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The Lard Works in Mysterious Ways: Ceramides in Nutrition-Linked Chronic Disease

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Abstract

Diet influences onset, progression, and severity of several chronic diseases, including heart failure, diabetes, steatohepatitis, and a subset of cancers. The prevalence and clinical burden of these obesity-linked diseases has risen over the past two decades. These metabolic disorders are driven by ectopic lipid deposition in tissues not suited for fat storage, leading to lipotoxic disruption of cell function and survival. Sphingolipids such as ceramides are among the most deleterious and bioactive metabolites that accrue, as they participate in selective insulin resistance, dyslipidemia, oxidative stress and apoptosis. This review discusses our current understanding of biochemical pathways controlling ceramide synthesis, production and action; influences of diet on ceramide levels; application of circulating ceramides as clinical biomarkers of metabolic disease; and molecular mechanisms linking ceramides to altered metabolism and survival of cells. Development of nutritional or pharmacological strategies to lower ceramides could have therapeutic value in a wide range of prevalent diseases.

Keywords

nutrition; chronic disease; ceramide; sphingolipids; lipotoxicity

1. INTRODUCTION

Chronic diseases are multifactorial by nature, with nutrition playing an important but enigmatic role. The importance of a healthy diet is most commonly associated with obesity-related diseases, wherein energy intake exceeds demand and excess calories are stored as triglycerides. While triglyceride storage itself is likely inert, the capacity of adipose depots to safely store these extra calories is finite. As a result, lipids can eventually accumulate in tissues not suited for fat storage, such as the heart, liver, vasculature, and pancreas. This ectopic lipid accumulation creates a lipotoxic state that primes the body for cardiometabolic disease development, including heart failure, atherosclerosis, nonalcoholic fatty liver disease

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(NAFLD), obesity related cancers, and diabetes. Extensive studies carried out over the past 25 years indicate that ceramides, a bioactive and lipotoxic member of the sphingolipid class, elicit many of the cellular changes that underly the aforementioned diseases. The discoveries regarding ceramides in metabolic pathology present exciting opportunities for understanding the evolutionary forces that promote nutrition-linked chronic disease development and for identifying novel therapeutic and predictive targets for clinical implementation.

While ceramides comprise a minor proportion of the whole-body lipidome, they play potent roles in nutrition-linked chronic disease. For example, overexpression of ceramide synthesizing genes or ablation of genes required for ceramide degradation worsens features of metabolic disease (13, 46), while genetic or pharmacological inhibition of ceramide synthesis prevents cardiometabolic diseases (12, 58, 59, 141). In vitro studies in the 1990s demonstrated the signaling capacity of ceramides via direct participation in the insulin-responsive glucose uptake pathway, providing the initial link between ceramides and insulin resistance (135). This finding has now been widely confirmed in rodent studies and prospective human cohort studies (12, 31, 32, 66, 86). Additional studies have shown relationships between serum ceramides and terminal consequences of the metabolic syndrome, such as diabetes and major adverse cardiac events (31, 66, 78, 86). Nonetheless, more work remains if we are to achieve widespread use of ceramides as biomarkers or develop ceramide-lowering strategies to treat these pathologies.

Herein we summarize the modes of ceramide synthesis and transport, the clinical utility of ceramides as biomarkers for nutrition-linked chronic diseases, the role of dietary sphingolipid intake and dietary patterns on ceramide profiles, and the molecular mechanisms of ceramides that drive the pathogenesis underlying metabolic disease. Moreover, we discuss the potential modes of ceramide-lowering strategies, including lifestyle interventions and findings from preclinical pharmacological targeting of the sphingolipid synthesis pathway, to mitigate obesity-coupled diseases.

2. CERAMIDE SYNTHESIS AND METABOLISM

The sphingolipid class encompasses 4,000–5,000 unique lipid species with reported structures (LIPID MAPS Structure Database, Reference 130), which are metabolized in elegant anabolic and catabolic pathways characterized within multiple organisms.

