2017.00556
106. Herzog C, Schmitz M, Levkau B, Herrgott I, Mersmann J, Larmann J, et al. Intravenous sphingosylphosphorylcholine protects ischemic and postischemic myocardial tissue in a mouse model of myocardial ischemia/reperfusion injury. *Mediat Inflamm.* (2010) 2010:425191. doi: 10.1155/2010/425191
107. Hofmann U, Burkard N, Vogt C, Thoma A, Frantz S, Ertl G, et al. Protective effects of sphingosine-1-phosphate receptor agonist treatment after myocardial ischaemia–reperfusion. *Cardiovasc Res.* (2009) 83:285–93. doi: 10.1093/cvr/cvp137
108. Fryer RM, Muthukumarana A, Harrison PC, Mazurek SN, Chen RR, Harrington KE, et al. The clinically-tested S1P receptor agonists, FTY720 and BAF312, demonstrate subtype-specific bradycardia (S1P1) and hypertension (S1P3) in rat. *PLoS ONE.* (2012) 7:e0052985. doi: 10.1371/journal.pone.0052985
109. Egom EEA, Ke Y, Musa H, Mohamed TM, Wang T, Cartwright E, et al. FTY720 prevents ischemia/reperfusion injury-associated arrhythmias in an *ex vivo* rat heart model via activation of Pak1/Akt signaling. *J Mol Cell Cardiol.* (2010) 48:406–14. doi: 10.1016/j.yjmcc.2009.10.009
110. Santos-Gallego CG, Vahl TP, Goliasch G, Picatoste B, Arias T, Ishikawa K, et al. Sphingosine-1-phosphate receptor agonist fingolimod increases myocardial salvage and decreases adverse postinfarction left ventricular remodeling in a porcine model of ischemia/reperfusion. *Circulation.* (2016) 133:954–66. doi: 10.1161/CIRCULATIONAHA.115.012427
111. Goltz D, Huss S, Ramadori E, Büttner R, Diehl L, Meyer R. Immunomodulation by splenectomy or by FTY 720 protects the heart against ischemia reperfusion injury. *Clin Exp Pharmacol Physiol.* (2015) 42:1168–77. doi: 10.1111/1440-1681.12465
112. Liu X, Wu J, Zhu C, Liu J, Chen X, Zhuang T, et al. Endothelial S1pr1 regulates pressure overload-induced cardiac remodelling through AKT-eNOS pathway. *J Cell Mol Med.* (2019) 24:2013–26. doi: 10.1111/jcmm.14900
113. Pan W, Yu J, Shi R, Yan L, Yang T, Li Y, et al. Elevation of ceramide and activation of secretory acid sphingomyelinase in patients with acute coronary syndromes. *Coronary Artery Dis.* (2014) 25:230–5. doi: 10.1097/MCA.0000000000000079
114. Modrzejewski W, Knapp M, Dobrzyn A, Musial W, Górski J. Ceramides and adhesive molecules in stable ischaemic heart disease. *Przeglad lekarski.* (2008) 65:131–4.
115. Cordis G, Yoshida T, Das D. HPTLC analysis of sphingomylein, ceramide and sphingosine in ischemic/reperfused rat heart. *J Pharm Biomed Anal.* (1998) 16:1189–93. doi: 10.1016/S0731-7085(97)00260-4
116. Knapp M, Lisowska A, Zabielski P, Musiał W, Baranowski M. Sustained decrease in plasma sphingosine-1-phosphate concentration and its accumulation in blood cells in acute myocardial infarction. *Prostaglandins Other Lipid Mediat.* (2013) 106:53–61. doi: 10.1016/j.prostaglandins.2013.10.001
117. Cui J, Engelman RM, Maulik N, Das DK. Role of ceramide in ischemic preconditioning. *J Am Coll Surg.* (2004) 198:770–7. doi: 10.1016/j.jamcollsurg.2003.12.016
118. Zhang DX, Fryer RM, Hsu AK, Zou A-P, Gross GJ, Campbell WB, et al. Production and metabolism of ceramide in normal and ischemic-reperfused myocardium of rats. *Basic Res Cardiol.* (2001) 96:267–74. doi: 10.1007/s003950170057
119. Beresewicz A, Dobrzyn A, Gorski J. Accumulation of specific ceramides in ischemic/reperfused rat heart; effect of ischemic preconditioning. *J Physiol Pharmacol.* (2002) 53:371–82.
120. Cavalli AL, Ligutti JA, Gellings NM, Castro E, Page M, Klepper R, et al. The role of TNFα and sphingolipid signaling in cardiac hypoxia: evidence that cardiomyocytes release TNFα and sphingosine. *Basic Appl Myol.* (2002) 12:167–75.
121. Jin Z-Q, Zhang J, Huang Y, Hoover HE, Vessey DA, Karliner JS. A sphingosine kinase 1 mutation sensitizes the myocardium to ischemia/reperfusion injury. *Cardiovasc Res.* (2007) 76:41–50. doi: 10.1016/j.cardiores.2007.05.029
122. Karliner JS, Honbo N, Summers K, Gray MO, Goetzl EJ. The lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid enhance survival during hypoxia in neonatal rat cardiac myocytes. *J Mol Cell Cardiol.* (2001) 33:1713–7. doi: 10.1006/jmcc.2001.1429
123. Vessey DA, Kelley M, Li L, Huang Y, Zhou H-Z, Zhu BQ, et al. Role of sphingosine kinase activity in protection of heart against ischemia reperfusion injury. *Med Sci Monit.* (2006) 12:BR318–24.
124. Vessey DA, Li L, Jin Z-Q, Kelley M, Honbo N, Zhang J, et al. A sphingosine kinase form 2 knockout sensitizes mouse myocardium to ischemia/reoxygenation injury and diminishes responsiveness to ischemic preconditioning. *Oxid Med Cell Longev.* (2011) 2011:961059. doi: 10.1155/2011/961059
125. Adamy C, Mulder P, Khouzami L, Andrieu-Abadie N, Defer N, Candiani G, et al. Neutral sphingomyelinase inhibition participates to the benefits of N-acetylcysteine treatment in post-myocardial infarction failing heart rats. *J Mol Cell Cardiol.* (2007) 43:344–53. doi: 10.1016/j.yjmcc.2007.06.010
126. Pavoine C, Pecker F. Sphingomyelinases: their regulation and roles in cardiovascular pathophysiology. *Cardiovasc Res.* (2009) 82:175–83. doi: 10.1093/cvr/cvp030
127. Klevstig M, Ståhlman M, Lundqvist A, Täng MS, Fogelstrand P, Adiels M, et al. Targeting acid sphingomyelinase reduces cardiac ceramide accumulation in the post-ischemic heart. *J Mol Cell Cardiol.* (2016) 93:69–72. doi: 10.1016/j.yjmcc.2016.02.019
128. Kikas P, Chalikias G, Tziakas D. Cardiovascular implications of sphingomyelin presence in biological membranes. *Eur Cardiol Rev.* (2018) 13:42–45. doi: 10.15420/ecr.2017:20:3
129. Deutschman DH, Carstens JS, Klepper RL, Smith WS, Page MT, Young TR, et al. Predicting obstructive coronary artery disease with serum sphingosine-1-phosphate. *Am Heart J.* (2003) 146:62–8. doi: 10.1016/S0002-8703(03)00118-2
130. Vessey DA, Li L, Kelley M, Zhang J, Karliner JS. Sphingosine can pre-and post-condition heart and utilizes a different mechanism from sphingosine 1-phosphate. *J Biochem Mol Toxicol.* (2008) 22:113–8. doi: 10.1002/jbt.20227
131. Vessey DA, Kelley M, Li L, Huang Y. Sphingosine protects aging hearts from ischemia/reperfusion injury: superiority to sphingosine 1-phosphate and ischemic pre-and post-conditioning. *Oxidati Med Cell Longev.* (2009) 2:146–51. doi: 10.4161/oxim.2.3.8622
132. Cuvillier O. Sphingosine in apoptosis signaling. *Biochim Biophys Acta.* (2002) 1585:153–62. doi: 10.1016/S1388-1981(02)00336-0
133. Taha TA, Mullen TD, Obeid LM. A house divided: ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. *Biochim Biophys Acta.* (2006) 1758:2027–36. doi: 10.1016/j.bbamem.2006.10.018
134. Pyne S. Cellular signaling by sphingosine and sphingosine 1-phosphate. In: *Phosphol Metabolism in Apoptosis:* Glasgow: Springer (2004). p. 245–68. doi: 10.1007/0-306-47931-1_13
135. Summers SA. Could ceramides become the new cholesterol? *Cell Metab.* (2018) 27:276–80. doi: 10.1016/j.cmet.2017.12.003
136. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, et al. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PLoS ONE.* (2015) 10:e0124844. doi: 10.1371/journal.pone.0124844
137. Cadby G, Melton PE, McCarthy NS, Giles C, Mellett NA, Huynh K, et al. Heritability of 596 lipid species and genetic correlation with cardiovascular traits in the busselton family heart study. *J Lipid Res.* (2020) 61:537–45. doi: 10.1194/jlr.RA119000594
138. Tabassum R, Rämö JT, Ripatti P, Koskela JT, Kurki M, Karjalainen J, et al. Genetic architecture of human plasma lipidome and its link to cardiovascular disease. *Nat Commun.* (2019) 10:4329. doi: 10.1038/s41467-019-11954-8
139. Bellis C, Kulkarni H, Mamtani M, Kent Jr JW, Wong G, Weir JM, et al. Human plasma lipidome is pleiotropically associated with cardiovascular risk factors and death. *Circul Cardiovasc Genet.* (2014) 7:854–63. doi: 10.1161/CIRCGENETICS.114.000600

140. Demirkan A, Pool R, Deelen J, Beekman M, Liu J, Harms AC, et al. Genome-wide association study of plasma triglycerides, phospholipids and relation to cardio-metabolic risk factors. *bioRxiv*. 2019:621334. doi: 10.1101/621334
141. Mantovani A, Dugo C. Ceramides and risk of major adverse cardiovascular events: a meta-analysis of longitudinal studies. *J Clin Lipidol.* (2020) 14:176–85. doi: 10.1016/j.jacl.2020.01.005
142. Javaheri A, Allegood JC, Cowart LA, Chirinos JA. Circulating ceramide 16:0 in heart failure with preserved ejection fraction. *J Am Coll Cardiol.* (2020) 75:2273–5. doi: 10.1016/j.jacc.2020.02.062
143. Tadic M, Cuspidi C, Calicchio F, Grassi G, Mancia G. Diagnostic algorithm for HFpEF: how much is the recent consensus applicable in clinical practice? *Heart Failure Rev.* (2020). doi: 10.1007/s10741-020-09966-4. [Epub ahead of print].
144. Fretts AM, Jensen P, Hoofnagle A, McKnight B, Sitlani C, Siscovick DS, et al. Abstract P492: circulating sphingolipids and risk of mortality: the cardiovascular health study. *Circulation.* (2020) 141(Suppl. 1):AP492. doi: 10.1161/circ.141.suppl_1.P492
145. Cantalupo A, Sasset L, Gargiulo A, Rubinelli L, Del Gaudio I, Benvenuto D, et al. Endothelial sphingolipid *de novo* synthesis controls blood pressure by regulating signal transduction and NO via ceramide. *Hypertension.* (2020) 75:1279–88. doi: 10.1161/HYPERTENSIONAHA.119.14507
146. Chun J, Brinkmann V. A mechanistically novel, first oral therapy for multiple sclerosis: the development of fingolimod (FTY720, Gilenya). *Discov Med.* (2011) 12:213.
147. Liu W, Zi M, Tsui H, Chowdhury SK, Zeef L, Meng Q-J, et al. A novel immunomodulator, FTY-720 reverses existing cardiac hypertrophy and fibrosis from pressure overload by targeting NFAT (nuclear factor of activated T-cells) signaling and periostin. *Circul Heart Failure.* (2013) 6:833–44. doi: 10.1161/CIRCHEARTFAILURE.112.000123
148. Turner N, Lim XY, Toop HD, Osborne B, Brandon AE, Taylor EN, et al. A selective inhibitor of ceramide synthase 1 reveals a novel role in fat metabolism. *Nat Commun.* (2018) 9:3615. doi: 10.1038/s41467-018-05613-7
149. Nicholls M. Plasma ceramides and cardiac risk. *Eur Heart J.* (2017) 38:1359–60. doi: 10.1093/eurheartj/ehx205
150. Poss AM, Holland WL, Summers SA. Risky lipids: refining the ceramide score that measures cardiovascular health. *Eur Heart J.* (2020) 41:381–2. doi: 10.1093/eurheartj/ehz525
151. Hilvo M, Meikle PJ, Pedersen ER, Tell GS, Dhar I, Brenner H, et al. Development and validation of a ceramide-and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. *Eur Heart J.* (2020) 41:371–80. doi: 10.1093/eurheartj/ehz387
152. Nwabuo CC, Duncan M, Xanthakis V, Peterson LR, Mitchell GF, McManus D, et al. Association of circulating ceramides with cardiac structure and function in the community: the framingham heart study. *J Am Heart Assoc.* (2019) 8:e013050. doi: 10.1161/JAHA.119.013050
153. Hammad SM, Hardin JR, Wilson DA, Twal WO, Nietert PJ, Oates JC. Race disparity in blood sphingolipidomics associated with lupus cardiovascular comorbidity. *PLoS ONE.* (2019) 14:e0224496. doi: 10.1371/journal.pone.0224496

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 29 September 2020
doi: 10.3389/fendo.2020.570628

Ceramides and Ceramide Scores: Clinical Applications for Cardiometabolic Risk Stratification

Mika Hilvo[1], *Vlad C. Vasile*[2], *Leslie J. Donato*[2], *Reini Hurme*[1] *and Reijo Laaksonen*[1,3]*

[1] Zora Biosciences Oy, Espoo, Finland, [2] Department of Laboratory Medicine and Pathology and Department of Cardiovascular Diseases Mayo Clinic College of Medicine, Rochester, MN, United States, [3] Finnish Cardiovascular Research Center Tampere, Tampere University, Tampere, Finland

Ceramides are bioactive lipids that have an important role in many cellular functions such as apoptosis and inflammation. During the past decade emerging clinical data have shown that ceramides are not only of great biochemical interest but may also have diagnostic utility. Ceramides have shown independent predictive value for negative cardiovascular outcomes as well as for the onset of type 2 diabetes. Based on abundant published data, risk score using the concentrations of circulating ceramides have been developed and adapted for routine clinical practice. Currently serum ceramides are used clinically as efficient risk stratifiers for primary and secondary prevention of atherosclerotic cardiovascular disease (CVD). A direct cause-effect relationship between CVD and ceramide has not been established to date. As ceramide-specific medications are being developed, conventional strategies such as lipid lowering agents and lifestyle interventions can be used to reduce overall risk. Ceramides can identify a very high-risk coronary heart disease category of patients in need for more intense medical attention, specifically those patients at higher risk as highlighted in the 2019 European Society of Cardiology guidelines for stable chronic coronary syndrome patients. In addition, the ceramide risk score may be used as a decision-making tool in primary prevention patients with moderate CVD risk. Finally, the ceramide risk score may have a unique utility as a motivational tool to increase patient's adherence to medical therapy and lifestyle changes.

Keywords: cardiovascular disease, coronary heart disease, stroke, heart failure, diabetes mellitus, ceramide, ceramide score

OPEN ACCESS

Edited by:
Scott A. Summers,
The University of Utah, United States

Reviewed by:
Tilla Worgall,
Columbia University, United States
Kyle Stephan McCommis,
Saint Louis University, United States

***Correspondence:**
Reijo Laaksonen
reijo.laaksonen@zora.fi

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *08 June 2020*
Accepted: *27 August 2020*
Published: *29 September 2020*

Citation:
Hilvo M, Vasile VC, Donato LJ, Hurme R and Laaksonen R (2020) Ceramides and Ceramide Scores: Clinical Applications for Cardiometabolic Risk Stratification. Front. Endocrinol. 11:570628. doi: 10.3389/fendo.2020.570628

INTRODUCTION

Ceramides are bioactive lipids that play important roles in many central processes of human cells, such as apoptosis and inflammation [for additional information see e.g., the following reviews (1–5)]. During the past decade many investigations have revealed a strong association between ceramides and cardiometabolic conditions. An increasing number of studies have shown a link between the development of diabetes and ceramide lipids (6–8). More recently, distinct serum ceramides have shown to be elevated in diabetes (9, 10), and some specific ceramides may predict incident diabetes mellitus type 2 (DM2) even years before the onset of the disease (11). Serum ceramide concentrations have been shown to predict cardiovascular atherosclerotic disease (CVD) such as coronary artery disease (CAD), stroke, as well as heart failure and atrial fibrillation (12–18). These associations were initially observed in small case-control studies or in investigations

comparing healthy controls to patients with stable CHD or ACS (12, 19). These promising findings led to a series of larger cohort studies and randomized clinical trials. The initial focus was on CHD but this was later expanded to other cardiovascular (CV) conditions.

The data obtained from these various studies has helped to identify the clinical utility for clinical testing of ceramides because serum ceramide measurements may provide independent and added-value to routinely used diagnostic and prognostic CVD tools. Several studies revealed that ceramides may provide important clinical value, yet the significance of individual ceramides and their mechanistic contribution to the disease pathogenesis has remained uncertain. This has hindered the development of clinical tests of single ceramides. Moreover, a panel test that includes the concentrations of several ceramide concentrations is likely cumbersome to apply to clinical decision making and is not an appealing diagnostic reporting option. To address these shortcomings ceramide-based clinical scores have been developed to aid in end-user comprehension and clinical adoption. The purpose of this review is to provide a summary of CVD- and diabetes-related ceramide data published as of spring 2020 and to depict how the results have been translated into clinical practice.

CERAMIDES IN CARDIOVASCULAR DISEASES

1) Ceramides in Coronary Heart Disease (CHD)

The association between ceramides and CHD has been shown in a number of case-control and cohort studies as well as clinical trials (12, 13, 19–23). Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), and Cer(d18:1/24:1), in addition to their ratios with Cer(d18:1/24:0) have been shown to predict the risk of myocardial infarcts (MIs) and CV death in apparently disease free subjects (21) as well as in patients with stable CHD or in secondary prevention after MIs (13). **Figure 1** shows Kaplan–Meier estimates of incident major adverse cardiovascular events (MACE) defined as a composite of CHD, ischemic stroke and heart failure (HF) for Cer(d18:1/18:0) and LDL-cholesterol quartiles.

The associations of ceramides with CVD appear stronger for fatal outcomes than non-fatal MIs (13–15, 21). MIs can be precipitated by a number of causes in addition to the traditional model of atherosclerotic plaque rupture (Type 1 MI). Type 2 MIs result from an increased demand or a decreased supply of oxygen which can occur in many conditions such as coronary endothelial dysfunction, coronary artery spasm, coronary artery embolus, tachyarryhthmias/bradyarrhythmias, anemia, respiratory failure, hypertension, or hypotension (24). High sensitive troponin assays can detect even minor ischemic changes seen in non ST-elevation MI or unstable angina. Thus, the phenotypic heterogeneity of MIs may dilute somewhat the prognostic performance of ceramides, although some authors argue that these lipids can provide more specific information to particular MI categories (13). Interestingly, two independent imaging studies have confirmed the localization and association of ceramides with the thin fibrotic plaques with necrotic core, supporting further the assumption that ceramides play an important role in plaque vulnerability in atherosclerotic CAD and thus may associate with fatal complications related to rupture prone inflammatory plaques (25, 26).

Importantly, distinct ceramide ratios improve risk stratification in patients with known CHD as compared to single ceramides or traditional lipid biomarkers. This comparison is illustrated in **Figure 2** which shows odds ratios for different ceramides and ceramide ratios as well as traditional lipid parameters in a cohort of stable CHD patients (13). Interestingly, Cer(d18:1/24:1)/Cer(d18:1/24:0) ratio appears consistently associated with CHD risk across studies. This is an intriguing finding since only one double-bond is separating Cer(d18:1/24:1) from Cer(d18:1/24:0) which are otherwise structurally identical. The etiology for the strong CVD risk association of this ceramide ratio still remains obscure.

2) Ceramides in Stroke

So far only a few reports have been published regarding ceramides and stroke. Gui et al. evaluated whether ceramides associate with ischemic stroke risk and clinical severity (16). They analyzed concentrations of plasma Cer(d18:1/16:0), Cer(d18:1/22:0), and Cer(d18:1/24:0), in 202 patients with acute ischemic stroke and 202 controls matched for age and sex. Plasma levels of all measured ceramides in stroke patients were significantly higher than in controls ($P < 0.001$). After adjustment for other risk factors, Cer(d18:1/16:0), Cer(d18:1/22:0), and Cer(d18:1/24:0) were associated with higher risk of ischemic stroke [odds ratios for one IQR increase: 2.15 (1.42–2.99); 2.90 (2.13–4.01), and 1.29 (1.10–1.69), respectively] (16). In patients with a minor stroke ($n = 103$), ceramide concentrations were lower than those observed in patients with moderate-to-high clinical severity ($P < 0.001$). Thus, the authors concluded that elevated plasma ceramide levels are predictors of both risk and severity at admission in ischemic stroke patients (16).

Fiedorowicz et al. assessed ceramide and sphingosine-1-phosphate (Sph-1-P) serum concentrations in patients with acute ischaemic stroke, transient ischemic attack (TIA), and age-matched neurological patients without cerebral ischaemia. They recognized two ratios, Sph-1-P/Cer(d18:1/24:1), and Cer(d18:1/24:0)/Cer(d18:1/24:1), with a solid diagnostic potential in ischaemic stroke. They furthermore found Sph-1-P/Cer(d18:1/24:1) ratio to be possibly useful in TIA diagnostics (17).

Based on these studies the relative strength of association between ceramides and stroke seems to be weaker than that of fatal CHD events. More dedicated studies on different stroke phenotypes are warranted since currently it is not entirely clear whether the strength of association is similar for atherosclerotic, thromboembolic and hemorrhagic strokes.

3) Ceramides in Heart Failure

Lemaitre et al. have recently reported associations of plasma ceramide and sphingomyelin species with incident heart failure

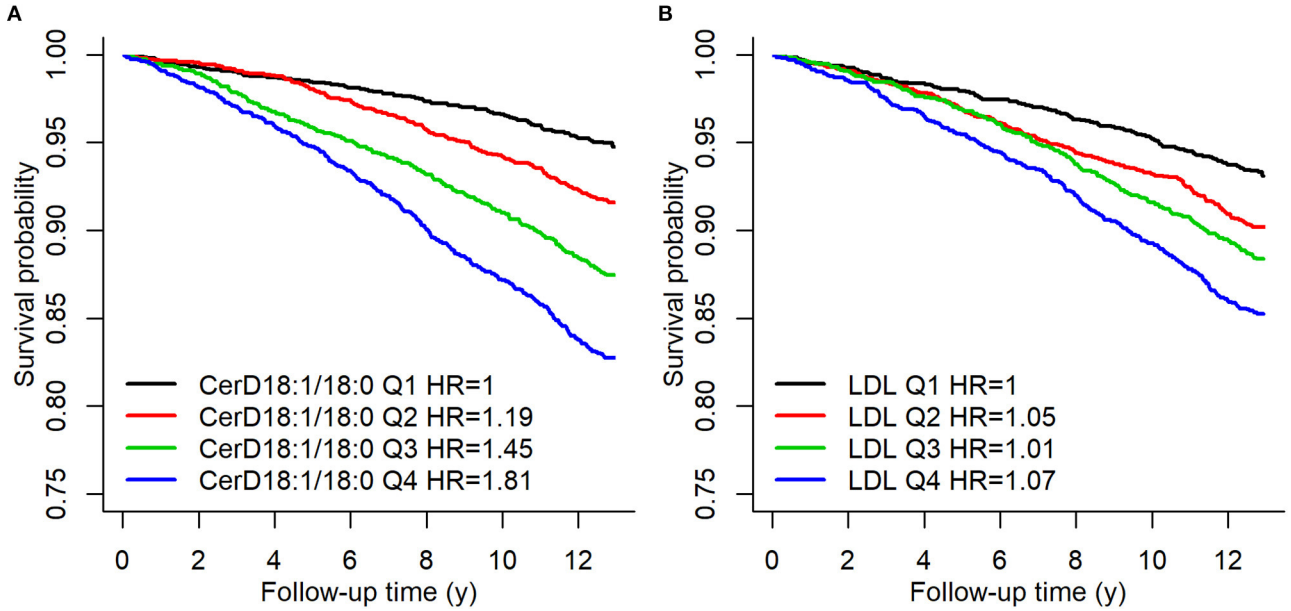

FIGURE 1 | Kaplan–Meier estimates of incident MACE Cer(d18:1/18:0) and LDL-C quartiles. A hazard ratio in the legend indicates the quartile specific hazard relative to the 1st quartile (models stratified for sex). Figure from Havulinna et al. (21). Figure reproduced under the terms of the Creative Commons Attribution License.

in the Cardiovascular Health Study (18). They examined eight components: ceramides and sphingomyelins that contain palmitic acid (Cer-16:0 and SM-16:0), arachidic acid (Cer-20:0 and SM-20:0), behenic acid (Cer-22:0 and SM-22:0), or lignoceric acid (Cer-24:0 and SM-24:0) in a study with a median follow-up of 9.4 years, where 1,179 cases of incident heart failure were reported among 4,249 study participants. In Cox regression analyses adjusted for risk factors, higher levels of Cer(d18:1/16:0) associated with higher risk of incident heart failure [hazard ratio for one SD increase:1.25 (95% CI, 1.16–1.36)]. In contrast, higher levels of Cer(d18:1/22:0) were associated with lower risk of heart failure in multivariable analyses further adjusted for Cer(d18:1/16:0) [hazard ratio, 0.85 (0.78–0.92)]. Therefore, this study identifies specific lipidomic biomarkers useful for determining risk of incident heart failure.

4) Ceramides in Atrial Fibrillation

Ceramides exhibit multiple biological activities that may influence the pathophysiological characteristics of atrial fibrillation (AF) among other CVDs. Jenssen et al. evaluated whether the length of the saturated fatty acid carried by ceramide or their sphingomyelin precursors were associated with incident AF risk among 4,206 Cardiovascular Health Study participants (mean age, 76 years; 40% men). They identified 1,198 incident AF cases over a median 8.7 years of follow-up. In adjusted Cox regression analyses, ceramides with very-long-chain saturated fatty acids were associated with reduced AF risk. In contrast, Cer(d18:1/16:0) was associated with increased AF risk with a hazard ratio of 1.31 (95% CI 1.03–1.66). Their findings suggest that several ceramides are associated with incident AF, and that these associations differ depending on the fatty acid. Ceramides with palmitic acid were associated with increased AF risk, whereas ceramides with very-long-chain saturated fatty acids were associated with reduced AF risk. The associations appeared to be independent of other risk factors and did not differ by subgroups such as age, sex, race, BMI, or prevalent coronary heart disease. Additional studies are needed to evaluate mechanistic linkages between ceramides and atrial fibrillation.

5) Ceramides in DM2

The mechanistic role of ceramides in insulin resistance has been reviewed recently (8, 27). Ceramide precursor molecules, dihydroceramides, are elevated years before the onset of type 2 diabetes mellitus (DM2) (28). Additionally, the ceramide ratio Cer(d18:1/18:0)/Cer(d18:1/16:0) predicts the onset of incident DM2 (11). The HR per standard deviation of this ceramide ratio in the population-based FINRISK 2002 study was 2.23 (95% CI 2.05–2.42) and the result remained significant even after adjusting for several traditional risk factors, including BMI, fasting glucose and HbA_{1c}. This finding was also validated in stable CAD patients in the WECAC cohort, where the HR was 1.81 (95% CI 1.53–2.14) (11). Thus, this ceramide ratio serves as a strong prognostic marker for the onset of incident DM2.

Taken together, clinical studies have consistently shown a direct and significant association between certain ceramide species and cardiometabolic outcomes. More data are needed to better understand the effect of the genetic contribution and different environmental factors on the circulating ceramide concentrations. In animal models, ceramide inhibitors have been shown to reduce atherosclerotic plaque formation, but studies in humans are currently lacking. Nevertheless, ceramides are clinically useful biomarkers aiding in clinical

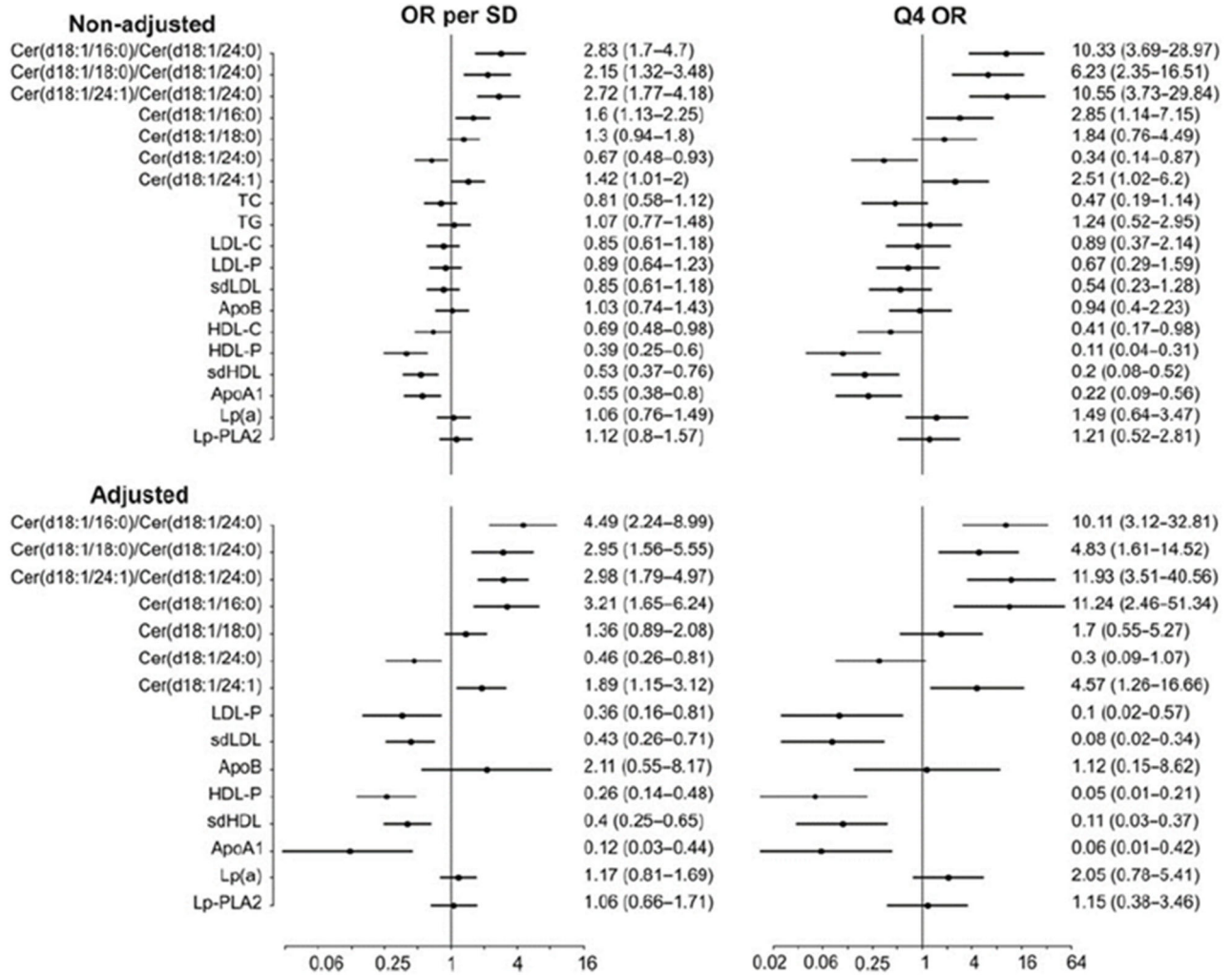

FIGURE 2 | CV death odds ratios for per standard deviation and 4th quartile for different lipid markers and ceramides in the COROGENE study. Adjustment is made for TC, TG, LDL-C, HDL-C, and CRP. Figure from Laaksonen et al. (13). Figure reproduced under the terms of the Creative Commons Attribution License.

decision making in patients suspected of atherosclerosis. Different ceramide-based scores, described below, have been developed to help clinical implementation of ceramide-based risk assessment.

FACTORS AFFECTING CIRCULATING CERAMIDE CONCENTRATIONS

Serum ceramide concentrations are influenced by certain drugs and lifestyle modifications, including dietary changes and exercise. As ceramides are found in circulating lipoprotein particles, such as LDL-C or HDL-C, their serum levels can be lowered with cholesterol lowering measures (29). It is not surprising that statins, ezetimibe, and PCSK9 inhibitors may lower serum ceramides (12, 29, 30). Furthermore, it has been reported that fenofibrate lowers ceramide concentrations significantly. Croyal et al. conducted analyses of samples collected from 102 patients with type 2 diabetes, enrolled in the FIELD trial, before and after fenofibrate treatment (200 mg/day) (31). They observed a significant decrease in plasma ceramides after fenofibrate treatment, independent of the lipid profile components.

Many studies have shown that metformin has potential to significantly lower ceramide levels in animals. Zabielski et al. concluded that the insulin-sensitizing action of metformin in skeletal muscle is associated with decreased 18-carbon acyl-chain-derived bioactive lipids including Cer(d18:0/18:0) and Cer(d18:1/18:0) in insulin-resistant muscle (32). The same group has also reported that high fat diet augmented the content and fractional synthesis rate (FSR) of diacylglycerol (DAG) and ceramides in the liver which was accompanied by systemic insulin resistance and inhibition of hepatic insulin signaling pathway under insulin stimulation (33). Metformin improved systemic insulin resistance and increased the hepatic insulin

signaling cascade and it lowered both the concentration and FSR of ceramides, DAG, and augmented acylcarnitine content and the expression of mitochondrial markers. The authors suggest that in the liver, the insulin sensitizing effect of metformin depends on increase of mitochondrial β-oxidation, which defends from hepatic buildup of both the ceramides and DAGs and preserves insulin sensitivity under HFD consumption (33). Recently, Marfella et al. observed that pathogenesis of human diabetic cardiomyopathy started with cardiomyocyte lipid accumulation following heart transplantation in diabetes mellitus recipients and that metformin use was associated with reduced ceramide and triglyceride accumulation independent of immunosuppressive therapy (34). Furthermore, insulin sensitizing thiazolidinediones have been found in mouse models to reduce ceramides in skeletal muscle (35). Later, Warshauer et al. observed in a single-center, randomized, double-blind, placebo-controlled trial comparing pioglitazone to placebo that pioglitazone in individuals with metabolic syndrome induced a potent decrease in plasma ceramides, and some of the changes correlated with changes in insulin resistance and adiponectin levels (36). Taken together, it appears that metformin and pioglitazone in addition to lipid lowering compounds have a significant potential to alter ceramide metabolism in ways that might be clinically beneficial. Recently Sodium-glucose cotransporter-2 (SGLT2) inhibitors have shown to reduce CV events in DM2 and HF patients (37, 38), but the possible linkage between ceramides and SGLT2 treatments remains to be evaluated.

Diet may also affect ceramide levels. Luukkonen et al. have shown that saturated fat intake induce insulin resistance and endotoxemia in addition to increasing multiple plasma ceramides in overweight subjects fed with an additional 1,000 kcal/day of saturated fat (39). Additionally, certain dietary interventions may lower circulating ceramide levels. Mathews et al. assessed the efficacy of a fruit and vegetable intervention on overall metabolic health, circulating ceramide supply and inflammatory status in young adults (40). They observed in this pilot study of 36 young adults participating in the 8-week free-living nutritional intervention that a short-term nutritional intervention can lower serum ceramide concentrations. Future studies with larger sample sizes is needed to better understand the effects of nutrients on distinct ceramide concentrations.

Interestingly, in the PREDIMED trial Wang et al. reported a positive association between baseline plasma ceramide concentrations and incident CVD. Importantly, they showed that a Mediterranean dietary intervention may alleviate potential harmful effects of raised plasma ceramide concentrations on CVD (41). Their study population consisted of 980 participants from the PREDIMED trial (Prevención con Dieta Mediterránea), including 230 incident cases of CVD and 787 randomly selected participants at baseline (including 37 overlapping cases) followed for $\leq$7.4 years. The participants were randomized to a Mediterranean diet supplemented with extra virgin olive oil, a Mediterranean diet supplemented with nuts, or a control diet. The traditional Mediterranean diet enriched with extra virgin olive oil or nuts showed the potential to alleviate the harmful effects of high ceramide concentrations on CVD outcomes. However, further studies are needed to replicate these intriguing results in other populations as well as to investigate potential mechanisms.

Reidy et al. have recently reviewed the relationship between skeletal muscle ceramides and insulin resistance in response to increased physical activity (42). Their review of the literature indicated that chronic exercise reduces ceramides in individuals with obesity, diabetes or hyperlipidemia. However, in metabolically healthy individuals increased physical activity can improve insulin sensitivity independent of changes in skeletal muscle ceramide content (42). Kasumov et al. studied 24 adults with obesity and normal glucose tolerance (NGT, $n = 14$) or diabetes ($n = 10$) before and after a 12-week supervised exercise-training program (5 days/week, 1 h/day, 80–85% of maximum heart rate) (43). Concentrations of Ceramides were similar in subjects with obesity and NGT and in subjects with diabetes, despite differences in glucose tolerance. Notably, exercise significantly reduced plasma concentrations of Cer(d18:1/14:0), Cer(d18:1/16:0), Cer(d18:1/18:1), and Cer(d18:1/24:0) in all subjects following the intervention ($P < 0.05$). Petrocelli et al. performed a reversed exercise study by evaluating the effect of bed rest on circulating ceramides (44). As acute bed rest places older adults at risk for health complications by disrupting homeostasis in many organ systems including the cardiovascular system, they hypothesized that circulating ceramides predictive of poor cardiometabolic outcomes would increase following 5-days of bed rest. In their study, 35 healthy younger and older men and women (young: $n = 13$, old: $n = 22$) underwent 5 days of controlled bed rest. The primary observations were that circulating ceramides decreased while ceramide ratios and the (CERT1) score (see below CERT1 score), associated with CV risk, increased primarily in older adults. It is noteworthy, that these findings were independent of changes in circulating lipoprotein levels (44).

In summary, ceramides are modifiable by certain drugs, lifestyle, and environmental factors. The significance of genetic factors affecting serum ceramide concentrations and ceramide metabolism at a cellular level still remain to be investigated. While serum ceramides are novel biomarkers of CVD, their potential causality has not yet been established. This would be important for promoting attempts to develop ceramide-targeted drugs. Initial data from animal experiments suggest that ceramide synthetic pathways may offer targets for drug development as inhibition of serine palmitoyltransferase (SPT) has shown to significantly reduce the development of atherosclerosis in a murine model (45–47); similarly dihydroceramide desaturase 1 (DES1) inhibition has proven to be beneficial in treatment of diabetes mellitus in mice (48).

CERAMIDE SCORE AS THE CLINICAL SOLUTION

CERT1 Score

Since panels of single ceramide species or other lipid combinations are rather cumbersome for clinical practice, CERT1 risk score was developed for clinical use based

on ceramide concentrations and their ratios (13, 21). To calculate the CERT1 score, Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) concentrations and their ratios to Cer(d18:1/24:0) are determined. The scoring system assigns 2 points to those with concentrations or ratios in the fourth quartile, 1 point to values in the third quartile, and 0 points to the first and second quartiles, with total CERT1 scores ranging from 0 to 12 (**Figure 3**).

Based on CERT1, patients are stratified into four risk categories (low–moderate–increased–high) (**Figure 3**) and a linear CVD risk increase is observed along with the increasing score both in patients with a stable CHD and acute coronary syndrome (ACS) (**Table 1**). When comparing the high to low risk category, there is a 4.2- and 6.0-fold relative risk increase in patients with stable CHD and ACS, respectively. When subjects were sorted according to their LDL-C concentrations and divided in quartiles, the high-risk patient quartile was not identified using only the LDL-C concentration, supporting the view that the ceramide score could improve risk stratification beyond LDL-C (13). The performance of the test was further validated in a large-scale population-based study (FINRISK 2002; $N = 8101$). In this cohort a 4.2-fold relative risk increase was observed when comparing the high to low risk category (21) (**Table 1**).

CERT2 Score

As phosphatidylcholines (PCs) have shown prognostic value for CV events (49), it was investigated whether the ceramide test score (CERT1) could be upgraded by adding distinct PCs to the score. Main drivers of the lipid species choice were analytical stability, ability to incorporate into the existing CERT1 assay, and statistical strength across a number of clinical cohorts. The novel ceramide test score, named CERT2, was developed in the WECAC study and validated in the LIPID and KAROLA studies, by selecting the test components in a stepwise manner (14). The original CERT1 score contained three single ceramides and three ceramide/ceramide ratios, whereas the CERT2 score had one ceramide/ceramide ratio, two ceramide/PC ratios and a single PC. Ceramide-PC ratio components of the CERT2 test showed higher HRs than the previously published ceramide–ceramide ratios, across all three studies (14).

The CERT2 risk estimation tool showed improved performance metrics and can be used to reliably stratify CHD patients for their risk of CV events, especially CV death (14). The performance of the CERT2 score, in addition to other CV biomarkers, was evaluated in three independent large cohorts of CHD patients. For CV death, the HRs (95% confidence interval) per standard deviation (SD) for CERT2 were 1.50 (1.35–1.68) in WECAC, 1.51 (1.38–1.65) in the LIPID trial, and 1.62 (1.32–2.00) in KAROLA. For all the investigated biomarkers, the HRs for CV events were lower than for CV death (14). Performance comparison of CERT2, CERT1, and other CVD biomarkers are provided in **Table 2**. This comparison shows that HR values of CERT2 exceed those of CERT1 and other markers, including the routinely used lipid parameters and high sensitivity C-reactive protein (hsCRP). Furthermore, in all three studies the risk for CV death and CV events increased along with increasing CERT2 score and risk group. For CV death, a 3.5- to 5.4-fold increase in risk was observed in different cohorts between the highest and lowest risk groups for CERT2, while the risk increase was more modest for CERT1 and for LDL-C which is widely used in clinical risk estimation assessments (14) (**Table 3**). One of the major advantages of this study was the inclusion of samples obtained from patients randomized to the placebo arm of the LIPID trial. This avoids confounding

SCORE CALCULATION

	Score component	Q1	Q2	Q3	Q4
CERT1	Cer(d18:1/16:0)	0	0	+1	+2
	Cer(d18:1/18:0)	0	0	+1	+2
	Cer(d18:1/24:1)	0	0	+1	+2
	Cer(d18:1/16:0) / Cer(d18:1/24:0)	0	0	+1	+2
	Cer(d18:1/18:0) / Cer(d18:1/24:0)	0	0	+1	+2
	Cer(d18:1/24:1) / Cer(d18:1/24:0)	0	0	+1	+2
CERT2	Cer(d18:1/24:1) / Cer(d18:1/24:0)	0	+1	+2	+3
	Cer(d18:1/16:0) / PC 16:0/22:5	0	+1	+2	+3
	Cer(d18:1/18:0) / PC 14:0/22:6	0	+1	+2	+3
	PC 16:0/16:0	0	+1	+2	+3

CERT1 / CERT2 SCORE: 0-12 points

RISK GROUP DETERMINATION

Risk group	CERT1	CERT2
Low risk	0-2	0-3
Moderate risk	3-6	4-6
Increased risk	7-9	7-8
High risk	10-12	9-12

FIGURE 3 | Calculation of CERT2 risk scores and determination of CVD risk groups (14). When determining the score for a subject, it is evaluated to which quartile the person belongs with regards to each score component. CERT1 has six components, and only quartiles 3 and 4 give points, while CERT2 has only 4 components but already the second quartile yields a risk point. Thus, the scale for both of these scores is the same, 0–12 points. Based on these the subject is categorized to one of the CVD risk categories. Figure reproduced under the terms of the Creative Commons Attribution License.