2.1. De Novo Ceramide Synthesis

Ceramides are produced in a conserved four-step biosynthetic pathway within the endoplasmic reticulum (ER), which commences with the rate-limiting condensation of a saturated long-chain acyl-CoA and amino acid to produce 3-ketosphinganine via serine palmitoyltransferase (SPT) (Figure 1a). Three genes (i.e., *SPTLC1, SPTLC2*, and *SPTLC3*) encode the essential subunits of the SPT complex, serving as heterodimers with differential tissue expression and specificity for acyl-CoA and amino acid substrates (47, 61). Additional components termed small subunits of SPT and orosomucoid-like proteins interact directly with the SPT complex substrate binding sites to confer additional regulation of substrate specificity and activity (1, 5, 22, 47, 49, 150). The SPT complex predominantly produces an 18-carbon sphingoid backbone produced by the condensation of

palmitoyl-CoA with serine but can incorporate alternative fatty acids (e.g., myristoyl-CoA, stearoyl-CoA) or initiate production of deoxysphingolipids by using alanine or glycine. The products of the SPT reaction are quickly acted upon by an NADPH-dependent reductase (i.e., 3-ketodihydrosphingosine reductase) to form the defining sphingoid scaffold dihydrosphingosine.

The next branch point adding considerable diversity to the sphingolipid pool is the third reaction catalyzed by one of six *n*-acyltransferases. These (dihydro)ceramide synthases (CERS1–6) produce dihydroceramides by adding a second, variable acyl chain to the sphingoid backbone. The CERS enzymes differ immensely in both tissue expression pattern and substrate specificity, producing dihydroceramides with variable acyl chain lengths spanning 14–34 carbons (87). Despite their unique gene origins, the CERS enzymes' primary sequences retain high homology, and substrate specificity is reportedly determined by a single ER luminal loop (137). Furthermore, all mammalian CERS, excepting CERS1, contain a conserved homeobox-like domain which has been shown in *Drosophila melanogaster* and cultured mammalian cells to mediate transcriptional regulation of lipase genes in response to intracellular fatty acid levels (9, 127). Transcriptional feedback of CERS enzymes has been observed, wherein knockdown or knockout of a specific CERS species elicits compensatory increases in alternative CERS isoforms and alterations in the composition of the cellular sphingolipidome (99, 113, 141). Additionally, the activity of CERS enzymes and the resulting diversity of the sphingolipid variable chain lengths is significantly affected by their formation of homo- or heterodimers (82).

In the ultimate step of the de novo ceramide synthesis cascade, dihydroceramide is converted to ceramide with the addition of a single 4,5-*trans*-double bond by the dihydroceramide desaturases (DES1–2), encoded by *DEGS1* and *DEGS2*. DES1 is expressed ubiquitously in tissues, whereas DES2 is localized to the skin and intestinal epithelium and contains bifunctionality as a C4-hydroxylase that produces phytoceramides (96). The double bond introduced at this step elicits a marked change in lipid bioactivity and pathological fate. Dihydroceramides are considered benign or beneficial, while ceramides are deleterious mediators of various stress responses that drive the pathogenesis underlying nutrition-linked chronic disease (12, 123, 124).

2.2. Metabolism and Catabolism of Sphingolipids

Ceramides are shuttled from the ER to the Golgi apparatus to form complex sphingolipid species through the addition of various head groups to the first position oxygen molecule (37). For example, sphingomyelin (SM) is synthesized in the luminal Golgi with the addition of a choline head-group by SM synthase (37). Glucosylceramides are formed in the *cis*-Golgi by glucosylceramide synthase, with further glycosylation to form lactosylceramide or monosialodihexosylganglioside occurring in the trans-Golgi (44, 74). Ceramide can be phosphorylated by ceramide kinase in the Golgi to form ceramide-1-phosphate (C1P) (38). The transfer of ceramides to distinct Golgi regions is important for the regulation of complex sphingolipid synthesis, as delivery of ceramides for SM or C1P synthesis seems to be mediated by the ATP-dependent ceramide transport protein, whereas glycosphingolipids are generated from vesicularly delivered ceramides (21, 35, 48).

In addition to de novo biosynthesis, a large portion of intracellular ceramides are generated via hydrolysis of complex sphingolipids or salvaged with reacylation of sphingoid bases (74). Several sphingomyelinase (SMase) enzymes act to cleave the phosphocholine head group from SM to yield ceramide and phosphocholine. This process is mediated by alkaline, neutral, and acid SMase enzymes and is important for the modulation of cellular stress responses and digestion of dietary sphingolipids (74, 114). Alkaline SMase is expressed predominantly in the liver and intestinal mucosa and dissociates from the plasma membrane to the intestinal lumen by the actions of pancreatic trypsin or bile salts to digest dietary SM (Figure 1b) (153). Four mammalian genes encode the neutral SMases (nSMase1–3 and MA-SMase), which share the same optimal pH but differ in subcellular location (2). The most well characterized is nSMase2, which is relevant to nutrition-related chronic diseases due to its activation in the plasma membrane by the inflammatory cytokine tumor necrosis factor-α (17). Acid SMase is posttranslationally modified and trafficked to produce lysosomal SMase, which operates at low pHs, or secretory SMase, which hydrolyzes SMs present in circulating lipoproteins (65).

Ceramides are deacylated by a family of ceramidases to form sphingosine and a free fatty acid of variable length. Similar to SMase enzymes, ceramidase nomenclature (i.e., acid, ASAH1; neutral, ASAH2; or alkaline, ACER1–3) denotes the enzymes' pH optima. ASAH2 is predominantly expressed in the gut epithelial brush border and plays a particular role in dietary ceramide digestion, as sphingosine is the only sphingolipid known to be readily absorbed by enterocytes (Figure 1b) (16). In addition to the ceramidase enzymes discussed above, adiponectin receptors (ADIPOR1 and 2) have ligand-activated ceramidase activity, which partially accounts for the insulin sensitizing effects of adiponectin (60, 129). Sphingosine formed from ceramide catabolism can be reacylated by CERS to form ceramides and complex sphingolipids, or phosphorylated by sphingosine kinases (SPHK1 and 2) to form the potent signaling molecule sphingosine-1-phosphate (S1P) (8, 122). In the gut, S1P is often fully degraded by S1P lyase to form phosphoethanolamine and a fatty aldehyde hexadecenal (144). Breakdown of glycosphingolipids by various glycosidases is important for dietary digestion and absorption of these lipids but is thought to occur less frequently extraintestinally and does not contribute to a significant fraction of ceramide regeneration (75, 131).

2.3. Dietary Sphingolipids

Sphingolipid content within foods is highly variable but is higher in dairy, eggs, fish, and soy products (146, 149). Consumption of dietary sphingolipids is not considered to contribute significantly to caloric intake (0.01–0.02% of intake by weight); however, the typical Western diet is estimated to provide approximately 0.3–0.4 grams of sphingolipid daily (146). While mammalian sources of dietary sphingolipid provide a broad spectrum of complex sphingolipids (e.g., SM, cerebro-sides, gangliosides, sulfatides) with SM as the predominant species, plant sources largely consist of a range of mono- and oligohexosylceramides with noncanonical sphingoid bases (i.e., d17:1, d18:1$^{\Delta 8}$, and d18:2$^{\Delta 4,8}$, reviewed in detail in 145, 146, 149). Thus, diets which differ in plant and animal product ingestion are likely reflected in relative abundance and diversity of dietary sphingolipid intake (159).

Dietary sphingolipids are differentially digested and absorbed according to class and composition of the sphingoid base and accessory acyl chain. As such, phosphosphingolipids, sphingosine, dihydrosphingosine, and SM are absorbed more readily than glycosphingolipids; sphingolipids with 16-carbon accessory chains are more readily taken up than very-long-chain species; and mammalian d18:1 sphingoid bases desaturated at the delta-4 position are selectively absorbed over alternative plant and fungal sources (33, 156). Dietary sphingolipids are not considered essential nutrients, and the extent to which digested sphingolipids are fully degraded and reassembled within enterocytes to contribute to the circulating and tissue lipidome is poorly understood (91); however, human and rodent studies have demonstrated that dietary sphingolipids effectively impair digestion and absorption of other dietary lipids (e.g., cholesterol, glycerolipids, free fatty acids) and may paradoxically confer some protection from systemic inflammation, insulin resistance, atherosclerosis, liver steatosis, and intestinal cancer (extensively reviewed in 103, 149, 156).