TABLE 1 | CERT1 and risk of CV death in primary prevention, stable CAD patients and ACS patients.

	Primary prevention			Stable CAD			ACS		
	Category	**Risk (%)[a]**	**Relative risk**	**Category**	**Risk (%)[b]**	**Relative risk**	**Category**	**Risk (%)[c]**	**Relative risk**
CERT1	0–2	1.2%	Ref.	0–2	2.7%	Ref.	0–2	1.6%	Ref.
	3–6	1.9%	1.6	3–6	4.8%	1.8	3–6	2.6%	1.7
	7–9	3.8%	3.2	7–9	6.9%	2.5	7–9	3.3%	2.1
	10–12	5.1%	4.3	10–12	11.4%	4.2	10–12	9.4%	6.0
LDL-C (mmol/L)	≤2.9	1.8%	Ref.	≤2.6	6.6%	Ref.	≤2.7	4.8%	Ref.
	2.9–3.8	2.2%	1.2	2.6–3.7	4.8%	0.7	2.7–3.7	2.9%	0.6
	3.8–4.7	2.8%	1.5	3.7–4.5	3.5%	0.5	3.7–4.5	1.1%	0.2
	≥4.7	3.4%	1.8	≥4.5	4.1%	0.6	≥4.5	1.1%	0.2

LDL-C was divided into groups in the same proportion as CERT1. Data from (13, 21).
[a]13-year risk, [b]5-year risk, [c]1-year risk.

TABLE 2 | Hazard ratios (HR) per standard deviation for the CERT scores predicting cardiovascular death and events, and comparison with other cardiovascular biomarkers in the WECAC cohort.

	CV death				CV events			
Variable	**HR (95% CI)[a]**	***p*-value**	**HR (95% CI)[b]**	***p*-value**	**HR (95% CI)[a]**	***p*-value**	**HR (95% CI)[b]**	***p*-value**
CERT2	1.50 (1.35, 1.68)	2.6E-13	1.44 (1.28, 1.63)	3.1E-09	1.36 (1.25, 1.48)	5.0E-12	1.29 (1.17, 1.42)	1.7E-07
CERT2-TnT	1.79 (1.59, 2.00)	<2.2E-16	1.63 (1.44, 1.85)	2.2E-14	1.53 (1.40, 1.68)	<2.2E-16	1.39 (1.26, 1.54)	7.9E-11
CERT1	1.27 (1.14, 1.41)	7.9E-06	1.23 (1.09, 1.38)	5.4E-04	1.24 (1.14, 1.35)	8.5E-07	1.18 (1.08, 1.30)	4.1E-04
LDL-C	1.05 (0.94, 1.17)	n.s.	1.15 (1.01, 1.30)	0.032	1.03 (0.94, 1.12)	n.s.	1.13 (1.02, 1.24)	0.015
HDL-C	0.81 (0.72, 0.91)	3.8E-04	0.95 (0.84, 1.07)	n.s.	0.83 (0.75, 0.91)	8.8E-05	0.94 (0.85, 1.04)	n.s.
TG	1.15 (1.04, 1.27)	0.005	1.07 (0.96, 1.19)	n.s.	1.15 (1.07, 1.24)	2.8E-04	1.08 (1.00, 1.18)	n.s.
ApoB	1.15 (1.03, 1.28)	0.009	1.33 (1.03, 1.72)	0.031	1.09 (1.00, 1.18)	n.s.	0.99 (0.80, 1.23)	n.s.
ApoA1	0.79 (0.71, 0.89)	8.6E-05	0.91 (0.74, 1.12)	n.s.	0.84 (0.76, 0.92)	1.6E-04	0.94 (0.80, 1.10)	n.s.
CRP	1.12 (1.05, 1.20)	0.001	1.10 (1.02, 1.19)	0.010	1.08 (1.02, 1.15)	0.013	1.06 (0.99, 1.14)	n.s.
TnT	1.43 (1.31, 1.55)	2.2E-16	1.30 (1.19, 1.43)	1.7E-08	1.35 (1.26, 1.45)	4.1E-16	1.23 (1.14, 1.34)	1.8E-07
Lpa	1.13 (1.02, 1.25)	0.020	1.13 (1.01, 1.26)	0.029	1.12 (1.03, 1.21)	0.010	1.10 (1.01, 1.19)	0.034
TMAO	1.06 (0.97, 1.16)	n.s.	1.02 (0.94, 1.12)	n.s.	1.04 (0.96, 1.12)	n.s.	1.01 (0.93, 1.10)	n.s.

n.s., not significant. Data from Hilvo et al. (14).
[a]Age as time scale, stratified by vitamin B intervention; [b]additionally adjusted for sex, statin, diabetes, hypertension, current smoking, previous MI, previous stroke, BMI, LDL-C, HDL-C, TG, CRP.
LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TG, triglycerides; ApoB, apolipoprotein B, ApoA1, apolipoprotein A1; hsCRP, high-sensitivity C-reactive protein; TnT, high-sensitivity troponin-T; Lp(a), lipoprotein(a); TMAO, trimethylamine N-oxide; NS, not significant.

effects caused by lipid lowering treatments. Importantly, the result obtained in the statin free population of the LIPID trial confirmed previous observations from the WECAC study and demonstrated the value CERT2 to assess risk in CHD (**Figure 4**). **Figure 5** shows the risk curves both for CERT2 and LDL-C in the placebo-treated patients in the LIPID trial. The risk curve for CERT2 increases linearly while the risk curve for LDL-C showed a slight risk increase only for the highest LDL-C levels > 5.2 mmol/l (200 mg/dL).

In the large-scale STABILITY trial of optimally treated secondary prevention patients, CERT2 was able to significantly improve HR for CV outcomes in a study population covering various geographical locations worldwide (15). There were differences in the score distribution by location, and further work is needed to delineate the factors behind this observed distribution difference. There were statistically significant associations between CERT2 and all CV outcomes including stroke and heart failure. For CERT2, the highest unadjusted hazard ratios (HRs) per SD were observed for CV death (HR, 1.57; 95% CI, 1.45–1.69), all-cause death (HR, 1.54; 95% CI, 1.45–1.64), and heart failure hospitalization (HR, 1.52; 95% CI, 1.35–1.70). For stroke the unadjusted HR was 1.29 (1.15–1.46).

In STABILITY the CERT2 score was associated significantly with smoking and multivascular disease as well as with multivessel CAD. Furthermore, patients with renal dysfunction had higher CERT2 scores, while the association with high blood pressure and DM was much weaker. In addition, CERT2 was significantly related with the levels of lipid biomarkers (LDL-C and triglyceride) and supported the view that ceramides are essential constituents of circulating lipoproteins. The CERT2 score was prognostic even after adjustment for LDL-C and triglyceride levels. This indicates that sphingolipids may be

TABLE 3 | Risk for CV death in different CERT score groups, and with respect to LDL-C.

	CERT2			CERT1			LDL-C		
	Group	Risk	Rel.risk	Group	Risk	Rel.risk	mmol/l	Risk	Rel.risk
WECAC	0–3	3.5%	Ref.	0–2	5.1%	Ref.	≤2.1	7.3%	Ref.
	4–6	6.4%	1.8	3–6	6.3%	1.2	2.1–3.2	7.7%	1.1
	7–8	7.8%	2.2	7–9	11.1%	2.2	3.2–4.2	8.1%	1.1
	9–12	15.1%	4.3	10–12	15.5%	3.0	≥4.2	6.6%	0.9
LIPID	0–3	4.3%	Ref.	0–2	5.9%	Ref.	≤3.2	8.8%	Ref.
	4–6	6.3%	1.5	3–6	8.7%	1.5	3.2–4.0	7.9%	0.9
	7–8	9.9%	2.3	7–9	8.9%	1.5	4.0–4.6	7.9%	0.9
	9–12	15.2%	3.5	10–12	14.8%	2.5	≥4.6	9.7%	1.1
KAROLA	0–3	2.6%	Ref.	0–2	3.5%	Ref.	≤2.4	7.6%	Ref.
	4–6	5.4%	2.1	3–6	7.0%	2.0	2.4–3.1	6.5%	0.9
	7–8	7.8%	3.0	7–9	11.8%	3.4	3.1–3.8	7.1%	0.9
	9–12	13.8%	5.4	10–12	12.7%	3.7	≥3.8	5.0%	0.7

For comparison, LDL-C was divided into groups (Q1-Q4) in the same proportion as CERT2. For the WECAC and KAROLA studies, the risk is for 10 years and for LIPID trial 6 years. Data from Hilvo et al. (14).
Ref. = reference category.

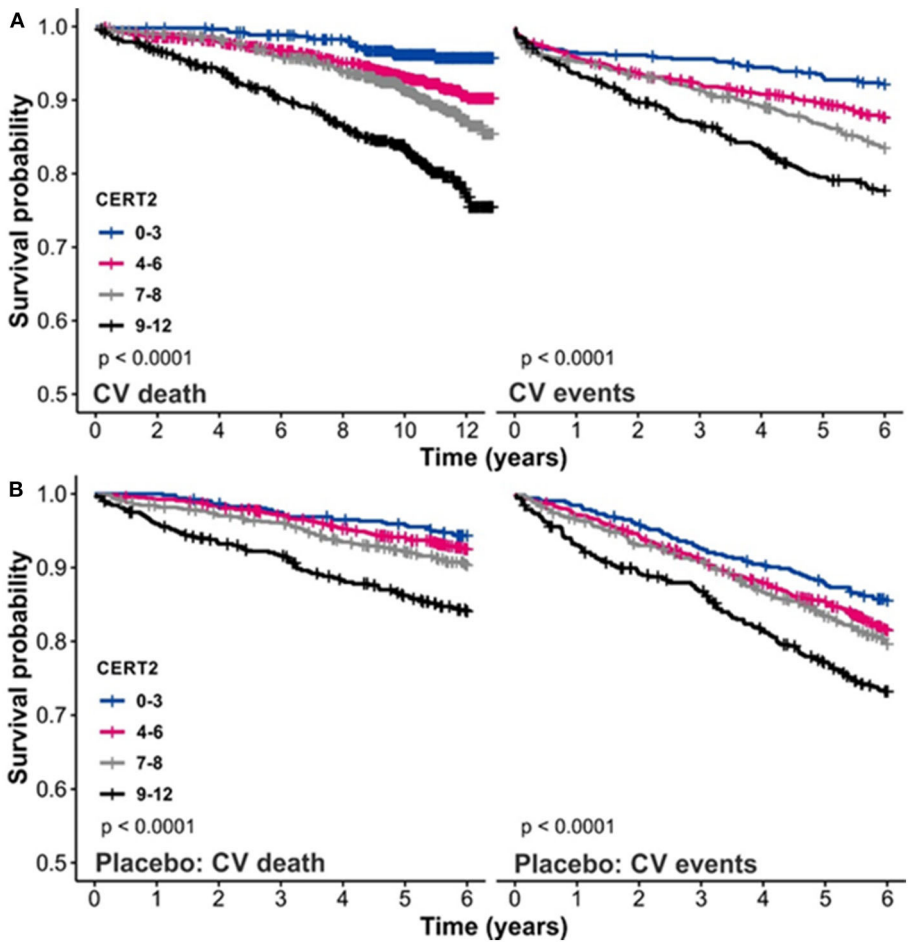

FIGURE 4 | Kaplan–Meier curves for CERT2 **(A)** in the WECAC cohort and **(B)** in the placebo treatment arm in the LIPID trial. Figure adapted from Hilvo et al. (14). Figure reproduced under the terms of the Creative Commons Attribution License.

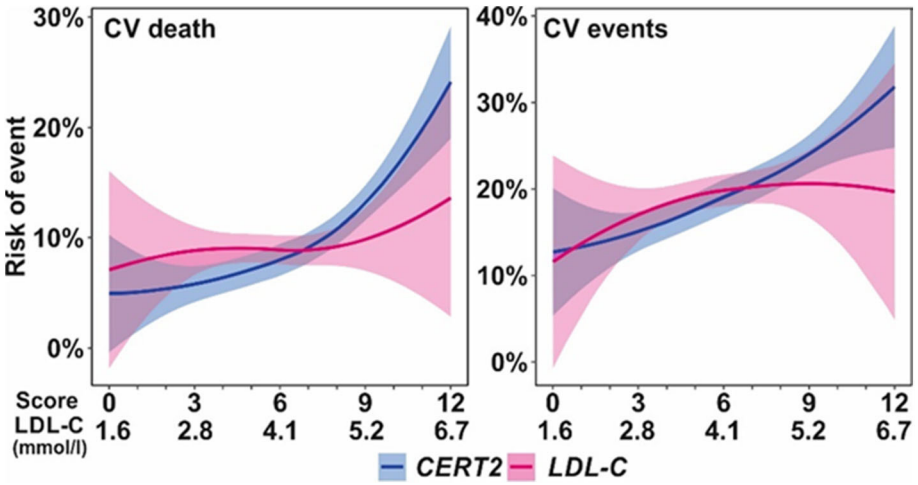

FIGURE 5 | Risk (6 years) curves for CERT2 and LDL-C in the LIPID trial placebo treatment arm. Figure adapted from Hilvo et al. (14). Figure reproduced under the terms of the Creative Commons Attribution License.

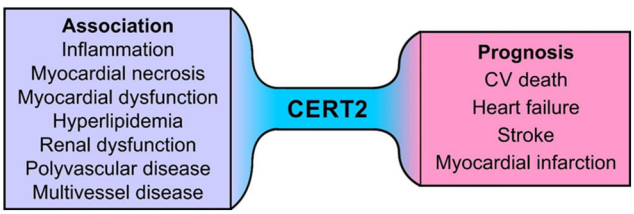

FIGURE 6 | Association of CERT2 with pathophysiological processes and cardiovascular outcomes.

important for cardiovascular disease beyond conventional lipids. The CERT2 score was also significantly associated with inflammatory markers (hs-CRP and IL-6), suggesting that certain ceramide levels might be a surrogate of vascular inflammation. These findings suggest that CERT2 could assess both plaque burden and inflammatory residual risk in patients with stable CHD. Higher CERT2 score was also associated with higher hs-TnT and NT-proBNP concentrations. These findings indicate that alterations in ceramide expression might be associated with, and possibly contribute to, myocardial injury and/or myocardial dysfunction. CERT2 score is a useful tool to determine residual risk in patients with stable CHD as it is associated with all CV events and reflects disturbances of several key mechanisms for CVD such as dyslipidemia, inflammation, myocardial injury, and renal dysfunction (15) (**Figure 6**).

Unbiased Machine Learning Based SIC Score

A new approach to identify associations between serum ceramides and coronary artery disease (CAD) was introduced by Poss et al. (50). They performed targeted lipidomics on serum samples from individuals with familial CAD ($n = 462$) and population-based controls ($n = 212$) to study the association between serum sphingolipids and CAD, using unbiased machine learning to find sphingolipids related with CAD. They observed that in this setting nearly every sphingolipid measured (30 out of 32) was significantly elevated in subjects with CAD compared to controls. The authors generated a novel sphingolipid-inclusive CAD risk score, termed SIC, that demarcates patients with CAD independently and more effectively than conventional clinical CVD biomarkers including serum LDL cholesterol and triglycerides (49). This new metric comprises several minor lipids that, according to authors, likely serve as measures of flux through the ceramide biosynthesis pathway rather than the abundant deleterious ceramides that are included in other ceramide-based scores. Despite the fact that this cross-sectional study used case and control samples collected from different sources and time points 10 years apart in 1980s and 1990s, the results suggest that comprehensive sphingolipid panels could also be considered as a measure of CVD. However, these findings need to be further validated in large-scale cohort studies. The use of a large panel of minor ceramides may pose practical issues in analytical validation for clinical use.

Diabetes Score (dScore)

Compared to the CVD risk scores, a different approach was taken when using the ceramide ratio Cer(d18:1/18:0)/Cer(d18:1/16:0) in a clinically applicable scoring system for predicting the onset of diabetes (dScore). In addition to the lipid ratio, this scoring system incorporates the patient's age, sex and BMI, and gives a 10-year absolute risk (scale 0–100) of developing DM2 (11). The subjects are grouped into risk categories: low risk is defined as <5%, moderate risk 5–15% and high risk more than 15% probability of developing DM2 in the next 10 years. It is noteworthy that the ceramide ratio, and consequently the dScore, was significantly reduced in persons with a weight loss of 5% or more (11), which is consistent with earlier dietary interventions showing that weight loss of a few kilograms is enough to reduce the risk of developing diabetes (51).

CLINICAL USE OF SCORES

The CERT1 ceramide score has been implemented in clinical use in private and public practice both in Finland and at Mayo Clinic in the USA. The score includes a single easy to understand readout for physicians as opposed to a list of multiple ceramide results each with separate reference values. The score calculation is based on ratios of distinct ceramides, which offers another advantage since an occasional variation in concentrations of single ceramides does not significantly affect the ratio. The score-based reporting system also allows an opportunity for graphical visualization of the results in a condensed form which is simple to understand and could be useful to share with patients. Ceramide laboratory measurements are performed by high-throughput robotic enabled mass spectrometry instrumentation. Mass spectrometry is classically associated with high-sensitivity requirements of hormonal measurements, vitamin D or immunosuppressants among other. The technology is highly robust, easy to set up and the quality controlled specific standards conform to all required regulatory standards. The cost and speed of daily operation is comparable to antibody-based assay solutions in high-volume laboratories and the equipment is becoming more routinely available in most high volume clinical laboratories.

The studies provide robust evidence that ceramide scores (CERT1, CERT2) can be used for risk stratification in both primary and secondary prevention settings. These scores allow for instance a rapid identification and stratification of residual risk in patients with known CHD who might benefit from more in-depth scrutiny and perhaps more aggressive therapy. Most risk prediction systems in clinical practice use age as part of the assessment. Age is most likely the most powerful biomarker for risk prognostication in general, but it has also significant limitations as it may delay timely intervention in younger and middle-aged individuals. Biomarkers that are not age-dependent allow an earlier detection of risk and prevention. Conversely, many elderly patients may be at relatively low risk despite their age-driven risk calculator scores. In these cases an age-independent biomarker may be useful as these patients might do well without aggressive interventions.

In the 2019 ESC/EAS Guidelines (52) for the management of dyslipidaemias the selection of intervention strategies depends on the cardiovascular risk (assessed e.g., with SCORE) and theconcentration of untreated low-density lipoprotein cholesterol. In many cases the selection between lifestyle modification or pharmacologic intervention is clear. In subjects at moderate CV risk, consideration of lipid lowering agents is recommended. Clinicians frequently evaluate patients with a moderate CV mortality risk (>1 to <5% in 10 years, assessed with SCORE) and LDL-C concentrations between 2.6 and 4.9 mmol/L (100 and 190 mg/dL) where the decision of initiation of a lipid lowering agent is not obvious or clearly recommended by guidelines. Especially in these subjects, additional risk assessment with CERT could be useful for risk stratification in order to identify the subgroup of patients at higher risk who may benefit from more aggressive intervention. This is also recapitulated in the US guidelines where patients deemed at intermediate risk by the ASCVD risk assessment calculator may be further stratified after the assessment of risk enhancers such as the coronary calcium (CAC) scoring (53). The latter testing, which is extensively used in primary preventive cardiology, has some important limitations as it does not capture information about the regional distribution of calcification within the coronary tree (54). Furthermore, CAC score does not incorporate information on the number, size of calcified coronary lesions or biology of calcification and atherosclerosis (54). Perhaps as a consequence of these limitations, events are observed in patients scored as CAC = 0, and many older patients with high CAC scores never experience events—pointing to potential room for improvement (54). Therefore, a simple blood test such as CERT or CERT2 could be clinically informative in a wide variety of patients.

In the latest ESC guideline for stable chronic coronary syndromes (sCCS) (55) the risk variability is described and emphasis is placed on the "very high risk" patients who carry a more than three percent annual mortality risk. Guidelines recommend the use of additional diagnostic tools to identify these patients at the highest risk. However, the recommended imaging methods may not always be available (e.g., stress echocardiography or MRI) and invasive angiography may not always be warranted. Thus, the identification of patients with CHD at highest risk can be significantly and efficiently improved by the addition of a blood test such as one of the ceramide risk scores. CERT has already been used clinically for secondary prevention both in Finland and the US. A combination of the newly developed CERT2 and high sensitivity troponin T (hsTNT) has already been suggested by Hilvo et al. to drastically improve prognostic performance (14).