2.4. Gut Microbial Sphingolipids

Dietary sphingolipids are incompletely digested and absorbed in the small intestine and travel to the colon, where they can be absorbed and assimilated into the microbial lipidome (84). Additionally, the rate limiting enzyme of de novo ceramide synthesis, SPT, is conserved in some bacterial species (157). Interestingly, whereas mammalian systems predominantly produce sphingoid bases with even-numbered acyl chains, microbial sphingolipids often incorporate odd-chain backbones (128). Rodent tracing studies, as well as observations of odd-chain base sphingolipid species in human circulation, suggest that bacterially derived sphingolipids can enter host circulation to influence metabolism (34, 70). Brown and colleagues (6) have reported a feedback mechanism for host and microbe sphingolipid homeostasis, in which sphingolipids produced by the abundant *Bacteroides* species are essential to subdue the accumulation of host-derived ceramides and resulting intestinal inflammation and disease. Gonzalez and colleagues (39) have produced a dossier of work implicating a farnesoid X receptor (FXR) gut-liver-ceramide axis, in which the microbial degradation of bile acids stimulates FXR activity and upregulates biosynthesis of ceramide in the enterocyte. Consequently, decreases in portal vein and systemic ceramides in mice lacking intestinal FXR downregulated hepatic lipogenesis and glucogenesis and promoted liver lipid oxidation and adipocyte browning to confer protection from obesity-related metabolic disease (29, 67, 155). Conversely, Kayser and colleagues (72) have recently reported that circulating sphingolipid levels in overweight and obese humans are inversely correlated with gut microbial diversity and bacterial expression of genes involved in bile acid degradation. Instead, resolution of gut dysbiosis and circulating ceramides with diet-induced weight loss was attributed to increased colonization of anti-inflammatory *Bifidobacterium* species and suppression of lipopolysaccharide biosynthesis. Thus, a knowledge gap exists regarding ways in which dietary patterns and nutrition-linked diseases alter gut microbiota and the intestinal sphingolipidome to influence whole-body metabolic health and disease.

3. CERAMIDES AS MARKERS OF DISEASE

Clinical care precision and effective resource partitioning through personalized medicine are aided via diagnostic, risk, prognostic, and predictive biomarkers (101). Ceramides are an ideal biomarker due to their sensitivity and specificity in association with discrete clinical outcomes and reliably detected presence in noninvasive body fluids, including serum, plasma, and urine. Moreover, ceramides are conditionally independent from already established lipid markers, triglycerides and cholesterol (109). While ceramides are proposed biomarkers for myriad metabolic conditions, they are most extensively studied in relation to cardiovascular disease (CVD) (Table 1). In fact, a ceramide score, CERT1, has been implemented clinically in both private and public practice in Finland as well as at the Cleveland and Mayo Clinics in the United States.

3.1. Individual Ceramides and Cardiovascular Disease

Associations between CVD and individual ceramides have been shown in numerous case-control and cohort studies, as well as clinical trials (3, 51, 78, 108, 136). Ceramide species Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), and Cer(d18:1/24:1) and their ratios to Cer(d18:1/24:0) have demonstrated predictive power for myocardial infarctions (MI) and cardiovascular death (51). Additionally, these same ceramide species and ratios predicted MI and cardiovascular death in patients with stable coronary heart disease or in secondary prevention following an MI (78). Interestingly, the associations between ceramides and CVD are stronger for recurrent events and fatal outcomes (3, 51, 78).

Far less is known regarding distinct ceramide species in association with heart failure and stroke. Lemaitre et al. (85) report associations between plasma ceramides in 1,179 cases of incident heart failure in the Cardiovascular Health Study. Specifically, Cer(d18:1/16:0) demonstrated a positive association, while Cer(d18:1/22:0) was inversely associated. Gui et al. (43) report increased levels of plasma Cer(d18:1/16:0), Cer(d18:1/22:0), and Cer(d18:1/24:0) in a matched case control study including 202 patients with acute ischemic stroke and 202 age- and sex-matched controls. Of note, ceramides were significantly higher in patients with moderate-to-high clinical severity ($n = 99$) than patients with minor stroke ($n = 103$), indicating the capacity for ceramide to predict both risk and severity of stroke. Further research in the areas of heart failure and stroke in association with ceramides may yield powerful diagnostic and risk predictive tools.

3.2. Ceramide-Based Scores and Cardiovascular Disease

Three predictive algorithms comprising ceramides have been published to date: Cardiac Event Risk Test 1 (CERT1) (51, 78), Cardiac Event Risk Test 2 (CERT2) (54), and sphingolipid-inclusive coronary artery disease score (SIC) (109). CERT1 is the most long-standing and established score, and it is used clinically in Finland and parts of the Unites States. This score, which was developed by Zora Biosciences and recently licensed by Quest Diagnostics, comprises Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) concentrations and each respective lipid's ratio to Cer(d18:1/24:0). These six score components are broken into quartiles, with the highest quartile assigned 2 points, the second-highest quartile 1 point, and the lower two quartiles 0 points (51, 78). Hence, the

score ranges from 0–12 points with discrete risk categories (0–2, low; 3–6, moderate; 7–9, increased; 10–12, high), demonstrating a positive linear relationship with CVD risk. Of note, this linear increase is not seen with conventional CVD marker low-density lipoprotein (LDL) cholesterol (56). The CERT1 score is an impressive clinical score, demonstrating a disease-severity dependent scale with promise for use in risk stratification. A revised iteration of the CERT1 score, CERT2, is composed of ceramide and phospholipid species selected in a stepwise fashion and validated in four large independent cohorts, including the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial, which includes participants from multiple geographical locations worldwide (54, 57). The third published ceramide-based score is SIC, which includes nonabundant sphingolipids in addition to the most prevalent ceramide species (109). SIC performs conditionally independently from and more effectively than conventional CVD markers, including LDL cholesterol and triglycerides, indicating that ceramides are robust, nonredundant, and novel biomarkers. However, SIC was generated in a case-control study and has not been validated in multiple cohorts or in a prospective study design. Ceramide-based scores are an important and rapidly translatable area of research, and they overcome the cumbersome nature of large sphingolipid panels by presenting an appealing and easily interpretable diagnostic reporting option. Of note, the improved disease prediction and risk stratification power yielded by ceramide-based scores has considerable potential to improve patient care and effective utilization of healthcare resources.

3.3. Ceramides in Association with Diabetes, Insulin Resistance, and Metabolic Syndrome

Ceramides are implicated as causal factors in insulin resistance and type 2 diabetes mellitus (T2DM) metabolism and are obligate intermediates in beta cell death (10). They are also potent prognostic markers of incident T2DM in mice and humans. Fretts et al. (32) recently identified significant associations of Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), and Cer(d18:1/22:0) with higher risk of incident diabetes in the Cardiovascular Health Study (n = 3,645), which comprises older adults with 26 years of follow-up. Accordingly, ceramides also associate with markers of glycemic control: insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and homeostatic model assessment of β-cell function (HOMA-β) in the Strong Heart Study (31, 86). Interestingly, SMs are associated with body mass index in the Strong Heart Study. Moreover, the ratio of Cer(d18:1/18:0)/Cer(d18:1/16:0) was predictive of incident diabetes in the FINRISK cohort, Western Norway Coronary Angiography Study, and interventional Prevent Metabolic Syndrome Trial (combined n = 11,760) (54). Notably, while not implicated as causative agents in diabetes, dihydroceramides may be more sensitive disease biomarkers than ceramides, as they are immediately adjacent to ceramides in the de novo synthesis pathway and considerably less abundant, rendering them a more sensitive readout of alterations in sphingolipid biosynthesis. In two independent cohorts, dihydroceramide species Cer(d18:0/18:0) and Cer(d18:0/22:0) predict diabetes up to 9 years before onset more potently than their corresponding ceramide species with the same acyl chain lengths (152). However, a majority of human cohort studies evaluating ceramides in diabetes do not measure dihydroceramides, and this finding has not been widely replicated. Mechanistic studies have definitively established the causal link between ceramides and diabetes development.