Different medical treatments may be prescribed in the future based on specific risks and such treatments may become truly personalized. It has been shown that cholesterol lowering agents such as ezetimibe or PCSK9 inhibitors result in higher absolute risk reductions in patients who have a higher baseline risk (56, 57). Ceramide scores could be used in the selection of CHD patients at greatest risk or in primary prevention patients for novel and patient-tailored individualized treatments. This approach appears appealing for lipid lowering drugs because the primary lipid target, LDL-cholesterol, or its proxy, total cholesterol, have rather limited association with outcome in elderly subjects (58–60) and in patients with established CHD (13, 14), thus for these patients ceramide scores could provide an instrument with added value for more tailored treatments.

Another potential field of interest for risk assessment could be antiplatelet therapy. Dual antiplatelet therapy is recommended and routinely used in secondary prevention after myocardial infarction. The duration of antiplatelet therapy after revascularization remains controversial and the risk of bleeding is of concern. Several factors are taken into consideration when assessing the risk of in-stent thrombosis vs. the risk of bleeding and clinicians often face difficult decisions not described directly in the guidelines when taking care of the complex cardiology patient. Ceramide scores could potentially be used in identifying patients at higher risk who might benefit the most from extended dual anti-thrombotic therapy. Finally, drugs that target ceramides directly may become available for clinical use in addition to

drugs that reduce circulating ceramide levels indirectly via HMG-CoA or PCSK9 inhibition. Additionally, lifestyle changes such as weight reduction have shown to reduce the ceramide-based diabetes risk score (11). As the CERT2 score contains also phosphatidylcholines containing omega-3 fatty acids it appears plausible that treatments such as EPA could significantly lower the risk, but this remains to be determined.

Despite rather comprehensive scientific evidence there are still significant barriers limiting more widespread use of ceramides in CVD risk assessment that are typical for any novel diagnostic test. First, the test must be widely available through multiple outlets. Currently, in the US CERT1 is offered only by one reference laboratory. Serving as a pilot for Europe as a whole, the test is available in nearly all private healthcare centers in Finland and has been incorporated into the portfolio of the public health care offering in that country. Second, most clinical outcomes studies using ceramides have been reported within only the last decade so most practicing clinicians are unaware of the clinical utility of ceramide testing. To overcome this challenge, physician education and dissemination of the scientific evidence to various groups of physicians is required. Widespread and effective dissemination is possible only for global marketing organizations and ceramides are lacking such support. Acceptance to ESC/EAS and AHA/ACC guidelines would be another avenue to more widespread clinical uptake. As described earlier in this review, there are certain conditions particularly in the recent ESC 2019 guidelines where we believe ceramide testing could well be justified. Third barrier not only for ceramides but for all new diagnostics is the stringent reimbursement policies of insurance companies as their requirements are often impractical for novel CVD tests and, thus, the established older tests are being reimbursed and used despite limitations of their clinical performance.

CONCLUSIONS

Ceramide scores are currently used in clinical practice to identify residual risk in patients with established CHD and may further be used in primary prevention to identify subjects at elevated risk who might benefit from more intensive prevention. CERT scores can be used for follow-up and to further motivate patients to initiate or continue medical and life-style recommendations.

AUTHOR CONTRIBUTIONS

RL wrote the manuscript. MH, VV, LJD, and RH critically revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Turpin-Nolan SM, Brüning JC. The role of ceramides in metabolic disorders: when size and localization matters. *Nat Rev Endocrinol.* (2020) 16:224–33. doi: 10.1038/s41574-020-0320-5
2. Albeituni S, Stiban J. Roles of ceramides and other sphingolipids in immune cell function and inflammation. *Adv Exp Med Biol.* (2019) 1161:169–91. doi: 10.1007/978-3-030-21735-8_15
3. Castro BM, Prieto M, Silva LC. Ceramide: a simple sphingolipid with unique biophysical properties. *Prog Lipid Res.* (2014) 54:53–67. doi: 10.1016/j.plipres.2014.01.004
4. Mullen TD, Obeid LM. Ceramide and apoptosis: exploring the enigmatic connections between sphingolipid metabolism and programmed cell death. *Anticancer Agents Med Chem.* (2012) 12:340–63. doi: 10.2174/187152012800228661
5. Gomez-Muñoz A, Presa N, Gomez-Larrauri A, Rivera IG, Trueba M, Ordoñez M. Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. *Prog Lipid Res.* (2016) 61:51–62. doi: 10.1016/j.plipres.2015.09.002
6. Galadari S, Rahman A, Pallichankandy S, Galadari A, Thayyullathil F. Role of ceramide in diabetes mellitus: evidence and mechanisms. *Lipids Health Dis.* (2013) 12:98. doi: 10.1186/1476-511X-12-98
7. Chaurasia B, Summers SA. Ceramides – lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab.* (2015) 26:538–50. doi: 10.1016/j.tem.2015.07.006
8. Sokolowska E, Blachnio-Zabielska A. The role of ceramides in insulin resistance. *Front Endocrinol.* (2019) 10:577. doi: 10.3389/fendo.2019.00577
9. Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, et al. Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the dallas heart study. *Diabetologia.* (2018) 61:2570–9. doi: 10.1007/s00125-018-4720-1
10. Lemaitre RN, Yu C, Hoofnagle A, Hari N, Jensen PN, Fretts AM, et al. Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: the strong heart family study. *Diabetes.* (2018) 67:1663–72. doi: 10.2337/db17-1449
11. Hilvo M, Salonurmi T, Havulinna AS, Kauhanen D, Pedersen ER, Tell GS, et al. Ceramide stearic to palmitic acid ratio predicts incident diabetes. *Diabetologia.* (2018) 61:1424–34. doi: 10.1007/s00125-018-4590-6
12. Tarasov K, Ekroos K, Suoniemi M, Kauhanen D, Sylvänne T, Hurme R, et al. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J Clin Endocrinol Metab.* (2014) 99:E45–52. doi: 10.1210/jc.2013-2559
13. Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur Heart J.* (2016) 37:1967–76. doi: 10.1093/eurheartj/ehw148
14. Hilvo M, Meikle PJ, Pedersen ER, Tell GS, Dhar I, Brenner H, et al. Development and validation of a ceramide- and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. *Eur Heart J.* (2020) 41:371–80. doi: 10.1093/eurheartj/ehz387
15. Hilvo M, Wallentin L, Ghukasyan Lakic T, Held C, Kauhanen D, Jylhä A, et al. Prediction of residual risk by ceramide-phospholipid score in patients with stable coronary heart disease on optimal medical therapy. *J Am Heart Assoc.* (2020) 9:e015258. doi: 10.1161/JAHA.119.015258
16. Gui YK, Li Q, Liu L, Zeng P, Ren RF, Guo ZF, et al. Plasma levels of ceramides relate to ischemic stroke risk and clinical severity. *Brain Res Bull.* (2020) 158:122–7. doi: 10.1016/j.brainresbull.2020.03.009
17. Fiedorowicz A, Kozak-Sykała A, Bobak Ł, Kałas W, Strzadała L. Ceramides and sphingosine-1-phosphate as potential markers in diagnosis of ischaemic stroke. *Neurol Neurochir Pol.* (2019) 53:484–91. doi: 10.5603/PJNNS.a2019.0063
18. Lemaitre RN, Jensen PN, Hoofnagle A, McKnight B, Fretts AM, King IB, et al. Plasma ceramides and sphingomyelins in relation to heart failure risk. *Circ Heart Fail.* (2019) 12:e005708. doi: 10.1161/CIRCHEARTFAILURE.118.005708
19. Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, et al. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler Thromb Vasc Biol.* (2011) 31:2723–32. doi: 10.1161/ATVBAHA.111.234096
20. Meeusen J, Donato L, Lueke A, Wendt P, Baudhuin L, Berger P, et al. Plasma ceramides independently predict coronary artery disease

and major adverse cardiovascular events*. *J Clin Lipidol.* (2016) 10:656–7. doi: 10.1016/j.jacl.2016.03.016
21. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, et al. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol.* (2016) 36:2424–30. doi: 10.1161/ATVBAHA.116.307497
22. Anroedh S, Hilvo M, Martijn Akkerhuis K, Kauhanen D, Koistinen K, Oemrawsingh R, et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J Lipid Res.* (2018) 59:1729–37. doi: 10.1194/jlr.P081281
23. Peterson LR, Xanthakis V, Duncan MS, Gross S, Friedrich N, Völzke H, et al. Ceramide remodeling and risk of cardiovascular events and mortality. *J Am Heart Assoc.* (2018) 7:7931. doi: 10.1161/JAHA.117.007931
24. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction 2018. *J Am Coll Cardiol.* (2018) 72:2231–64. doi: 10.1093/eurheartj/ehy655
25. Pan W, Dong H, Sun R, Zhao L, Sun M, Li L, et al. Plasma ceramides in relation to coronary plaque characterization determined by optical coherence tomography. *J Cardiovasc Transl Res.* (2020) 1—10. doi: 10.1007/s12265-020-09978-3. [Epub ahead of print].
26. Uchida Y, Uchida Y, Kobayashi T, Shirai S, Hiruta N, Shimoyama E, et al. Detection of ceramide, a risk factor for coronary artery disease, in human coronary plaques by fluorescent angioscopy. *Circ J.* (2017) 81:1886–93. doi: 10.1253/circj.CJ-17-0363
27. Yaribeygi H, Bo S, Ruscica M, Sahebkar A. Ceramides and diabetes mellitus: an update on the potential molecular relationships. *Diabet Med.* (2020) 37:11–9. doi: 10.1111/dme.13943
28. Wigger L, Cruciani-Guglielmacci C, Nicolas A, Denom J, Fernandez N, Fumeron F, et al. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep.* (2017) 18:2269–79. doi: 10.1016/j.celrep.2017.02.019
29. Hilvo M, Simolin H, Metso J, Ruuth M, Öörni K, Jauhiainen M, Laaksonen R, et al. PCSK9 inhibition alters the lipidome of plasma and lipoprotein fractions. *Atherosclerosis.* (2018) 269:159–65. doi: 10.1016/j.atherosclerosis.2018.01.004
30. Ng TWK, Ooi EMM, Watts GF, Chan DC, Weir JM, Meikle PJ, et al. Dose-dependent effects of rosuvastatin on the plasma sphingolipidome and phospholipidome in the metabolic syndrome. *J Clin Endocrinol Metab.* (2014) 99:E2335–40. doi: 10.1210/jc.2014-1665
31. Croyal M, Kaabia Z, León L, Ramin-Mangata S, Baty T, Fall F, et al. Fenofibrate decreases plasma ceramide in type 2 diabetes patients: a novel marker of CVD? *Diabetes Metab.* (2018) 44:143–9. doi: 10.1016/j.diabet.2017.04.003
32. Zabielski P, Chacinska M, Charkiewicz K, Baranowski M, Gorski J, Blachnio-Zabielska AU. Effect of metformin on bioactive lipid metabolism in insulin-resistant muscle. *J Endocrinol.* (2017) 233:329–40. doi: 10.1530/JOE-16-0381
33. Zabielski P, Hady HR, Chacinska M, Roszczyc K, Gorski J, Blachnio-Zabielska AU. The effect of high fat diet and metformin treatment on liver lipids accumulation and their impact on insulin action. *Sci Rep.* (2018) 8:7249. doi: 10.1038/s41598-018-25397-6
34. Marfella R, Amarelli C, Cacciatore F, Balestrieri ML, Mansueto G, D'Onofrio N, et al. Lipid accumulation in hearts transplanted from nondiabetic donors to diabetic recipients. *J Am Coll Cardiol.* (2020) 75:1249–62. doi: 10.1016/j.jacc.2020.01.018
35. Planavila A, Alegret M, Sánchez RM, Rodríguez-Calvo R, Laguna JC, Vázquez-Carrera M. increased akt protein expression is associated with decreased ceramide content in skeletal muscle of troglitazone-treated mice. *Biochem Pharmacol.* (2005) 69:1195–204. doi: 10.1016/j.bcp.2005.01.015
36. Warshauer JT, Lopez X, Gordillo R, Hicks J, Holland WL, Anuwe E, et al. Effect of pioglitazone on plasma ceramides in adults with metabolic syndrome. *Diabetes Metab Res Rev.* (2015) 31:734–44. doi: 10.1002/dmrr.2662
37. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet.* (2019) 393:31–9. doi: 10.1016/S0140-6736(18)32590-X
38. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med.* (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303
39. Luukkonen PK, Sädevirta S, Zhou Y, Kayser B, Ali A, Ahonen L, et al. Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars. *Diabet Care.* (2018) 41:1732–9. doi: 10.2337/dc18-0071
40. Mathews AT, Famodu OA, Olfert MD, Murray PJ, Cuff CF, Downes MT, et al. Efficacy of nutritional interventions to lower circulating ceramides in young adults: FRUVEDomic pilot study. *Physiol Rep.* (2017) 5:e13329. doi: 10.14814/phy2.13329
41. Wang DD, Toledo E, Hruby A, Rosner BA, Willett WC, Sun Q, et al. Plasma ceramides, mediterranean diet, and incident cardiovascular disease in the PREDIMED trial (prevención con dieta mediterránea). *Circulation.* (2017) 135:2028–40. doi: 10.1161/CIRCULATIONAHA.116.024261
42. Reidy PT, Mahmassani ZS, McKenzie AI, Petrocelli JJ, Summers SA, Drummond MJ. Influence of exercise training on skeletal muscle insulin resistance in aging: spotlight on muscle ceramides. *Int J Mol Sci.* (2020) 21:1514. doi: 10.3390/ijms21041514
43. Kasumov T, Solomon TPJ, Hwang C, Huang H, Haus JM, Zhang R, et al. Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes. *Obesity.* (2015) 23:1414–21. doi: 10.1002/oby.21117
44. Petrocelli JJ, McKenzie AI, Mahmassani ZS, Reidy PT, Stoddard GJ, Poss AM, et al. Ceramide biomarkers predictive of cardiovascular disease risk increase in healthy older adults after bed rest. *J Gerontol Ser A.* (2020) doi: 10.1093/gerona/glaa072. [Epub ahead of print].
45. Park T-S, Rosebury W, Kindt EK, Kowala MC, Panek RL. Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic ApoE-deficient mice. *Pharmacol Res.* (2008) 58:45–51. doi: 10.1016/j.phrs.2008.06.005
46. Hojjati MR, Li Z, Jiang X-C. Serine palmitoyl-CoA transferase (SPT) deficiency and sphingolipid levels in mice. *Biochim Biophys Acta.* (2005) 1737:44–51. doi: 10.1016/j.bbalip.2005.08.006
47. Hojjati MR, Li Z, Zhou H, Tang S, Huan C, Ooi E, et al. Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J Biol Chem.* (2005) 280:10284–289. doi: 10.1074/jbc.M412348200
48. Chaurasia B, Tippetts TS, Monibas RM, Liu J, Li Y, Wang L, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science.* (2019) 365:386–92. doi: 10.1126/science.aav3722
49. Mundra PA, Barlow CK, Nestel PJ, Barnes EH, Kirby A, Thompson P, et al. Large-scale plasma lipidomic profiling identifies lipids that predict cardiovascular events in secondary prevention. *JCI Insight.* (2018) 3:121326. doi: 10.1172/jci.insight.121326
50. Poss AM, Maschek JA, Cox JE, Hauner BJ, Hopkins PN, Hunt SC, et al. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. *J Clin Invest.* (2020) 130:1363–76. doi: 10.1172/JCI131838
51. Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, et al. The Finnish Diabetes Prevention Study (DPS): lifestyle intervention and 3-year results on diet and physical activity. *Diabet Care.* (2003) 26:3230–6. doi: 10.2337/diacare.26.12.3230
52. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J.* (2020) 41:111–88. doi: 10.1093/eurheartj/ehz455
53. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the american college of cardiology/american heart association task force on clinical practice guidelines. *Circulation.* (2019) 139:e1082–e1143. doi: 10.1161/CIR.0000000000000699
54. Blaha MJ, Mortensen MB, Kianoush S, Tota-Maharaj R, Cainzos-Achirica M. Coronary artery calcium scoring: is it time for a change in methodology? *JACC Cardiovasc Imaging.* (2017) 10:923–37. doi: 10.1016/j.jcmg.2017.05.007
55. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J.* (2020) 41:407–77. doi: 10.1093/eurheartj/ehz425

56. Qamar A, Giugliano RP, Bohula EA, Park J-G, Jarolim P, Murphy SA, et al. Biomarkers and clinical cardiovascular outcomes with ezetimibe in the IMPROVE-IT Trial. *J Am Coll Cardiol.* (2019) 74:1057–68. doi: 10.1016/j.jacc.2019.06.038
57. Sabatine MS, De Ferrari GM, Giugliano RP, Huber K, Lewis BS, Ferreira J, et al. Clinical benefit of evolocumab by severity and extent of coronary artery disease. *Circulation.* (2018) 138:756–66. doi: 10.1161/CIRCULATIONAHA.118.034309
58. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. *Ann Intern Med.* (1971) 74:1. doi: 10.7326/0003-4819-74-1-1
59. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA.* (1986) 256:2823–8. doi: 10.1001/jama.1986.03380200061022
60. Sniderman AD, Islam S, McQueen M, Pencina M, Furberg CD, Thanassoulis G, et al. Age and cardiovascular risk attributable to apolipoprotein b, low-density lipoprotein cholesterol or non-high-density lipoprotein cholesterol. *J Am Heart Assoc.* (2016) 5:e003665. doi: 10.1161/JAHA.116.003665

Conflict of Interest: Zora Biosciences Oy holds patent disclosures related for the diagnostic and prognostic use of ceramides and phospholipids in CVD. MH, RH, and RL are employees. RH and RL are shareholders of Zora Biosciences Oy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 02 October 2020
doi: 10.3389/fendo.2020.569250

The Role of Ceramides in Diabetes and Cardiovascular Disease Regulation of Ceramides by Adipokines

Bianca C. Field[1], *Ruth Gordillo*[1] *and Philipp E. Scherer*[1,2]*

[1] Touchstone Diabetes Center, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, United States, [2] Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, United States

Metabolic dysfunction is intertwined with the pathophysiology of both diabetes and cardiovascular disease. Recently, one particular lipid class has been shown to influence the development and sustainment of these diseases: ceramides. As a subtype of sphingolipids, these species are particularly central to many sphingolipid pathways. Increased levels of ceramides are known to correlate with impaired cardiovascular and metabolic health. Furthermore, the interaction between ceramides and adipokines, most notably adiponectin and leptin, appears to play a role in the pathophysiology of these conditions. Adiponectin appears to counteract the detrimental effects of elevated ceramides, largely through activation of the ceramidase activity of its receptors. Elevated ceramides appear to worsen leptin resistance, which is an important phenomenon in the pathophysiology of obesity and metabolic syndrome.

Keywords: leptin, adiponectin, adiponectin receptors, sphingolipids, ceramidase, adipokine

OPEN ACCESS

Edited by:
Scott A. Summers,
The University of Utah, United States

Reviewed by:
Gary Sweeney,
York University, Canada
Xavier Prieur,
INSERM U1087 L'unité de recherche de l'institut du thorax, France
William Louis Holland,
The University of Utah, United States

***Correspondence:**
Philipp E. Scherer
Philipp.Scherer@utsouthwestern.edu

Specialty section:
This article was submitted to Diabetes: Molecular Mechanisms, a section of the journal Frontiers in Endocrinology

Received: *03 June 2020*
Accepted: *09 September 2020*
Published: *02 October 2020*

Citation:
Field BC, Gordillo R and Scherer PE (2020) The Role of Ceramides in Diabetes and Cardiovascular Disease Regulation of Ceramides by Adipokines. Front. Endocrinol. 11:569250. doi: 10.3389/fendo.2020.569250

INTRODUCTION

Sphingolipids are by far the most structurally diverse class of lipid molecules. The structural complexity of sphingolipids stems from variation in three components of these lipids: a long chain base, an amide-linked fatty acid, and a head group (20). They are signaling molecules involved in diverse cellular functions, from control of the cell cycle to degradation of plasma membrane proteins (21). They are involved in the most devastating inborn errors and degenerative diseases, including cancer, Alzheimer's disease and dementia (22). In recent years, ceramides, a subgroup of sphingolipids, have received increased attention for their role in myriad pathophysiologic mechanisms, including those underlying cancer (23–25), inflammation (26), depression (27), and neurodegenerative disorders (28). Additionally, more recent studies have highlighted their increasingly important role in obesity and diabetes (9, 29–31) as well as cardiovascular disease (CVD) (32). Preventing aberrant ceramide deposition can prevent or ameliorate a variety of cardiometabolic pathologies, including insulin resistance, CVD (atherosclerosis, heart failure), and hepatic steatosis (33, 34), as well as mitochondrial dysfunction (**Figure 1**). Here, we will focus on ceramides' role in diabetes and CVD, two of the leading causes of death in the United States (35) and worldwide (36).

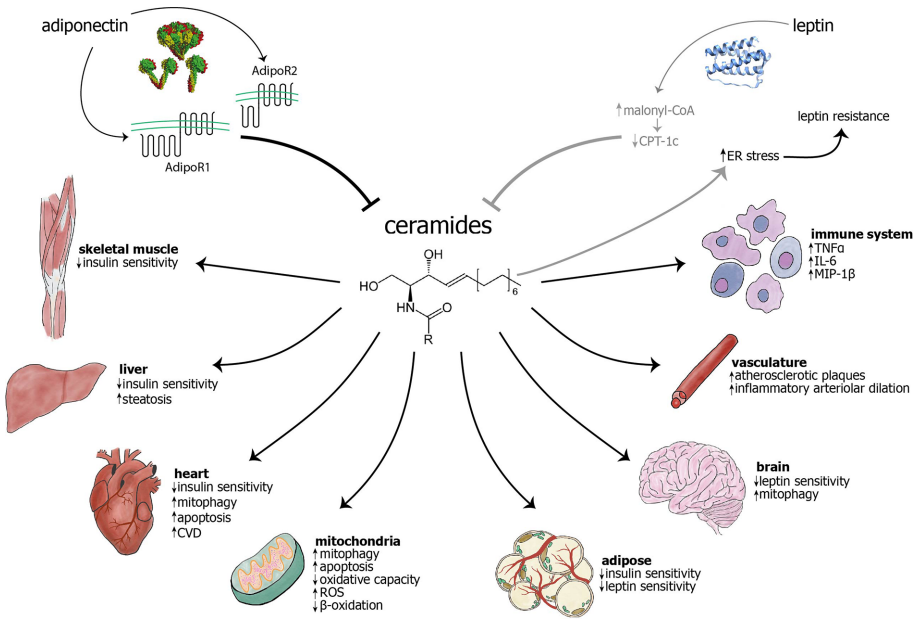

FIGURE 1 | The adipokines adiponectin and leptin have largely inhibitory effects on ceramide levels, while ceramides are known to cause myriad effects in cellular components, different cell types, and organs. Adiponectin inhibits ceramides through the intrinsic ceramidase activity of its receptors (1), AdipoR1 and AdipoR2. The mechanism of leptin's action on ceramides remains unknown, although it has been hypothesized that leptin may exert its influence through inhibition of carnitine palmitoyltransferase-1c (CPT-1c) by malonyl-CoA (2). Conversely, ceramide accumulation may cause ER stress and subsequent leptin resistance (3–6). The previous two relationships are visualized in gray to indicate that they have not been extensively studied and characterized. Ceramides are known to act on mitochondria and cause increased mitophagy, apoptosis, and generation of reactive oxygen species (ROS), as well as decreased β-oxidation and overall oxidative capacity. Ceramides have been shown to cause mitophagy in cardiomyocytes and neurons, among others (not shown) (7). Ceramides decrease insulin sensitivity in skeletal muscle, liver, heart, and adipose (8, 9). Their accumulation may cause decreased leptin sensitivity in the brain and adipose tissue. In the vasculature, ceramide accumulation leads to increased atherosclerotic plaques and inflammatory arteriolar dilation (10–13). They can also lead to increased cardiovascular disease (14, 15), hepatic steatosis (16), and increased inflammation in immune cells (17). As opposed to other targets, skin largely benefits from increased ceramides, with increased cell differentiation and wound healing, as well as decreased senescence (18, 19). AdipoR, adiponectin receptor; CPT-1c, carnitine palmitoyltranferase 1c; CVD, cardiovascular disease; ER, endoplasmic reticulum; IL-6, interleukin-6; MIP-1, macrophage inflammatory protein 1β; ROS, reactive oxygen species; TNFα, tumor necrosis factor α.

INTRODUCTION TO CERAMIDES

Ceramide Biochemistry

Ceramides constitute a simple subtype of sphingolipids composed of a variable-length fatty acid and an amino group (a sphingoid base, usually sphingosine). Their unique structure, highly hydrophobic with a small side chain, enable them to function well both as a structural membrane component and signaling molecule (20, 37). Under physiologic conditions, they range from short chain (C6:0) to very long chain (VLC) (C26:0), although longer chain ceramides are more commonly found in mammalian cells. Ceramide biosynthesis can occur through three distinct pathways: *de novo*, sphingomyelin hydrolysis, or sphingolipid salvage. *De novo* synthesis occurs in the endoplasmic reticulum (ER) and involves ceramide synthases (CerS) as well as serine palmitoyl-transferase (SPT), which catalyzes the rate-limiting step and is inhibited by the drug myriocin. In another pathway, sphingomyelinase (SMase) hydrolyzes sphingomyelin to form ceramide. Finally, in the salvage pathway, alkaline ceramidase (ACER), which is inhibited by the drug D-erythro-MAPP, and acid ceramidase (AC) work in the endolysosomal system to regenerate ceramide from sphingosine (38, 39).

Sphingomyelinases and ceramidases exist in three forms: acid, neutral, and alkaline. Their subtype depends on their subcellular localization, function, and optimal pH. Generally, acid SMases and ceramidases function in lysosomes, neutral enzymes along the plasma membrane, and alkaline enzymes in the Golgi or ER (40). Furthermore, CerSs, important in both *de novo* and salvage pathways, preferentially catalyze certain fatty acyl-CoA substrates in order to make different ceramide subtypes (39, 40). Because ceramides are the center of a web of sphingolipid biosynthesis reactions, it can be difficult to prove causation between particular metabolites and enzymes (38).

Dihydroceramides are the precursor metabolites of ceramides in the *de novo* sphingolipid biosynthesis pathway. CerS form dihydroceramides by condensing sphinganine and acyl-CoA, and they are a family of six isoforms (CerS1-6) that each display specificity for certain acyl-CoA chain lengths. Ceramides are formed from dihydroceramides by the action of the enzyme dihydroceramide desaturase (41, 42).

Ceramides as Cell Membrane Components

Ceramides are key to the structural integrity the plasma and mitochondrial membranes. While they are restricted to the

membrane in which they are formed, they can flip-flop between membrane leaflets (43), although this movement occurs slowly (44). This incorporation can change membrane configuration and relative hydrophobicity of the plasma membrane-cytosol interface, changing the affinity for the membrane of a number of proteins, which in turn affect the activity of membrane enzymes (45).

Ceramides in Circulation

Clinical studies show that circulating ceramides primarily originate in the liver, a major site of *de novo* ceramide synthesis (17, 46), although adipocytes may also contribute to circulating ceramides in some species (33). They circulate at a concentration of about 10μM and are primarily transported in circulation by lipoproteins (17, 46, 47), while exosomal transport may also significantly contribute (48). One study, using thin layer and high-performance liquid chromatography (TLC and HPLC, respectively), found that plasma ceramides are primarily (~80%) carried by very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), with the remaining ceramide being carried by albumin (~15%) and high-density lipoprotein (HDL; ~5%) (46). Another study, using fast performance liquid chromatography (FPLC), separated lipoprotein fractions and found that ceramides were primarily carried by LDL (60.3 +/−6.7%) and to a lesser extent by HDL (24.1+/−7.4%), and VLDL (15.6+/−9.9%). However, ceramides comprised a small fraction of total lipids carried by these lipoproteins (LDL and VLDL ~0.6%; HDL ~0.1%). While the distribution of ceramide species carried by these lipoprotein classes were largely similar, LDL and VLDL carried more similar proportions of specific ceramide species (less C16:0, more C24:0), while HDL carried others (more C16:0, less C24:0) (47). Additionally, recent research has found that ceramide species are also transported in exosomes from adipocytes and endothelial cells (48).

Ceramides in Mitochondria

Ceramides are a normal component of mitochondrial membranes, and they are necessary for normal mitochondrial function. Purified mitochondria and mitochondria-associated membranes have been shown to generate ceramides *in vitro*, and specific ceramide synthases (CerS2, CerS4, and CerS6) are present in the inner and outer mitochondrial membranes (49). Furthermore, normal biological mechanisms can be mediated by ceramides. For instance, CerS6-generated ceramides may mediate pruning of oligodendrocytes during normal brain development (50).

While ceramides are necessary for normal mitochondrial functions, accumulation of excess ceramides have been shown to induce mitochondrial dysfunction (51, 52). Increased mitochondrial ceramide content has been shown to have numerous deleterious effects, including inhibition of mitochondrial oxidative capacity, depletion of ATP, inhibition of the electron transport chain, and enhanced generation of reactive oxygen species (51, 53–55). Additionally, studies show that ceramides can alter or nearly eliminate the respiration-dependent mitochondrial membrane potential (51, 56, 57). They disrupt the normal structure of the inner and outer mitochondrial membranes, increasing their permeability independent of cytochrome c and electron transfer activity (51). One study in a mouse model of ischemia showed that ceramide accumulation leads to inhibition of the respiratory chain (58). In mouse models of diet-induced obesity (DIO), pharmacologic or genetic depletion of ceramides increases mitochondrial complex activity [complex IV in subcutaneous white adipose tissue (sWAT), complexes II and IV in brown adipose tissue (BAT)], as well as respiration in sWAT, BAT, and brain (59). Pharmacological depletion of ceramides also decreased the respiratory exchange ratio (RER) in models of DIO (60). Several ceramide lipid storage diseases, including Fabry disease and neuronal ceroid lipofuscinosis, are associated with dysfunction in the mitochondrial respiratory chain (7). Other sphingolipid storage diseases cause inhibition of mitochondrial biogenesis (61).

Ceramides in Mitophagy and Apoptosis

Beyond causing mitochondrial dysfunction, ceramides are known to regulate mitophagy (62). Accumulation of ceramide species in yeast led to increased mitochondrial fission and mitophagy (63). Similarly, in mouse and human cardiomyocytes, VLC ceramides produced by CerS2 led to increased mitophagy, along with increased oxidative stress and mitochondrial dysfunction (56). Ceramide-induced mitophagy can also help explain some pathophysiology and treatment mechanisms. For example, one study found that cigarette smoke caused mitophagy through increased levels of C16 ceramide, triggering necroptosis (64). Another study found that in glioblastoma cells, a plant agglutinin that is used to inhibit glioblastoma growth induced mitophagy-dependent cell death *via* increased ceramides (65).

Ceramides also have an established role in the induction of apoptosis (66), and their effects on mitochondria appear to be important in this process (52, 67). Apoptotic stimuli activate CerS in the mitochondrial membranes, indicating a role for ceramides in mitochondria-induced apoptosis (53). Mitochondria can function as sensors for cellular stress by monitoring local levels of specific lipids, including sphingolipids (45). This function facilitates the intrinsic apoptotic pathway. Formation of ceramide channels on the outer mitochondrial membrane leads to apoptosis, with ceramide species C16, C18, and C20 being most important in this process (52). Ceramides also work synergistically with the apoptotic protein Bax to induce mitochondrial outer membrane permeabilization in yeast (55). Furthermore, C2 ceramide was found to alter mitochondrial membrane potential and cause mitochondrial fragmentation, leading to apoptosis in cultured cardiomyocytes (57). Some interventions, including overexpression of Bcl-2 (68–71) and pharmacological inhibition of mitochondrial membrane depolarization (72, 73), have also prevented ceramide-induced apoptosis through prevention of mitochondrial dysfunction (74).

Ceramides may need to localize specifically to mitochondria to induce apoptosis. One group found that increased ceramides due to sphingomyelinase overexpression only induced apoptosis if the enzyme was targeted to mitochondria, but not if it was targeted to

other cell compartments, including the inner plasma membrane, cytoplasm, ER, Golgi, and nucleus. Despite a resultant increase in whole-cell ceramide content, there was no induction of apoptosis when increased ceramides were targeted to these compartments (75). Furthermore, another group found that specific ceramide enzymes localize to the inner and outer membranes, respectively, suggesting that there exist distinct pools of ceramides (76).

Ceramides in Metabolic Syndrome

Development of insulin resistance is a key step in the progression of metabolic syndrome, obesity, and diabetes, and it occurs when increasing levels of insulin are required to stimulate insulin-induced glucose uptake, particularly in myocytes and adipocytes. Studies have shown that plasma, adipose, and skeletal muscle ceramides are elevated in patients with obesity and type 2 diabetes mellitus (T2DM) (17, 77, 78), as well as in adipose, hepatic, skeletal muscle, and plasma samples from animal models of insulin resistance (17, 79–81). Moreover, ceramide levels in plasma, adipose, liver, and skeletal muscle negatively correlate with severity of insulin sensitivity in humans and in animal models (17, 77, 78, 81, 82). This is supported by the evidence that ceramides decrease the activity of Akt, which is a downstream effector of insulin signaling as well as of apoptosis and cell proliferation (24). Furthermore, adipose ceramide levels are higher in patients with increased hepatic lipid accumulation than in those with healthy livers (77). Reduced ceramide content prevents lipotoxicity induced by ceramide accumulation, preserving insulin and glucagon secretion (83). Ceramide bound to LDL also increases insulin resistance in skeletal myocytes and increases expression of inflammatory genes in macrophages, including IL-6 and TNFα (17). Clinical studies further prove that skeletal muscle ceramide content negatively correlates with insulin sensitivity (82, 84).

Relative amounts of particular ceramide species in insulin resistant states vary among sources. In one clinical study, skeletal muscle ceramides C16:0 and C18:3 were most strongly negatively correlated with insulin sensitivity (82). However, another clinical study showed that plasma C18:0 was most increased in obese T2DM participants over their healthy controls, but C24:0 was most abundant (78). More research is needed to clarify the relative impacts of specific ceramide species on insulin resistance.

Work by Summers and colleagues shows that ceramide production is an important factor in the development of insulin resistance through glucocorticoids and saturated fatty acids, but not through unsaturated fatty acids (16, 85). Glucocorticoids induce production of sphingolipid metabolites by promoting expression of the enzymes AC and glucosylceramide synthase (GCS), which produce ceramides through the salvage pathway. While Holland et al. did not measure or report an increase in acid sphingomyelinase activity, Cinque et al. demonstrated that dexamethasone, a glucocorticoid, induced thymocyte apoptosis by sequential activation of nuclear phosphoinositide specific phospholipase C-beta (PI-PLCβ) and acid sphingomyelinase (aSMase). Therefore, increased activity of both AC and aSMase, induced by glucocorticoids, could cause increased ceramide levels (86, 87). Saturated fatty acids promote ceramide accumulation in liver and skeletal muscle, as well as induction of insulin resistance (16). Conversely, reduction of ceramides has been shown to reverse the metabolic consequences of ceramide deposition in tissues. Induced overexpression of AC in mice, under the control of the adiponectin promoter, led to decreased ceramides in visceral and subcutaneous fat, liver, and serum, particularly ceramide species C16:0 and C18:0 (16, 88). After induction of AC expression, mice had reduced hepatic steatosis and improved insulin signaling and glucose metabolism, as shown by improvements in oral glucose tolerance tests and insulin tolerance tests, as well as increased glucose infusion rates and decreased hepatic glucose output during hyperinsulinemic-euglycemic clamps (88).

Ceramides' induction of mitochondrial dysfunction appears to play a role in the development of metabolic syndrome and its associated conditions. Several studies specifically implicated ceramides produced by the enzyme ceramide synthase 6 (CerS6). One group found that ob/ob mice, which congenitally lack leptin expression, have elevated CerS6 in their liver, BAT, and sWAT, but not gonadal white adipose tissue (gWAT) (89), and another found that C16:0 ceramide from CerS6 inhibits mitochondrial beta-oxidation in BAT and liver (90). Another study found that in mice with high-fat diet (HFD)–induced weight gain, only CerS6 increased in mitochondria and mitochondria-associated membranes, not ceramide synthase 5 (CerS5). CerS6 also interacts with mitochondrial fission factor (MFF) to promote mitochondrial fission, and knockout of either of these proteins normalizes mitochondrial structure altered by HFD (91). In ob/ob and DIO mice, but not in db/db mice, which lack the leptin receptor, Raichur et al. found that these mice have elevated C16:0 ceramide in liver and plasma. They also found that these ablation of CerS6 and subsequent lowering of C16:0 ceramide by 50% in ob/ob and DIO mice corrected high blood glucose and insulin resistance, and also caused 25% weight loss without a change in food intake (89).

Evidence also points to roles for other CerS in metabolic dysfunction. In diabetic cardiomyopathy, ceramide synthase 2 (CerS2)- and CerS5-derived ceramides, including VLC ceramides, led to increased mitophagy and insulin resistance (56). In DIO mice, ceramide synthase 1 (CerS1) and C18:0 are elevated in skeletal muscle, and knockout of CerS1 globally as well as specifically from skeletal muscle improves insulin sensitivity through increased fibroblast growth factor 21 (FGF21) in muscle (92). The role of ceramides in metabolic syndrome will be discussed further in later sections examining the interaction of ceramides with adiponectin and leptin, respectively.

Ceramides in Cardiovascular Disease

Ceramides have a well-established role in the development of CVDs, including atherosclerosis (15, 93), cardiomyopathy (15, 94, 95), heart failure (15, 96), myocardial infarction (14, 15), and stroke (14, 15). In particular, dihydroceramides, the precursors of ceramides in the *de novo* biosynthetic pathway of sphingolipids, are implicated in the development of CVD (15).

A substantial body of literature in rodent models of CVD indicates that ceramides are not only biomarkers of cardiovascular health, but also likely play a causative role in

metabolic and CVD (8, 29, 94, 97–100). Studies in rodent models reveal that pharmacological inhibition of ceramide synthesis prevents heart failure secondary to ischemic cardiomyopathy and also prevents ventricular remodeling, fibrosis, and macrophage infiltration following myocardial infarction (94, 97–99). Moreover, such ceramide-lowering interventions also resolve many conditions underlying CVDs, including dyslipidemia, insulin resistance, hypertension, atherosclerosis, and hepatic steatosis (10–12, 16, 101–104). Manipulations of the *de novo* ceramide synthesis pathway further suggest that certain ceramide species are deleterious, whereas others are benign or beneficial (90, 92, 105, 106). Those containing the C16 or C18 acyl chain (90, 92, 105) and a double bond (i.e., ceramides, not dihydroceramides) (101) in the sphingolipid backbone are particularly harmful. Finally, studies in rodents reveal that ceramide degradation is a primary means by which adiponectin receptors, which have ceramidase activity (1), exert their antidiabetic, cardioprotective, and insulin-sensitizing actions (1, 107, 108). Cumulatively, these data identify ceramides as some of the more toxic metabolites that accumulate in metabolic distress.

Other authors have reported the existence of a strong correlation between CVD and increased dihydroceramide levels. Both dihydroceramides and ceramides correlate with the release of the inflammatory cytokine interleukin 6 (IL-6), but only dihydroceramides correlates with macrophage inflammatory protein 1β (MIP-1β) release (13, 15). Studies have also shown that dihydroceramide levels are elevated in patients with rheumatoid arthritis (109), patients with left ventricular assist devices (110) and hypertensive rats (13–15, 41, 111, 112).

Elevated levels of dihydroceramides have been found in atherosclerotic plaques (13) as well as in models of brain hypoxia (111, 112). What role this increase in dihydroceramides plays in plaque stability is still debatable, since the extracellular addition of dihydroceramides to human aortic smooth muscle cells did not cause apoptosis, whereas addition of ceramides did (13). After stroke, a key component of tissue injury results from ischemia and subsequent reperfusion, during which ceramides increase in mitochondria and the ER due to *de novo* synthesis (58, 74). Myocardial biopsies from patients with heart failure reveal increased levels of ceramide content. A common treatment for heart failure is implantation of a left ventricular assist device, which compensates for defects due to heart failure, leading to a reduction in mechanical load on the cardiac tissue. This intervention both lowered myocardial ceramide levels and improved whole-body and cardiac insulin sensitivity (113).

A meta-analysis of three prospective clinical studies in Finland, Switzerland, and Norway that followed patients with CVD found that ceramide levels, particularly certain ceramide ratios, were significantly positively correlated with cardiovascular death, independent of other lipid markers, including cholesterol, and C-reactive protein (114). It also found that ratios of long-chain ceramide species (C18:1/C16:0 and C18:1/C18:0) were more strongly correlated with negative cardiovascular outcomes than ratios of VLC ceramide species (C18:1/C24:0) were (114). Furthermore, ratios of ceramide species were shown to be more significant than gross changes (114, 115). Laaksonen et al. found that the ratios of (C18:1/C24:0)/(C18:1/C16:0) and (C18:1/C24:0)/(C18:1/C16:0) negatively correlate with coronary heart disease (CHD) and mortality. Another more recent study found that serum sphingolipids are markers of coronary artery disease, independent of cholesterol (100), further solidifying the strong correlation between these species and negative cardiac outcomes. Thus, plasma ceramide levels can be used as clinical biomarkers to predict risk of cardiovascular death.

Ceramides are also strongly implicated in mitochondrial dysfunction found in CVDs. In cultured cardiomyocytes, C2 ceramide was found to alter mitochondrial membrane potential and cause mitochondrial fragmentation, leading to apoptosis (57). Increased ceramides and resulting apoptosis were also found in cardiomyocytes of mouse models of various types of cardiomyopathy (94, 116). A mouse model of type 1 diabetic cardiomyopathy showed increased ceramide accumulation in myocardium, as well as diastolic dysfunction (117). In a mouse model of type 2 diabetic cardiomyopathy, CerS2- and CerS5-derived ceramides, including VLC ceramides, led to increased mitophagy and lipotoxic cardiomyocyte hypertrophy (56). In rats, mitochondria from reperfused cardiac tissue contained higher ceramide levels than controls did, specifically in the detergent-resistant portion of the outer mitochondrial membrane (118). This correlation was also found in human subjects with CVD. Myocardial tissue from heart failure patients revealed increased ceramide content as well as reduced PGC-1α expression, which indicates impaired oxidative respiration in mitochondria (113). Across species, from mice to rats to humans, ceramides are strongly linked to CVD through mitochondrial dysfunction.