Further epidemiological studies, particularly prospective cohorts with diverse populations, are necessary to fully interrogate the predictive and prognostic utility of ceramide, and potentially dihydroceramide, as biomarkers in diabetes.

3.4. Ceramides and Obesity-Related Cancers

Elevated body weight and corresponding metabolic changes are associated with certain cancers, including colorectal, breast, endometrial, and liver cancers (4). The role of ceramides in cancer is paradoxical, as proapoptotic ceramides prevent tumor growth but also participate in the metabolic milieu (e.g., insulin resistance and dyslipidemia) driving onset of obesity-related cancers. An additional layer of complexity lies within differences in interpretation of ceramide concentrations in circulation and in tumor tissue. In colorectal cancer, concentrations of circulating Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/18:1), and Cer(d18:1/24:1), but not hexosyl-ceramides or SMs, were significantly associated with stage IV colorectal cancer (121). In breast cancer, higher ceramide concentrations in tumors have been associated with less aggressive breast cancer by the Ki67 index and nuclear grade; however, higher expression of de novo ceramide synthesizing genes is associated with poorer outcomes (98). In the setting of ovarian cancer, an insulin resistance–related disease, Cer(d18:1/16:0), Cer(d18:1/18:1), and Cer(d18:1/18:0) are elevated in plasma of patients with advanced ovarian cancer (76). In hepatocellular carcinoma, ceramides are decreased in tumor tissue compared with adjacent nontumor tissue, yet they are increased in circulation. Furthermore, plasma Cer(d18:1/16:0) correlates with markers of hepatocellular injury (41, 77). Moreover, ceramides are proposed as an early indicator of response to radiotherapy for hepatocellular carcinoma (28). Circulating ceramides, specifically Cer(d18:1/16:0) and Cer(d18:1/24:1), are also proposed as early (before weight loss) markers of cancer cachexia (97). Additional studies with larger sample sizes and prospective designs are merited to elucidate the relationships between tumor and circulating ceramide and cancer incidence, severity, and outcome.

3.5. Origins of Circulating Ceramides that Serve as Biomarkers of Cardiometabolic Disease

As ceramide scores become more widely reported in the literature and utilized in the clinic, it is important to determine why distinct ceramide species associate with discrete disease outcomes (91, 160). For example, why is Cer(d18:1/16:0) positively associated with cardiovascular mortality, while Cer(d18:1/24:0) is inversely associated? Multiple explanations exist, including the tissue sources of particular ceramide species and unique biological roles of specific ceramide species (113, 141). The CERS enzymes that add the variable acyl chain to the sphingoid backbone have unique tissue distributions and substrate specificities that cause particular tissues to be rich in specific acyl chains (Figure 2). For example, muscle has abundant CERS1 and therefore contains predominately Cer(d18:1/18:0) ceramides. Likewise, circulating and intramuscular Cer(d18:1/18:0) is tightly linked with insulin resistance and diabetes, which aligns with muscle ceramides and their role in blunting insulin-stimulated glucose uptake in the muscle in the insulin resistant state (54, 142, 152). The liver predominately expresses CERS2, with resulting biosynthesis of very long chain ceramides, including Cer(d18:1/24:0), Cer(d18:1/24:1), and Cer(d18:1/26:0). Likewise, Cer(d18:1/16:0) is enriched in adipose tissue, which primarily

expresses CERS6. Cross-talk between organs is apparent in preclinical models, as genetic lowering of ceramide in liver or adipose attenuates sphingolipid levels in the alternate organ and improves glycemic control, liver steatosis, and adipose morphology (12, 154).