Interestingly, specific diets may even play a role in ceramide-induced mitochondrial function and cardiomyopathy. Mice that developed diabetic cardiomyopathy due to HFD made from milk fat, rather than from lard, had higher levels of C14 ceramide in cardiac tissue. These mice on milk fat-based HFD also developed cardiac dysfunction more quickly than mice on a lard-based HFD. Treatment with myriocin reversed the previously abnormal electrocardiogram parameters in mice fed a milk fat-based HFD, and it also prevented development of left ventricular and cardiomyocyte hypertrophy (119). Cumulatively, these studies of animal models and patients strongly implicate ceramides in the development and maintenance of CVD.

ADIPONECTIN AND CERAMIDES

Adiponectin is a prevalent adipokine that acts on many tissues and organs, including the heart, liver, and kidney (120–122). Its actions are correlated with better metabolic health, resulting in improved insulin sensitivity, reduced inflammation, and enhanced cell survival (122, 123). In congenitally leptin-deficient ob/ob mice, which have severe diabetes and metabolic dysfunction (124), adiponectin overexpression was able to reverse their phenotype, restoring normal glucose and insulin levels (125). This dramatic shift underscores the powerful,

beneficial role of adiponectin in metabolic health. It has also been shown to improve both alcoholic and non-alcoholic hepatic steatosis (126). Adiponectin promotes expansion of adipose tissue in a benign manner, retaining metabolic health by preventing aberrant lipid accumulation, as occurs in the liver in hepatic steatosis (125, 127, 128).

Ceramidase Activity of Adiponectin Receptors

After the discovery of adiponectin, the most common adiponectin receptors, AdipoR1 and AdipoR2, were cloned (129). They were later classified as part of the progestin and adiponectin receptor (PAQR) family (130). A fungal member of the PAQR family, Izh2p, was found to be inhibited by drugs affecting sphingolipid metabolism, including myriocin, which inhibits SPT and D-erythro-MAPP, which inhibits ACER (131). Later, discovery of the protein crystal structure and *in vitro* work established that AdipoRs are seven-transmembrane receptors with topology opposite that of GPCRs. These receptors have basal ceramidase activity that is increased by adiponectin binding. They have been shown to bind many anionic, but not neutral, phospholipids and sphingolipids, including species such as ceramide-1-phosphate and dihydroceramide-1-phosphate (132). Moreover, AdipoR2 was purified bound to a molecule of C18 free fatty acid, and a ceramide binding pose was identified by computer simulations. Using fluorophore-labeled C18:0, it was confirmed by fluorescence spectroscopy that ceramide binds AdipoR2 (1, 133). Adiponectin receptors can hydrolyze short ceramides and long ceramides, but C18:0 is hydrolyzed most efficiently. Adiponectin binding increases the basal AdipoR2 ceramidase activity 20-fold as shown by ultra-performance liquid chromatography mass spectrometry (UPLC-MS) (1). While this enzymatic activity is slow, other work has shown that intermembrane enzymes tend to be slower than their soluble counterparts (134). Due to the ceramidase activity of its receptors, adiponectin is hypothesized to have an important role in ceramide metabolism.

Adiponectin receptors have long been a potential target for amelioration of obesity-associated metabolic dysfunction (135), and its intrinsic ceramidase activity makes it a pharmacologic target for therapeutic lowering of ceramides as well. Kadowaki and colleagues developed a candidate drug for this purpose: an orally administered small-molecule agonist of adiponectin receptors 1 and 2 called AdipoRon that binds to both adiponectin receptors at low micromolar concentration (136). This compound has been shown to activate the ceramidase activity of adiponectin receptors, indicating its potential as a ceramide-lowering compound (137, 138).

FGF21-Adiponectin-Ceramide Axis

Another protein, FGF21, has been implicated in the signaling of adiponectin and ceramides. This protein is known to have antidiabetic properties, as it causes weight loss and improved insulin sensitivity (139). Our group, as well as Lin and colleagues, found that adiponectin secretion in mice is increased by FGF21 (107, 140). We also found that increased FGF21, in mice and cell culture, lowered circulating ceramides, and that mice lacking FGF21 have impaired adiponectin production and increased circulating ceramides. Despite similar circulating FGF21 levels, mice lacking adiponectin and leptin did not respond to FGF21 as compared to ob/ob mice, which significantly lowered hepatic ceramides in response to FGF21. Furthermore, ceramides were increased in DIO mice (107). In another mouse model, an adipocyte-specific knockout of a serine palmitoyl transferase (SPT) subunit resulted in lower ceramide levels and increased FGF21, consistent with this proposed axis (59). In yet another model, depletion of ceramides from suppression of CerS1 globally and in skeletal muscle resulted in improved skeletal muscle insulin sensitivity that was mediated through FGF21 (92).

CLINICAL EFFECTS OF CERAMIDE-INDUCED DYSFUNCTION AND ADIPONECTIN

Insulin Resistance

In female type 2 diabetic children and adolescents, as well as healthy controls, plasma adiponectin and plasma ceramide levels are inversely correlated. These obese diabetic patients had increased levels of ceramide species, including C18:0, C20:0, C22:0, and C24:1, as compared to their lean, non-diabetic counterparts (141). These findings were replicated in adult obese diabetic patients as compared to obese non-diabetic adult controls. Controlling for obesity, diagnosis of type 2 diabetes correlated with increased sphingolipids, dihydroceramides (total, dhC16:0, and dhC24:0), and ceramides (total and C16:0) (59). Dihydroceramides were elevated in patients up to nine years prior to their diabetes diagnosis, demonstrating the value of dihydroceramides as an early sign of metabolic dysfunction (142).

One study analyzed the metabolic health of women with polycystic ovarian syndrome (PCOS), a common gynecological condition linked to metabolic syndrome and insulin resistance (143). In this study, women with PCOS had 25% reduced whole-body insulin sensitivity and 40% lower circulating adiponectin levels. Importantly, their skeletal muscle showed a 300% increase in ceramide levels, further underlining the inverse relationship between circulating adiponectin and ceramide levels (144).

Cardiovascular Disease

Numerous clinical studies have found correlation between ceramides and CVD. The Dallas Heart Study found that in plasma, adiponectin was inversely correlated with saturated fatty acid chain ceramides only, not unsaturated species. Furthermore, patients with unfavorable lipid profiles were more likely to have short chain ceramides, both saturated and monounsaturated. Importantly, total cholesterol and LDL, two long-accepted metrics of cardiovascular and metabolic health, were strongly positively correlated with increased circulating ceramides (145). In fact, one study found that circulating ceramides may be an early indicator of cardiovascular changes in healthy adults of all ages, and that their plasma levels were

inversely correlated with adiponectin levels (115). They are also inversely correlated with the 6-minute walk test, a clinical diagnostic criterion that correlates with aerobic capacity (146). Another study showed that accumulation of ceramides led to arteriolar dilation by inflammatory mediators, while overexpression of ceramidase, exogenous adiponectin, or exogenous sphingosine-1-phosphate (a byproduct of ceramide degradation) led to healthy arteriolar dilation (147). This is important because dysfunction in small vessels often causes or contributes to chronic diseases and their complications, including diabetes mellitus and coronary artery disease (148–151).

PRECLINICAL STUDIES OF ADIPONECTIN AND CERAMIDES

The clinical studies detailed above collectively show that adiponectin and ceramides levels are negatively correlated, and they strongly suggest a causative link between adiponectin signaling and ceramide metabolism. However, they are unable to probe mechanisms more directly. To this end, studies conducted in cell culture and animal models provide greater insight into the relationship between ceramides and this adipokine.

Adiponectin and Ceramides

This hypothesized relationship has been investigated with the use of transgenic mice and cells (152). In DIO and ob/ob mice, ceramides are elevated (107) and can be lowered by administration of exogenous adiponectin (108). When adiponectin was overexpressed in adipocytes of DIO mice, the animals remained insulin sensitive with low plasma ceramide levels, despite their increased adiposity. With this overexpression, mice even remained euglycemic when pancreatic beta cells were killed, impairing insulin secretion (108). Even indirect elevation of circulating adiponectin led to increased sphingosine and sphinganine in the liver, demonstrating increased ceramidase activity (153). Conversely, elimination of adiponectin production from adipocytes led to increased ceramide deposition in the liver (108). Specifically, ceramide species C16:0, C18:0, C24:0, and C24:1 were elevated, while ceramide breakdown products sphingosine and sphingosine-1-phosphate (S1P) were lowered (154). Blocking ceramide production with SPT inhibitor myriocin in DIO mice led to increased levels of circulating high molecular weight adiponectin (59). This inverse relationship between adiponectin and ceramides has also been shown in rats (155, 156) and dolphins.

Interestingly, a somewhat different relationship between adiponectin and ceramides is seen in the skin. In these cells, adiponectin plays a beneficial role, aiding in cell differentiation, wound healing, and cell senescence (18, 157–159). Hong et al. found that in response to adiponectin administration, ceramide levels, as well as sphingosine and S1P levels, increased in human epidermal keratinocytes. The authors hypothesized that ceramide levels are elevated either because adiponectin administration also led to increases in overall lipid synthesis through activation of nuclear hormone receptors, or because of inhibition of catabolic action by other ceramidases, which was not assessed (19).

Adiponectin Receptors and Ceramides

As previously mentioned, adiponectin receptors 1 and 2 are thought to have ceramidase activity. AdipoR1 and AdipoR2 overexpression in adipocytes in gonadal, mesenteric, and subcutaneous adipose tissue (gWAT, mWAT, and sWAT, respectively) increased ceramidase activity (137). Conversely, cultured cells lacking these receptors had impaired ceramidase activity and increased lipid-induced cell death that could not be rescued by adiponectin administration (108). When these receptors are overexpressed in the liver, ceramidase activity increases (108). This hepatic overexpression also leads to lower ceramide species in mWAT and sWAT, indicating communication between these organs (137). In contrast, in their absence, mouse embryonic fibroblasts have impaired ceramidase activity (108). A synthetic adiponectin receptor agonist was developed that can stimulate AdipoR effects in the absence of adiponectin (136), and its injection into wildtype mice showed a comparable increase in ceramidase activity as compared to mice overexpressing AdipoR1 or AdipoR2 in hepatocytes (137).

Exogenous adiponectin administration is able to reverse the diabetic phenotype of ob/ob mice (125). Furthermore, reduction of ceramides has been shown to increase insulin sensitivity. However, the question remained whether specifically the ceramidase action of adiponectin receptors is required to improve insulin sensitivity in leptin-deficient mice. Holland et al. found that overexpression of AdipoR2, but not AC, was able to rescue glucose homeostasis in leptin-deficient mice. Adiponectin receptors lower a larger variety of ceramide species, including deoxyceramides, which are possibly more cytotoxic because of the limited pathways for metabolizing these alanine (rather than serine) derived sphingolipids, than does AC (137).

LEPTIN AND CERAMIDES

Leptin is another major adipokine that plays a prominent role in feeding and adipose homeostasis, as well as in reproduction, maintenance of bone mass, immunity, etc. (160, 161). Its release from adipose conveys a satiety signal to the hypothalamus, leading to lower food intake (162). The diabetic phenotype of ob/ob mice is ameliorated by administration of exogenous leptin. However, mice lacking the leptin receptor, known as db/db mice, do not respond to leptin as they cannot detect it (124, 163).

Leptin Resistance and Ceramides

Leptin resistance is most extreme in the db/db mouse, but in mice and humans, it occurs in DIO as well (163–165). Hyperleptinemia has been shown to be necessary for the development of leptin resistance (166). These high levels of leptin have been shown to increase expression of SOCS3 in the hypothalamus, which blocks transduction of leptin signaling, causing leptin resistance (167, 168). Recently, our group has shown that reversing hyperleptinemia by reducing circulating

leptin via genetic and pharmacological means restores hypothalamic sensitivity to leptin, leading to reduced weight gain and increased insulin sensitivity. This intervention leads to increased expression of LepR and POMC in the hypothalamus and subsequent improvement in metabolic phenotype (169, 170). Other research indicates that ceramide levels may play a role in modulating leptin sensitivity as well.

Several studies have directly linked ceramides to leptin resistance. One study showed that administration of myriocin, an inhibitor of *de novo* ceramide synthesis, to obese mice (both DIO and ob/ob) led to reduced levels of leptin expression in epididymal fat. Myriocin treatment had no effect on SOCS3 expression in leptin-deficient ob/ob mice, but it decreased SOCS3 expression in DIO mice. Together, these results indicate that lowering ceramide levels increases leptin sensitivity and that this increased sensitivity cannot occur in the absence of leptin signaling. Furthermore, administration of ceramides to 3T3-L1 adipocytes led to increased SOCS3 expression, further indicating that elevated ceramides induce leptin resistance (60). A recent study replicated this in rats, showing that administration of ceramides induced leptin resistance. Conversely, downregulation of acid sphingomyelinase, which produces ceramide, ameliorated leptin resistance in rats, as indicated by increased expression of leptin receptor and decreased expression of SOCS3 (171).

Peripheral Effects of Leptin and Ceramides

Several studies indicate that leptin can reduce ceramide levels, but its ability to do so may depend on leptin sensitivity. One study in Sprague-Dawley rats found that exercise-induced lowering of muscle ceramide content, which was initially elevated from HFD, precede any improvement in leptin or insulin sensitivity in skeletal muscle (172). From these data, the authors hypothesize that ceramides may be an important determinant of insulin sensitivity, and the same conclusion could be made regarding ceramides' impact on leptin sensitivity as well. In another rat model, Wistar rats, leptin caused decreased total ceramide levels in gWAT, as well as decreased expression of genes encoding several ceramide synthesis enzymes, including SPT, LASS2, LASS4, SMPD1, and SMPD2 (173). In Zucker diabetic fatty (ZDF) rats, which have hyperleptinemia and leptin resistance, administration of leptin failed to block upregulation of SPT, resulting in increased ceramide content. However, with increased leptin sensitivity (conferred by delivery of leptin receptor cDNA to islet cells), leptin was able to block SPT mRNA expression (174). This indicates a relationship between leptin and ceramides that is dependent upon leptin sensitivity.

Just as leptin has been shown to reduce ceramides, some studies show that ceramides have a similar, reciprocal regulatory effect on leptin. Treatment of 3T3-L1 pre-adipocytes with ceramide-1-phosphate decreases leptin secretion (175). In DIO mice, which have hyperleptinemia and resultant leptin resistance, treatment with myriocin lead to lower circulating ceramides and decreased gene expression of leptin and SOCS3 in gWAT (60). Reduction of leptin levels has been shown to increase leptin sensitivity, and SOCS3 is known mediator of leptin resistance (167–169, 176). Therefore, reduced expression of leptin and SOCS3 secondary to ceramide reduction may enable increased leptin sensitivity.

However, the relationship between ceramides and leptin is still not entirely clear, as some studies have shown contradictory data. In both the DIO and ob/ob mouse models of obesity, which have high and nonexistent circulating levels of leptin respectively, total plasma and hepatic ceramide levels are increased (108, 177). These studies indicate that regardless of leptin levels, ceramides are increased in models of obesity and diabetes. In gonadal white adipose (gWAT) of leptin-deficient ob/ob mice, while C14 ceramide was increased, total ceramide levels as well as those of C18:1, C24:0, and C24:1 were decreased, showing that ceramides do not respond uniformly to the absence of leptin (177). In adipocytes, knocking out a subunit of SPT (leading to lowered adipose and circulating ceramides), led to lower levels of circulating leptin (59). Therefore, further research is necessary to clarify the relationship between leptin and ceramides, in states of both leptin sensitivity and leptin resistance.

Cardiovascular Effects of Leptin and Ceramides

Ceramide accumulation and leptin resistance can induce dysfunction in cardiovascular tissue as well. Leptin levels correlate with cardiovascular dysfunction in male patients, independent of degree of obesity and hypertension (178). One possible explanation of this correlation is demonstrated by one study, which showed that cardiac contractile dysfunction was exacerbated by accumulation of ceramides in cardiac muscle, which potentiated the contractile effects of leptin (179). Another study showed that decreasing ceramides in rat aortic endothelial cells, through downregulation of acid sphingomyelinase, improved leptin sensitivity (171). These studies suggest that ceramides contribute to leptin resistance in cardiovascular tissues.

Central Effects of Leptin and Ceramides

Recent studies have shown that ceramide effects in the hypothalamus contribute to leptin resistance. It has been shown that palmitate, a lipid long known to cross the blood-brain barrier (180) and also induce inflammation (181) does so in the hypothalamus partially through ceramide synthesis (182). Mice subjected to intravenous emulsions of saturated fatty acids had ceramides accumulate in the hypothalamus, indicating a hypothalamic role for ceramide detection and regulation (108). Infusion of a ceramide analog directly into the hypothalamic arcuate nucleus, a brain region that regulates many neuroendocrine functions including feeding (183), of Sprague-Dawley rats suppressed leptin-induced anorectic effects, indicating an inverse relationship between ceramide levels and leptin sensitivity. Physiologic data supports this relationship as well, as fasting (a state of low leptin) increases ceramides, and refeeding (high leptin) decreases ceramides in the arcuate nucleus (2). Conversely, lowering hypothalamic ceramide levels through administration of myriocin increased leptin-induced anorectic effects. In the arcuate nucleus, mRNA expression of SPT, the enzyme driving *de novo* ceramide synthesis, was comparable to

expression of AgRP and POMC, which are both crucial to the arcuate's role in food intake (2). Such significant levels of expression of a key ceramide synthesis enzyme points to an important role for ceramides in this region.

Gao et al. propose that mechanistically, leptin reduces ceramide content through modulation of malonyl-CoA and carnitine palmitoyltransferase 1c (CPT-1c). They hypothesize that leptin induces its anorectic effects in part by increasing malonyl-CoA, which in turn inhibits CPT-1c, leading to decreased *de novo* ceramide synthesis. CPT-1c may act as a transporter of palmitoyl-CoA into the ER, enabling its use as a substrate for ceramide synthesis. Therefore, inhibition of CPT-1c through leptin-induced increases in malonyl-CoA would in turn lower ceramide levels (2). This group found further evidence of ceramide regulation by CPT-1c in hippocampal dendrites, bolstering evidence for this relationship between CPT-1c and ceramides (184).

Another study found that in Zucker rats, a strain known to have leptin resistance, an accumulation of ceramides in the mediobasal hypothalamus led to ER stress and lipotoxicity (185). ER stress has previously been shown to cause leptin resistance (3–5). The authors postulated that ceramides induce ER stress through prevention of proper protein folding. Relief of this ceramide-induced ER stress led to subsequent leptin sensitization, indicating bidirectional control of leptin sensitivity by ceramides (6). However, another study found that central administration of leptin caused decreased ceramide levels in the plasma membrane and Golgi, but not the ER of Wistar rat eWAT (173). Therefore, it is unclear whether ER stress is the true mediator of ceramides' effect on leptin sensitivity.

SUMMARY AND FUTURE DIRECTIONS

Over the past few decades, studies have begun to show the significant impact of ceramides and adipokines on metabolic health, particularly in diabetes and CVD. Higher levels of ceramides correlate with low levels of adiponectin as well as with leptin and insulin resistance, indicating that these sphingolipids contribute to dysfunction in cardiovascular, hepatic, and adipose tissue. Cumulatively, these studies suggest that monitoring ceramide levels could allow better assessment of cardiovascular and metabolic disease progression and/or severity, and that ceramides are a possible target for future therapeutic intervention in cardiometabolic pathologies.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

FUNDING

The authors are supported by US National Institutes of Health (NIH) grants R01-DK55758, RC2-DK118620, P01-DK088761, R01-DK099110 and P01-AG051459 (PS). PS was also supported by an unrestricted research grant from the Novo Nordisk Foundation.

REFERENCES

1. Vasiliauskaité-Brooks I, Sounier R, Rochaix P, Bellot G, Fortier M, Hoh F, et al. Structural insights into adiponectin receptors suggest ceramidase activity. *Nature* (2017) 544(7648):120–3. doi: 10.1038/nature21714
2. Gao S, Zhu G, Gao X, Wu D, Carrasco P, Casals N, et al. Important roles of brain-specific carnitine palmitoyltransferase and ceramide metabolism in leptin hypothalamic control of feeding. *Proc Natl Acad Sci USA* (2011) 108 (23):9691–6. doi: 10.1073/pnas.1103267108
3. Hosoi T, Sasaki M, Miyahara T, Hashimoto C, Matsuo S, Yoshii M, et al. Endoplasmic reticulum stress induces leptin resistance. *Mol Pharmacol* (2008) 74(6):1610–9. doi: 10.1124/mol.108.050070
4. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, et al. Endoplasmic Reticulum Stress Plays a Central Role in Development of Leptin Resistance. *Cell Metab* (2009) 9(1):35–51. doi: 10.1016/j.cmet.2008.12.004
5. Won JC, Jang P-G, Namkoong C, Koh EH, Kim SK, Park J-Y, et al. Central Administration of an Endoplasmic Reticulum Stress Inducer Inhibits the Anorexigenic Effects of Leptin and Insulin. *Obesity* (2009) 17(10):1861–5. doi: 10.1038/oby.2009.194
6. Contreras C, López M. Ceramide sensing in the hippocampus: The lipostatic theory and Ockham's razor. *Mol Metab* (2014) 3(2):90–1. doi: 10.1016/j.molmet.2013.12.004
7. Kogot-Levin A, Saada A. Ceramide and the mitochondrial respiratory chain. *Biochimie* (2014) 100(1):88–94. doi: 10.1016/j.biochi.2013.07.027
8. Holland WL, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: New insights from in vivo manipulation of sphingolipid metabolism. *Endocr Rev* (2008) 29(4):381–402. doi: 10.1210/er.2007-0025
9. Xia JY, Morley TS, Scherer PE. The adipokine/ceramide axis: Key aspects of insulin sensitization. *Biochimie* (2014) 96(1):130–9. doi: 10.1016/j.biochi.2013.08.013
10. Glaros EN, Kim WS, Quinn CM, Jessup W, Rye KA, Garner B. Myriocin slows the progression of established atherosclerotic lesions in apolipoprotein E gene knockout mice. *J Lipid Res* (2008) 49(2):324–31. doi: 10.1194/jlr.M700261-JLR200
11. Glaros EN, Kim WS, Wu BJ, Suarna C, Quinn CM, Rye K-A, et al. Inhibition of atherosclerosis by the serine palmitoyl transferase inhibitor myriocin is associated with reduced plasma glycosphingolipid concentration. *Biochem Pharmacol* (2007) 73(9):1340–6. doi: 10.1016/j.bcp.2006.12.023
12. Park TS, Rosebury W, Kindt EK, Kowala MC, Panek RL. Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic ApoE-deficient mice. *Pharmacol Res* (2008) 58(1):45–51. doi: 10.1016/j.phrs.2008.06.005
13. Edsfeldt A, Dunér P, Ståhlman M, Mollet IG, Asciutto G, Grufman H, et al. Sphingolipids contribute to human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol* (2016) 36(6):1132–40. doi: 10.1161/ATVBAHA.116.305675
14. Meeusen JW, Donato LJ, Bryant SC, Baudhuin LM, Berger PB, Jaffe AS. Plasma Ceramides. *Arterioscler Thromb Vasc Biol* (2018) 38(8):1933–9. doi: 10.1161/ATVBAHA.118.311199
15. Magaye RR, Savira F, Hua Y, Kelly DJ, Reid C, Flynn B, et al. The role of dihydrosphingolipids in disease. *Cell Mol Life Sci* (2019) 76(6):1107–34. doi: 10.1007/s00018-018-2984-8
16. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of Ceramide Synthesis Ameliorates Glucocorticoid-, Saturated-Fat-, and Obesity-Induced Insulin Resistance. *Cell Metab* (2007) 5(3):167–79. doi: 10.1016/j.cmet.2007.01.002
17. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, et al. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes* (2013) 62 (2):401–10. doi: 10.2337/db12-0686
18. Kawai K, Kageyama A, Tsumano T, Nishimoto S, Fukuda K, Yokoyama S, et al. Effects of adiponectin on growth and differentiation of human

keratinocytes-Implication of impaired wound healing in diabetes. *Biochem Biophys Res Commun* (2008) 374(2):269–73. doi: 10.1016/j.bbrc.2008.07.045
19. Hong SP, Seo HS, Shin K-O, Park K, Park BC, Kim MH, et al. Adiponectin Enhances Human Keratinocyte Lipid Synthesis via SIRT1 and Nuclear Hormone Receptor Signaling. *J Invest Dermatol* (2019) 139(3):573–82. doi: 10.1016/j.jid.2018.08.032
20. Pruett ST, Bushnev A, Hagedorn K, Adiga M, Haynes CA, Sullards MC, et al. Biodiversity of sphingoid bases ("sphingosines") and related amino alcohols. *J Lipid Res* (2008) 49(8):1621–39. doi: 10.1194/jlr.R800012-JLR200
21. Welsch CA, Roth LWA, Goetschy JF, Movva NR. Genetic, biochemical, and transcriptional responses of Saccharomyces cerevisiae to the novel immunomodulator FTY720 largely mimic those of the natural sphingolipid phytosphingosine. *J Biol Chem* (2004) 279(35):36720–31. doi: 10.1074/jbc.M406179200
22. Pralhada Rao R, Vaidyanathan N, Rengasamy M, Mammen Oommen A, Somaiya N, Jagannath MR. Sphingolipid Metabolic Pathway: An Overview of Major Roles Played in Human Diseases. *J Lipids* (2013) 2013:1–12. doi: 10.1155/2013/178910
23. Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. *Sci (80-).* (1993) 259(5102):1769–71. doi: 10.1126/science.8456305
24. Zhou H, Summers SA, Birnbaum MJ, Pittman RN. Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. *J Biol Chem* (1998) 273(26):16568–75. doi: 10.1074/jbc.273.26.16568
25. Nganga R, Oleinik N, Ogretmen B. Mechanisms of Ceramide-Dependent Cancer Cell Death. *Adv Cancer Res* (2018) 140:1–25. doi: 10.1016/bs.acr.2018.04.007
26. Gomez-Muñoz A, Presa N, Gomez-Larrauri A, Rivera IG, Trueba M, Ordoñez M. Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. *Prog Lipid Res* (2016) 61:51–62. doi: 10.1016/j.plipres.2015.09.002
27. Dinoff A, Herrmann N, Lanctôt KL. Ceramides and depression: A systematic review. *J Affect Disord* (2017) 213:35–43. doi: 10.1016/j.jad.2017.02.008
28. Wang G, Bieberich E. Sphingolipids in neurodegeneration (with focus on ceramide and S1P). *Adv Biol Regul* (2018) 70:51–64. doi: 10.1016/j.jbior.2018.09.013
29. Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol Metab* (2015) 26(10):538–50. doi: 10.1016/j.tem.2015.07.006
30. Aburasayn H, Al Batran R, Ussher JR. Targeting ceramide metabolism in obesity. *Am J Physiol Metab* (2016) 311(2):E423–35. doi: 10.1152/ajpendo.00133.2016
31. Fang Z, Pyne S, Pyne NJ. Ceramide and sphingosine 1-phosphate in adipose dysfunction. *Prog Lipid Res* (2019) 74:145–59. doi: 10.1016/j.plipres.2019.04.001
32. Cogolludo A, Villamor E, Perez-Vizcaino F, Moreno L. Ceramide and Regulation of Vascular Tone. *Int J Mol Sci* (2019) 20(2):411. doi: 10.3390/ijms20020411
33. Apostolopoulou M, Gordillo R, Koliaki C, Gancheva S, Jelenik T, De Filippo E, et al. Specific Hepatic Sphingolipids Relate to Insulin Resistance, Oxidative Stress, and Inflammation in Nonalcoholic Steatohepatitis. *Diabetes Care* (2018) 41(June):1235–43. doi: 10.2337/dc17-1318
34. Gorden DL, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, et al. Biomarkers of NAFLD progression: A lipidomics approach to an epidemic. *J Lipid Res* (2015) 56(3):722–36. doi: 10.1194/jlr.P056002
35. Hales CM, Carroll MD, Fryar CD, Ogden CL. *Prevalence of Obesity Among Adults and Youth: United States, 2015-2016* (2017). Available at: https://www.cdc.gov/nchs/data/databriefs/db288.pdf (Accessed October 10, 2019).
36. World Health Organization G. *Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016* (2018). Available at: https://www.who.int/healthinfo/global_burden_disease/estimates/en/ (Accessed May 12, 2020).
37. Castro BM, Prieto M, Silva LC. Ceramide: A simple sphingolipid with unique biophysical properties. *Prog Lipid Res* (2014) 54(1):53–67. doi: 10.1016/j.plipres.2014.01.004
38. Hannun YA, Obeid LM. Many ceramides. *J Biol Chem* (2011) 286 (32):27855–62. doi: 10.1074/jbc.R111.254359
39. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat Rev Mol Cell Biol* (2008) 9(2):139–50. doi: 10.1038/nrm2329
40. Hernández-Corbacho MJ, Salama MF, Canals D, Senkal CE, Obeid LM. Sphingolipids in mitochondria. *Biochim Biophys Acta - Mol Cell Biol Lipids* (2017) 1862(1):56–68. doi: 10.1016/j.bbalip.2016.09.019
41. Liu A, Chu YJ, Wang X, Yu R, Jiang H, Li Y, et al. Serum Metabolomics Study Based on LC-MS and Antihypertensive Effect of Uncaria on Spontaneously Hypertensive Rats. *Evidence-Based Complement Altern Med* (2018) 2018:1–11. doi: 10.1155/2018/9281946
42. Levy M, Futerman AH. Mammalian ceramide synthases. *IUBMB Life* (2010) 62(5):347–56. doi: 10.1002/iub.319
43. López-Montero I, Rodriguez N, Cribier S, Pohl A, Vélez M, Devaux PF. Rapid transbilayer movement of ceramides in phospholipid vesicles and in human erythrocytes. *J Biol Chem* (2005) 280(27):25811–9. doi: 10.1074/jbc.M412052200
44. Simon CG, Holloway PW, Gear ARL. Exchange of C16-ceramide between phospholipid vesicles. *Biochemistry* (1999) 38(44):14676–82. doi: 10.1021/bi991537w
45. Tomassini B, Testi R. Mitochondria as sensors of sphingolipids. *Biochimie* (2002) 84(2-3):123–9. doi: 10.1016/S0300-9084(02)01377-9
46. Lightle S, Tosheva R, Lee A, Queen-Baker J, Boyanovsky B, Shedlofsky S, et al. Elevation of ceramide in serum lipoproteins during acute phase response in humans and mice: Role of serine-palmitoyl transferase. *Arch Biochem Biophys* (2003) 419(2):120–8. doi: 10.1016/j.abb.2003.08.031
47. Wiesner P, Leidl K, Boettcher A, Schmitz G, Liebisch G. Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J Lipid Res* (2009) 50(3):574–85. doi: 10.1194/jlr.D800028-JLR200
48. Crewe C, Joffin N, Rutkowski JM, Kim M, Zhang F, Towler DA, et al. Endothelial-to-Adipocyte Extracellular Vesicle Axis Governed by Metabolic State. *An Cell* (2018) 175(3):695–708.e13. doi: 10.1016/j.cell.2018.09.005
49. Bionda C, Portoukalian J, Schmitt D, Rodriguez-Lafrasse C, Ardail D. Subcellular compartmentalization of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? *Biochem J* (2004) 382(2):527–33. doi: 10.1042/BJ20031819
50. Novgorodov SA, Chudakova DA, Wheeler BW, Biewlawski J, Kindy MS, Obeid LM, et al. Developmentally regulated ceramide synthase 6 increases mitochondrial Ca2+ loading capacity and promotes apoptosis. *J Biol Chem* (2011) 286(6):4644–58. doi: 10.1074/jbc.M110.164392
51. Di Paola M, Cocco T, Lorusso M. Ceramide interaction with the respiratory chain of heart mitochondria. *Biochemistry* (2000) 39(22):6660–8. doi: 10.1021/bi9924415
52. Colombini M. Ceramide channels and mitochondrial outer membrane permeability. *J Bioenerg Biomembr* (2017) 49(1):57–64. doi: 10.1007/s10863-016-9646-z
53. Ueda N. Ceramide-Induced Apoptosis in Renal Tubular Cells: A Role of Mitochondria and Sphingosine-1-Phoshate. *Int J Mol Sci* (2015) 16 (12):5076–124. doi: 10.3390/ijms16035076
54. Gudz TI, Tserng KY, Hoppel CL. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. *J Biol Chem* (1997) 272(39):24154–8. doi: 10.1074/jbc.272.39.24154
55. Ganesan V, Perera MN. Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis* (2010) 15:553–62. doi: 10.1007/s10495-009-0449-0
56. Law BA, Liao X, Moore KS, Southard A, Roddy P, Ji R, et al. Lipotoxic very-long-chain ceramides cause mitochondrial dysfunction, oxidative stress, and cell death in cardiomyocytes. *FASEB J* (2018) 32(3):1403–16. doi: 10.1096/fj.201700300R
57. Parra V, Eisner V, Chiong M, Criollo A, Moraga F, Garcia A, et al. Changes in mitochondrial dynamics during ceramide-induced cardiomyocyte early apoptosis. *Cardiovasc Res* (2008) 77(2):387–97. doi: 10.1093/cvr/cvm029
58. Yu J, Novgorodov SA, Chudakova D, Zhu H, Bielawska A, Bielawski J, et al. JNK3 signaling pathway activates ceramide synthase leading to

mitochondrial dysfunction. *J Biol Chem* (2007) 282(35):25940–9. doi: 10.1074/jbc.M701812200
59. Chaurasia B, Kaddai VA, Lancaster GI, Henstridge DC, Sriram S, Galam DLA, et al. Adipocyte Ceramides Regulate Subcutaneous Adipose Browning, Inflammation, and Metabolism. *Cell Metab* (2016) 24(6):820–34. doi: 10.1016/J.CMET.2016.10.002
60. Yang G, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol - Endocrinol Metab* (2009) 297(1): E211–24. doi: 10.1152/ajpendo.91014.2008
61. Yambire KF, Fernandez-Mosquera L, Steinfeld R, Mühle C, Ikonen E, Milosevic I, et al. Mitochondrial biogenesis is transcriptionally repressed in lysosomal lipid storage diseases. *Elife* (2019) 8:1–29. doi: 10.7554/eLife.39598
62. Fugio LB, Coeli-Lacchini FB, Leopoldino AM. Sphingolipids and Mitochondrial Dynamic. *Cells* (2020) 9(3):581. doi: 10.3390/cells9030581
63. Teixeira V, Medeiros TC, Vilaça R, Pereira AT, Chaves SR, Côrte-Real M, et al. Ceramide signalling impinges on Sit4p and Hog1p to promote mitochondrial fission and mitophagy in Isc1p-deficient cells. *Cell Signal* (2015) 27(9):1840–9. doi: 10.1016/j.cellsig.2015.06.001
64. Mizumura K, Justice MJ, Schweitzer KS, Krishnan S, Bronova I, Berdyshev EV, et al. Sphingolipid regulation of lung epithelial cell mitophagy and necroptosis during cigarette smoke exposure. *FASEB J* (2018) 32(4):1880–90. doi: 10.1096/fj.201700571R
65. Panda PK, Naik PP, Meher BR, Das DN, Mukhopadhyay S, Praharaj PP, et al. PUMA dependent mitophagy by Abrus agglutinin contributes to apoptosis through ceramide generation. *Biochim Biophys Acta - Mol Cell Res* (2018) 1865(3):480–95. doi: 10.1016/j.bbamcr.2017.12.002
66. Dany M, Gencer S, Nganga R, Thomas RJ, Oleinik N, Baron KD, et al. Targeting FLT3-ITD signaling mediates ceramide-dependent mitophagy and attenuates drug resistance in AML. *Blood* (2016) 128(15):1944–58. doi: 10.1038/nrc.2017.96
67. Colombini M. Ceramide channels and their role in mitochondria-mediated apoptosis. *Biochim Biophys Acta - Bioenerg* (2010) 1797(6-7):1239–44. doi: 10.1016/j.bbabio.2010.01.021
68. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, et al. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J Exp Med* (1995) 182(2):367–77. doi: 10.1084/jem.182.2.367
69. Petit PX, Gendron MC, Schrantz N, Métiviers D, Kroemer G, Maciorowska Z, et al. Oxidation of pyridine nucleotides during Fas- and ceramide-induced apoptosis in Jurkat cells: correlation with changes in mitochondria, glutathione depletion, intracellular acidification and caspase 3 activation. *Biochem J* (2001) 353(2):357–67. doi: 10.1042/0264-6021:3530357
70. Zhang I, Alter N, Reed JC, Borner C, Obeid LM, Hannun YA. Bcl-2 interrupts the ceramide-mediated pathway of cell death. *Proc Natl Acad Sci U S A* (1996) 93(11):5325–8. doi: 10.1073/pnas.93.11.5325
71. Geley S, Hartmann BL, Kofler R. Ceramides induce a form of apoptosis in human acute lymphoblastic leukemia cells that is inhibited by Bcl-2, but not by CrmA. *FEBS Lett* (1997) 400(1):15–8. doi: 10.1016/S0014-5793(96)01284-7
72. Stoica BA, Movsesyan VA, Lea IV PM, Faden AI. Ceramide-induced neuronal apoptosis is associated with dephosphorylation of Akt, BAD, FKHR, GSK-3β, and induction of the mitochondrial-dependent intrinsic caspase pathway. *Mol Cell Neurosci* (2003) 22(3):365–82. doi: 10.1016/S1044-7431(02)00028-3
73. Pastorino JG, Simbula G, Yamamoto K, Glascott PA, Rothman RJ, Farber JL. The Cytotoxicity of Tumor Necrosis Factor Depends on Induction of the Mitochondrial Permeability Transition. *J Biol Chem* (1996) 271(47):29792–8. doi: 10.1074/jbc.271.47.29792
74. Novgorodov SA, Gudz TI. Ceramide and mitochondria in ischemia/reperfusion. *J Cardiovasc Pharmacol* (2009) 53(3):198–208. doi: 10.1097/FJC.0b013e31819b52d5
75. Birbes H, Bawab SE, Hannun YA, Obeid LM. Selective hydrolysis of a mitochondrial pool of sphingomyelin induces apoptosis. *FASEB J* (2001) 15(14):2669–79. doi: 10.1096/fj.01-0539com
76. Novgorodov SA, Wu BX, Gudz TI, Bielawski J, Ovchinnikova TV, Hannun YA, et al. Novel pathway of ceramide production in mitochondria: Thioesterase and neutral ceramidase produce ceramide from sphingosine and acyl-CoA. *J Biol Chem* (2011) 286(28):25352–62. doi: 10.1074/jbc.M110.214866
77. Kolak M, Westerbacka J, Velagapudi VR, Wågsäter D, Yetukuri L, Makkonen J, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes* (2007) 56(8):1960–8. doi: 10.2337/db07-0111
78. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, DeFronzo RA, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* (2009) 58(2):337–43. doi: 10.2337/db08-1228
79. Turinsky J, O'Sullivan DM, Bayly BP. 1,2-Diacylglycerol and Ceramide Levels in Insulin-resistant Tissues of the Rat in Vivo. *J Biol Chem* (1990) 265(28):16880–5.
80. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA* (2001) 98(13):7522–7. doi: 10.1073/pnas.121164498
81. Brozinick JT, Hawkins E, Hoang Bui H, Kuo M-S, Tan B, Kievit P, et al. Plasma sphingolipids are biomarkers of metabolic syndrome in non-human primates maintained on a Western-style diet. *Int J Obes* (2013) 37:1064–70. doi: 10.1038/ijo.2012.191
82. Straczkowski M, Kowalska I, Nikolajuk A, Dzienis-Straczkowska S, Kinalska I, Baranowski M, et al. Relationship between Insulin Sensitivity and Sphingomyelin Signaling Pathway in Human Skeletal Muscle. *Diabetes* (2004) 53(5):1215–21. doi: 10.2337/diabetes.53.5.1215
83. Kusminski CM, Bickel PE, Scherer PE. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discovery* (2016) 15(9):639–60. doi: 10.1038/nrd.2016.75
84. Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, et al. Ceramide Content Is Increased in Skeletal Muscle from Obese Insulin-Resistant Humans. *Diabetes* (2004) 53(1):25–31. doi: 10.2337/diabetes.53.1.25
85. Summers SA, Nelson DH. A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X, and Cushing's syndrome. *Diabetes* (2005) 54(3):591–602. doi: 10.2337/diabetes.54.3.591
86. Cifone MG, Migliorati G, Parroni R, Marchetti C, Millimaggi D, Santoni A, et al. Dexamethasone-induced thymocyte apoptosis: Apoptotic signal involves the sequential activation of phosphoinositide-specific phospholipase C, acidic sphingomyelinase, and caspases. *Blood* (1999) 93(7):2282–96. doi: 10.1182/blood.v93.7.2282
87. Cinque B, Fanini D, Di Marzio L, Palumbo P, La Torre C, Donato V, et al. Involvement of cPLA2 inhibition in dexamethasone-induced thymocyte apoptosis. *Int J Immunopathol Pharmacol* (2008) 21(3):539–51. doi: 10.1177/039463200802100307
88. Xia JY, Holland WL, Kusminski CM, Sun K, Sharma AX, Pearson MJ, et al. Targeted Induction of Ceramide Degradation Leads to Improved Systemic Metabolism and Reduced Hepatic Steatosis. *Cell Metab* (2015) 22(2):266–78. doi: 10.1016/j.cmet.2015.06.007
89. Raichur S, Brunner B, Bielohuby M, Hansen G, Pfenninger A, Wang B, et al. The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Mol Metab* (2019) 21(January):36–50. doi: 10.1016/j.molmet.2018.12.008
90. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab* (2014) 20(4):678–86. doi: 10.1016/j.cmet.2014.08.002
91. Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, et al. CerS6-Derived Sphingolipids Interact with Mff and Promote Mitochondrial Fragmentation in Obesity. *Cell* (2019) 177(6):1536–1552.e23. doi: 10.1016/j.cell.2019.05.008
92. Turpin-Nolan SM, Hammerschmidt P, Chen W, Jais A, Timper K, Awazawa M, et al. CerS1-Derived C18:0 Ceramide in Skeletal Muscle Promotes Obesity-Induced Insulin Resistance. *Cell Rep* (2019) 26(1):1–10.e7. doi: 10.1016/j.celrep.2018.12.031
93. Bismuth J, Lin P, Yao Q, Chen C. Ceramide: A common pathway for atherosclerosis? *Atherosclerosis* (2008) 196(2):497–504. doi: 10.1016/j.atherosclerosis.2007.09.018