The majority of circulating sphingolipids are trafficked within lipoproteins and are present in very-low-density lipoprotein, LDL, and high-density lipoprotein (HDL) (45, 119) (Figure 2). Per particle, very-low-density lipoproteins have the highest concentration of SM, ceramide, and S1P, emphasizing the major contribution of hepatic-derived ceramides in circulation (45). Accordingly, apolipoprotein B- (ApoB)-containing lipoprotein deficiencies observed in abetalipoproteinemia or microsomal transfer protein knockout mice confer an 80–90% reduction in plasma ceramides, but not glycosphingolipids (64). Microsomal transfer protein is also essential for packaging of lipids into intestinally derived chylomicrons. Early rat feeding studies with radiolabeled SM or dihydrosphingosine indicate absorption of sphingolipid-derived fatty acids into lymph triglycerides and lecithin (102), and human trials have detected sphingolipids in intestinally derived chylomicrons (83). As such, a portion of sphingolipids shuttled within ApoB-containing lipoproteins could be supplied by enterocytes.

Due to the relative abundance of circulating lipoproteins, most SM is carried within LDL and the larger HDL subfraction HDL2 (45). Ceramide, glycosphingolipids, and dihydrosphingosine are predominantly carried within LDL, sphingosine and dihydroS1P within the smaller HDL3 subfraction, and S1P within HDL3 and bound to albumin (45, 119). While lipoprotein sphingolipid proportions may mirror their tissue of origin, sphingolipids are likely modified within circulation, although these mechanisms are incompletely characterized. SM within LDL can be metabolized to ceramide by secretory SMase (23) (Figure 1). S1P is exported via the transporter Spinster2 (100), and its delivery to and uptake from HDL may be facilitated by phospholipid transfer protein and apolipoprotein M, respectively (15, 158). Yet, little else is known regarding the delivery of sphingolipids to tissues via lipoprotein lipase, lipoprotein endocytosis, or alternative mechanisms.

An emerging field of research regarding lipid transport via small extracellular vesicles (sEVs) has provided an additional method of sphingolipid shuttling aside from lipoprotein packaging and transport. Thus far, sphingolipids have been quantified in sEVs derived from adipose tissue, skeletal muscle, heart, and the endothelium (7, 20, 143). Indeed, ceramides may themselves play a role in sEV sphingolipid shuttling via stimulation of nSMase-dependent formation and release of exosomes (126). The consequences of sphingolipid delivery to distal tissue compartments via sEVs has not been determined but may play a role in whole-body sphingolipid metabolism and cardiometabolic pathogenesis.

4. NUTRITION-RELATED INTERVENTIONS AND CIRCULATING CERAMIDES

Though ceramide-based risk scores are utilized in the clinic, evidence-based recommendations are nonexistent for patients deemed high risk to manage their hyperceramidemia (132). Exploration of lifestyle interventions, including diet, weight loss,

and metabolic surgery, and their effects on circulating ceramide profiles, will contribute to the effective implementation of ceramide-based clinical algorithms and successful ceramide-associated cardiometabolic disease risk mitigation.

4.1. Dietary Patterns and Ceramides

In the past decade, a series of human studies have been conducted to probe for impacts of dietary intake on circulating sphingolipids. An emerging pattern suggests that the degree of unsaturation of dietary lipids modulates the circulating sphingolipidome, although there is little consensus regarding which specific sphingolipids are significantly altered and in which direction (26, 73, 88, 90, 115, 120, 139, 147). Perhaps the most consistent pairing of dietary intervention and lipid outcome in human studies is with palmitate supplementation, which increases long-chain (139) or total circulating ceramides (73, 115). Additionally, overfeeding studies in which fat consumption is raised to 40–60% of energy intake have reported increases in total intramuscular (19) or circulating ceramides (52, 90). Conversely, interventions that increase polyunsaturated fat intake decrease circulating ceramides (79, 115, 140) or shift the balance of accessory chains toward a higher proportion of very-long-chain ceramides (92, 120, 147). These observations suggest that ceramide levels may be induced by increasing the availability of de novo sphingolipid synthesis substrates (e.g., palmitate) or increasing the overall lipid load. Moreover, sphingolipid metabolism may be altered by the anti-inflammatory properties of certain dietary polyunsaturated fats (e.g., docosahexaenoic acid) or their direct actions on expression of ceramide biosynthesis machinery (25, 69).

Interestingly, preliminary studies have linked consumption of dairy, typically high in saturated fat and sphingolipid content, to improvements in circulating sphingolipids (14, 36, 83). Several metabolic improvements with milk SM or polar lipid mixture feeding have been observed in rodent studies, including resolution of high-fat-diet-induced dyslipidemia, gut dysbiosis, liver steatosis, and adipose inflammation (104). Le Barz et al. (83) examined the effects of milk polar lipid feeding in postmenopausal women and ileostomy patients. Despite the 4-week dietary supplementation with milk-derived SM and ceramide, serum Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) paradoxically decreased. Furthermore, ceramide and SM content of intestinally derived chylomicrons decreased, whereas their concentrations in ileal efflux and fecal material increased. Together, these data suggest that dietary composition may significantly impact sphingolipid absorption and/or enterocyte and microbial sphingolipid metabolism to modulate circulating ceramides. Furthermore, reports of neutral or inverse associations of dairy intake with CVD and mortality risk may be partially explained by favorable alterations in sphingolipid homeostasis (138).

At present, we lack experimental evidence and scientific consensus on dietary approaches to modulate ceramide levels and associated disease risk. Future studies must address important questions regarding the mechanisms by which diet affects ceramide synthesis or gut metabolism, which nutrients most significantly impact sphingolipid metabolism, and how these interactions perform within larger dietary patterns. Current studies are limited by differences in lipid measurement and target resolution. Although the majority

of human diet studies cited herein utilized targeted platforms for sphingolipidomics, many quantified only a select few ceramide species to compare between diet groups (Table 2). Future investigations may benefit from inclusion of a wider range of sphingolipid species. Additionally, large studies have typically been observational, and most reports, including interventional trials, rely heavily on self-reported intake data. Lastly, studies conducted thus far primarily represent predominantly white populations from the US and northern Europe, which limits translatability to the black and indigenous populations that experience the highest rates of nutrition-linked chronic disease. Further research is required to distinguish signatures of diverse cultural, racial, and socioeconomic dietary patterns in the context of circulating ceramides and disease risk.

4.2. Ceramides with Weight Loss or Metabolic Surgery

Circulating ceramides are elevated in obese individuals with T2DM or NAFLD (50, 66, 89, 151), and moderate weight loss of 3–5% is a currently recommended therapy to reduce cardiometabolic comorbidities of obesity (116). Yet, it remains unknown whether metabolic improvements observed with clinically significant weight loss are associated with reductions in ceramide levels. Several studies have investigated the impact of weight loss on tissue and circulating ceramides. Kayser et al. (72) reported significant decreases in serum dihydroceramides, d18:1 ceramides, d18:2 ceramides, and SM by 47%, 35%, 39%, and 26%, respectively, in nondiabetic obese adults after 6 weeks of caloric restriction. Promrat and colleagues (112) also reported significant decreases in serum ceramides of obese patients with steatohepatitis after a 1-year diet, exercise, and behavioral weight loss intervention. Alternatively, Dube et al. (27) reported that exercise more effectively decreased intramuscular ceramides than diet-induced weight loss. Translation of these findings, although promising, is limited by differences in methods for sphingolipid quantification, weight loss intervention and duration, and patient population, as well as limited sample size (combined $n = 96$). Considerably more evidence is required to delineate the impacts of diet and lifestyle-related weight loss interventions on levels of circulating ceramides and attenuation of cardiometabolic disease risk.

Bariatric surgery is a durable, long-term weight loss intervention that elicits metabolic improvements, including remission of diabetes, that exceed those achieved by nonsurgical weight loss. Thus far, seven small studies have investigated the effects of bariatric surgery, primarily Roux-en-Y gastric bypass, on circulating (40, 53, 62, 71, 95, 106) or intramuscular