94. Park TS, Hu Y, Noh HL, Drosatos K, Okajima K, Buchanan J, et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res* (2008) 49(10):2101–12. doi: 10.1194/jlr.M800147-JLR200
95. Walls SM, Cammarato A, Chatfield DA, Ocorr K, Harris GL, Bodmer R. Ceramide-Protein Interactions Modulate Ceramide-Associated Lipotoxic Cardiomyopathy. *Cell Rep* (2018) 22(10):2702–15. doi: 10.1016/j.celrep.2018.02.034
96. Peterson LR, Xanthakis V, Duncan MS, Gross S, Friedrich N, Völzke H, et al. Ceramide remodeling and risk of cardiovascular events and mortality. *J Am Heart Assoc* (2018) 7(10):1–11. doi: 10.1161/JAHA.117.007931
97. Lee SY, Kim JR, Hu Y, Khan R, Kim S-J, Bharadwaj KG, et al. Cardiomyocyte specific deficiency of serine palmitoyltransferase subunit 2 reduces ceramide but leads to cardiac dysfunction. *J Biol Chem* (2012) 287(22):18429–39. doi: 10.1074/jbc.M111.296947
98. Park TS, Goldberg IJ. Sphingolipids, Lipotoxic Cardiomyopathy, and Cardiac Failure. *Heart Fail Clin* (2012) 8(4):633–41. doi: 10.1016/j.hfc.2012.06.003
99. Ussher JR, Koves TR, Cadete VJJ, Zhang L, Jaswal JS, Swyrd SJ, et al. Inhibition of De Novo Ceramide Synthesis Reverses Diet-Induced Insulin Resistance and Enhances Whole-Body Oxygen Consumption. *Diabetes* (2010) 59(10):2453–64. doi: 10.2337/db09-1293
100. Poss AM, Maschek JA, Cox JE, Hauner BJ, Hopkins PN, Hunt SC, et al. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. *J Clin Invest* (2020) 130(3):1363–76. doi: 10.1172/JCI131838
101. Chaurasia B, Tippetts TS, Monibas RM, Liu J, Li Y, Wang L, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Sci (80-)* (2019) 365(6451):386–92. doi: 10.1126/science.aav3722
102. Hojjati MR, Li Z, Zhou H, Tang S, Huan C, Ooi E, et al. Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J Biol Chem* (2005) 280(11):10284–9. doi: 10.1074/jbc.M412348200
103. Park TS, Panek RL, Rekhter MD, Mueller SB, Rosebury WS, Robertson A, et al. Modulation of lipoprotein metabolism by inhibition of sphingomyelin synthesis in ApoE knockout mice. *Atherosclerosis* (2006) 189(2):264–72. doi: 10.1016/j.atherosclerosis.2005.12.029
104. Bharath LP, Ruan T, Li Y, Ravindran A, Wan X, Nhan JK, et al. Ceramide-initiated protein phosphatase 2A activation contributes to arterial dysfunction in vivo. *Diabetes* (2015) 64(11):3914–26. doi: 10.2337/db15-0244
105. Hla T, Kolesnick R. C16:0-ceramide signals insulin resistance. *Cell Metab* (2014) 20(5):703–5. doi: 10.1016/j.cmet.2014.10.017
106. Raichur S, Wang ST, Chan PW, Li Y, Chaing J, Chaurasia B, et al. CerS2 haploinsufficiency inhibits β-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab* (2014) 20(4):687–95. doi: 10.1016/j.cmet.2014.09.015
107. Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM, et al. An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. *Cell Metab* (2013) 17(5):790–7. doi: 10.1016/j.cmet.2013.03.019
108. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* (2011) 17(1):55–63. doi: 10.1038/nm.2277
109. Fang L, Mundra PA, Fan F, Galvin A, Weir JM, Wong G, et al. Plasma lipidomic profiling in patients with rheumatoid arthritis. *Metabolomics* (2016) 12(8):1–10. doi: 10.1007/s11306-016-1086-6
110. Fine B, Marx A, Topkara V, Gomez EA, Vunjak-Novakovic G, Colombo P. An Integrated Analysis of Metabolomics After Left Ventricular Assist Device Implantation. *J Hear Lung Transplant* (2017) 36(4):S93. doi: 10.1016/j.healun.2017.01.235
111. Testai FD, Kilkus JP, Berdyshev E, Gorshkova I, Natarajan V, Dawson G. Multiple sphingolipid abnormalities following cerebral microendothelial hypoxia. *J Neurochem* (2014) 131(4):530–40. doi: 10.1111/jnc.12836
112. Testai FD, Xu H-L, Kilkus J, Suryadevara V, Gorshkova I, Berdyshev E, et al. Changes in the metabolism of sphingolipids after subarachnoid hemorrhage. *J Neurosci Res* (2015) 93(5):796–805. doi: 10.1002/jnr.23542
113. Chokshi A, Drosatos K, Cheema FH, Ji R, Khawaja T, Yu S, et al. Ventricular assist device implantation corrects myocardial lipotoxicity, reverses insulin resistance, and normalizes cardiac metabolism in patients with advanced heart failure. *Circulation* (2012) 125(23):2844–53. doi: 10.1161/CIRCULATIONAHA.111.060889
114. Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur Heart J* (2016) 37:1967–76. doi: 10.1093/eurheartj/ehw148
115. Petrocelli JJ, McKenzie AI, Mahmassani ZS, Reidy PT, Stoddard GJ, Poss AM, et al. Ceramide Biomarkers Predictive of Cardiovascular Disease Risk Increase in Healthy Older Adults After Bed Rest. *J Gerontol Biol Sci* (2020) 75(9):1663–70. doi: 10.1093/gerona/glaa072
116. Chiu HC, Kovacs A, Ford DA, Hsu F-F, Garcia R, Herrero P, et al. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest* (2001) 107(7):813–22. doi: 10.1172/JCI10947
117. Basu R, Oudit GY, Wang X, Zhang L, Ussher JR, Lopaschuk GD, et al. Type 1 diabetic cardiomyopathy in the Akita (Ins2WT/C96Y) mouse model is characterized by lipotoxicity and diastolic dysfunction with preserved systolic function. *Am J Physiol - Hear Circ Physiol* (2009) 297(6):2096–108. doi: 10.1152/ajpheart.00452.2009
118. Martínez-Abundis E, Correa F, Pavón N, Zazueta C. Bax distribution into mitochondrial detergent-resistant microdomains is related to ceramide and cholesterol content in postischemic hearts. *FEBS J* (2009) 276(19):5579–88. doi: 10.1111/j.1742-4658.2009.07239.x
119. Russo SB, Baicu CF, Van Laer A, Geng T, Kasiganesan H, Zile MR, et al. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. *J Clin Invest* (2012) 122(11):3919–30. doi: 10.1172/JCI63888
120. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* (1995) 270(45):26746–9. doi: 10.1074/jbc.270.45.26746
121. Turer AT, Scherer PE. Adiponectin: mechanistic insights and clinical implications. *Diabetologia* (2012) 55(9):2319–26. doi: 10.1007/s00125-012-2598-x
122. Wang ZV, Scherer PE. Adiponectin, the past two decades. *J Mol Cell Biol* (2016) 8(2):93–100. doi: 10.1093/jmcb/mjw011
123. Scherer PE. The many secret lives of adipocytes: implications for diabetes. *Diabetologia* (2019) 62(2):223–32. doi: 10.1007/s00125-018-4777-x
124. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chair BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. *Sci (80-)* (1995) 269(5223):543–6. doi: 10.1126/science.7624777
125. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* (2007) 117(9):2621–37. doi: 10.1172/JCI31021
126. Xu A, Wang Y, Keshaw H, Xu LY, Lam KSL, Cooper GJS. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* (2003) 112(1):91–100. doi: 10.1172/jci17797
127. Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, et al. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. *Nat Med* (2012) 18(10):1539–51. doi: 10.1038/nm.2899
128. Scherer PE. The multifaceted roles of adipose tissue - Therapeutic targets for diabetes and beyond: The 2015 banting lecture. *Diabetes* (2016) 65(6):1452–61. doi: 10.2337/db16-0339
129. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* (2003) 423(6941):762–9. doi: 10.1038/nature01705
130. Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, et al. PAQR proteins: A novel membrane receptor family defined by an ancient 7-transmembrane pass motif. *J Mol Evol* (2005) 61(3):372–80. doi: 10.1007/s00239-004-0375-2
131. Villa NY, Kupchak BR, Garitaonandia I, Smith JL, Alonso E, Alford C, et al. Sphingolipids function as downstream effectors of a fungal PAQF. *Mol Pharmacol* (2009) 75(4):866–75. doi: 10.1124/mol.108.049809
132. Ye J, Bian X, Lim J, Medzhitov R. Adiponectin and related C1q/TNF-related proteins bind selectively to anionic phospholipids and sphingolipids. *Proc Natl Acad Sci U S A* (2019) 117(29):776690. doi: 10.1101/776690
133. Tanabe H, Fujii Y, Okada-Iwabu M, Iwabu M, Nakamura Y, Hosaka T, et al. Crystal structures of the human adiponectin receptors. *Nature* (2015) 520(7547):312–6. doi: 10.1038/nature14301

134. Langosch D, Scharnagl C, Steiner H, Lemberg MK. Understanding intramembrane proteolysis: From protein dynamics to reaction kinetics. *Trends Biochem Sci* (2015) 40(6):318–27. doi: 10.1016/j.tibs.2015.04.001
135. Holland WL, Scherer PE. Ronning after the adiponectin receptors. *Science* (2013) 342(6165):1460–1. doi: 10.1126/science.1249077
136. Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K-I, Matsuda K, et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature* (2013) 503(7477):493–9. doi: 10.1038/nature12656
137. Holland WL, Xia JY, Johnson JA, Sun K, Pearson MJ, Sharma AX, et al. Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced ceramidase signaling in lipid and glucose homeostasis. *Mol Metab* (2017) 6(3):267–75. doi: 10.1016/j.molmet.2017.01.002
138. Choi SR, Lim JH, Kim MY, Kim EN, Kim Y, Choi BS, et al. Adiponectin receptor agonist AdipoRon decreased ceramide, and lipotoxicity, and ameliorated diabetic nephropathy. *Metabolism* (2018) 85:348–60. doi: 10.1016/j.metabol.2018.02.004
139. Owen BM, Mangelsdorf DJ, Kliewer SA. Tissue-specific actions of the metabolic hormones FGF15/19 and FGF21. *Trends Endocrinol Metab* (2015) 26(1):22–9. doi: 10.1016/j.tem.2014.10.002
140. Lin Z, Tian H, Lam KSL, Lin S, Hoo RCL, Konishi M, et al. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab* (2013) 17(5):779–89. doi: 10.1016/j.cmet.2013.04.005
141. Lopez X, Goldfine AB, Holland WL, Gordillo R, Scherer PE. Plasma ceramides are elevated in female children and adolescents with type 2 diabetes. *J Pediatr Endocrinol Metab* (2013) 26(9–10):995–8. doi: 10.1515/jpem-2012-0407
142. Wigger L, Cruciani-Guglielmacci C, Nicolas A, Denom J, Fernandez N, Fumeron F, et al. Plasma Dihydroceramides Are Diabetes Susceptibility Biomarker Candidates in Mice and Humans. *Cell Rep* (2017) 18(9):2269–79. doi: 10.1016/j.celrep.2017.02.019
143. De Groot PCM, Dekkers OM, Romijn JA, Dieben SWM, Helmerhorst FM. PCOS. coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. *Hum Reprod Update* (2011) 17(4):495–500. doi: 10.1093/humupd/dmr001
144. Hansen SL, Svendsen PF, Jeppesen JF, Hoeg LD, Andersen NR, Kristensen JM, et al. Molecular Mechanisms in Skeletal Muscle Underlying Insulin Resistance in Women Who Are Lean With Polycystic Ovary Syndrome. *J Clin Endocrinol Metab* (2019) 104(5):1841–54. doi: 10.1210/jc.2018-01771
145. Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, et al. Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the Dallas Heart Study. *Diabetologia* (2018) 61(12):2570–9. doi: 10.1007/s00125-018-4720-1
146. Mänttäri A, Suni J, Sievänen H, Husu P, Vähä-Ypyä H, Valkeinen H, et al. Six-minute walk test: a tool for predicting maximal aerobic power (VO2max) in healthy adults. *Clin Physiol Funct Imaging* (2018) 38(6):1038–45. doi: 10.1111/cpf.12525
147. Schulz ME, Katunaric B, Hockenberry JC, Gutterman DD, Freed JK. Manipulation of the Sphingolipid Rheostat Influences the Mediator of Flow-Induced Dilation in the Human Microvasculature. *J Am Heart Assoc* (2019) 8(17):e013153. doi: 10.1161/JAHA.119.013153
148. Camici PG, Crea F. Coronary Microvascular Dysfunction. *N Engl J Med* (2007) 356(8):830–40. doi: 10.1056/NEJMra061889
149. Chao CYL, Cheing GLY. Microvascular dysfunction in diabetic foot disease and ulceration. *Diabetes Metab Res Rev* (2009) 25(7):604–14. doi: 10.1002/dmrr.1004
150. Karaca Ü, Schram MT, Houben AJHM, Muris DMJ, Stehouwer CDA. Microvascular dysfunction as a link between obesity, insulin resistance and hypertension. *Diabetes Res Clin Pract* (2014) 103(3):382–7. doi: 10.1016/j.diabres.2013.12.012
151. Stirban A. Microvascular Dysfunction in the Context of Diabetic Neuropathy. *Curr Diabetes Rep* (2014) 14(11):1–9. doi: 10.1007/s11892-014-0541-x
152. Wang ZV, Mu J, Schraw TD, Gautron L, Elmquist JK, Zhang BB, et al. PANIC-ATTAC: A mouse model for inducible and reversible β-cell ablation. *Diabetes* (2008) 57(8):2137–48. doi: 10.2337/db07-1631
153. Sharma AX, Quittner-Strom EB, Lee Y, Johnson JA, Martin SA, Yu X, et al. Glucagon Receptor Antagonism Improves Glucose Metabolism and Cardiac Function by Promoting AMP-Mediated Protein Kinase in Diabetic Mice. *Cell Rep* (2018) 22(7):1760–73. doi: 10.1016/j.celrep.2018.01.065
154. Xia JY, Sun K, Hepler C, Ghaben AL, Gupta RK, An YA, et al. Acute loss of adipose tissue-derived adiponectin triggers immediate metabolic deterioration in mice. *Diabetologia* (2018) 61(4):932–41. doi: 10.1007/s00125-017-4516-8
155. Blachnio-Zabielska AU, Hady HR, Markowski AR, Kurianiuk A, Karwowska A, Górski J, et al. Inhibition of Ceramide De Novo Synthesis Affects Adipocytokine Secretion and Improves Systemic and Adipose Tissue Insulin Sensitivity. *Int J Mol Sci* (2018) 19(12):3995. doi: 10.3390/ijms19123995
156. Sobolesky PM, Harrell TS, Parry C, Venn-Watson S, Janech MG. Feeding a modified fish diet to bottlenose dolphins leads to an increase in serum adiponectin and sphingolipids. *Front Endocrinol (Lausanne)* (2016) 7:33 (APR):33. doi: 10.3389/fendo.2016.00033
157. Chang J, Li Y, Huang Y, Lam KSL, Hoo RLC, Wong WT, et al. Adiponectin prevents diabetic premature senescence of endothelial progenitor cells and promotes endothelial repair by suppressing the p38 MAP kinase/p16INK4A signaling pathway. *Diabetes* (2010) 59(11):2949–59. doi: 10.2337/db10-0582
158. Shibata S, Tada Y, Asano Y, Hau CS, Kato T, Saeki H, et al. Adiponectin Regulates Cutaneous Wound Healing by Promoting Keratinocyte Proliferation and Migration via the ERK Signaling Pathway. *J Immunol* (2012) 189(6):3231–41. doi: 10.4049/jimmunol.1101739
159. Jin T, Kim MJ, Heo WI, Park KY, Choi SY, Lee M-K, et al. Adiponectin corrects premature cellular senescence and normalizes antimicrobial peptide levels in senescent keratinocytes. *Biochem Biophys Res Commun* (2016) 477(4):678–84. doi: 10.1016/j.bbrc.2016.06.119
160. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* (1994) 371:425–32. doi: 10.1038/372425a0
161. Friedman J. Leptin at 20: An overview. *J Endocrinol* (2014) 223(1):T1–8. doi: 10.1530/JOE-14-0405
162. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. *Nature* (1996) 382:250–2. doi: 10.1038/382250a0
163. Cui H, López M, Rahmouni K. The cellular and molecular bases of leptin and ghrelin resistance in obesity. *Nat Rev Endocrinol* (2017) 13(6):338–51. doi: 10.1038/nrendo.2016.222
164. El-Haschimi K, Pierroz DD, Hileman SM, Bjørbæk C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* (2000) 105(12):1827–32. doi: 10.1172/JCI9842
165. Enriori PJ, Evans AE, Sinnayah P, Cowley MA. Leptin Resistance and Obesity. *Obesity* (2006) 14(S8):254S–8S. doi: 10.1038/oby.2006.319
166. Knight ZA, Hannan KS, Greenberg ML, Friedman JM. Hyperleptinemia Is Required for the Development of Leptin Resistance. Stadler K, ed. *PloS One* (2010) 5(6):e11376. doi: 10.1371/journal.pone.0011376
167. Bjørbæk C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* (1998) 1(4):619–25. doi: 10.1016/S1097-2765(00)80062-3
168. Bjørbæk C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem* (1999) 274(42):30059–65. doi: 10.1074/jbc.274.42.30059
169. Zhao S, Zhu Y, Schultz RD, Li N, He Z, Zhang Z, et al. Partial Leptin Reduction as an Insulin Sensitization and Weight Loss Strategy. *Cell Metab* (2019) 30(4):706–719.e6. doi: 10.1016/j.cmet.2019.08.005
170. Zhao S, Li N, Zhu Y, Straub L, Zhang Z, Wang M-Y, et al. Partial leptin deficiency confers resistance to diet-induced obesity in mice. *Mol Metab* (2020) 100995:1–12. doi: 10.1016/j.molmet.2020.100995
171. Cai B, Lu Y, Xu M. Acid sphingomyelinase downregulation alleviates vascular endothelial leptin resistance in rats. *Acta Pharmacol Sin* (2019) 41:650–60. doi: 10.1038/s41401-019-0328-3
172. Ritchie IRW, Gulli RA, Stefanyk LE, Harasim E, Chabowski A, Dyck DJ. Restoration of skeletal muscle leptin response does not precede the exercise-induced recovery of insulin-stimulated glucose uptake in high-fat-fed rats. *Am J Physiol Integr Comp Physiol* (2011) 300(2):R492–500. doi: 10.1152/ajpregu.00602.2010

173. Bonzón-Kulichenko E, Schwudke D, Gallardo N, Moltó E, Fernández-Agulló T, Shevchenko A, et al. Central Leptin Regulates Total Ceramide Content and Sterol Regulatory Element Binding Protein-1C Proteolytic Maturation in Rat White Adipose Tissue. *Endocrinology* (2009) 150(1):169–78. doi: 10.1210/en.2008-0505
174. Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. *J Biol Chem* (1998) 273(49):32487–90. doi: 10.1074/jbc.273.49.32487
175. Ordoñez M, Presa N, Dominguez-Herrera A, Trueba M, Gomez-Muñoz A. Regulation of adipogenesis by ceramide 1-phosphate. *Exp Cell Res* (2018) 372 (2):150–7. doi: 10.1016/j.yexcr.2018.09.021
176. Howard JK, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. *Trends Endocrinol Metab* (2006) 17(9):365–71. doi: 10.1016/j.tem.2006.09.007
177. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: A potential mechanism for cardiovascular and metabolic risk. *Diabetes* (2006) 55(9):2579–87. doi: 10.2337/db06-0330
178. Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, Petrocelli A, Carella C, et al. Plasma Leptin Level Is Associated With Myocardial Wall Thickness in Hypertensive Insulin-Resistant Men. *Hypertension* (1999) 34(5):1047–52. doi: 10.1161/01.HYP.34.5.1047
179. Ren J, Relling DP. Leptin-induced suppression of cardiomyocyte contraction is amplified by ceramide. *Peptides* (2006) 27(6):1415–9. doi: 10.1016/j.peptides.2005.11.022
180. Dhopeshwarkar GA, Mead JF. Uptake and transport of fatty acids into the brain and the role of the blood-brain barrier system. *Adv Lipid Res* (1973) 11:109–42. doi: 10.1016/b978-0-12-024911-4.50010-6
181. Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A, et al. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol - Endocrinol Metab* (2009) 296(5):1003–12. doi: 10.1152/ajpendo.90377.2008
182. Sergi D, Morris AC, Kahn DE, McLean FH, Hay EA, Kubitz P, et al. Palmitic acid triggers inflammatory responses in N42 cultured hypothalamic cells partially via ceramide synthesis but not via TLR4. *Nutr Neurosci* (2020) 23 (4):321–34. doi: 10.1080/1028415X.2018.1501533
183. Valassi E, Scacchi M, Cavagnini F. Neuroendocrine control of food intake. *Nutr Metab Cardiovasc Dis* (2008) 18(2):158–68. doi: 10.1016/j.numecd.2007.06.004
184. Carrasco P, Sahún I, McDonald J, Ramírez S, Jacas J, Gratacós E, et al. Ceramide levels regulated by carnitine palmitoyltransferase 1C control dendritic spine maturation and cognition. *J Biol Chem* (2012) 287 (25):21224–32. doi: 10.1074/jbc.M111.337493
185. Contreras C, González-García I, Martínez-Sánchez N, Seoane-Collazo P, Jacas J, Morgan DA, et al. Central ceramide-induced hypothalamic lipotoxicity and ER stress regulate energy balance. *Cell Rep* (2014) 9 (1):366–77. doi: 10.1016/j.celrep.2014.08.057

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership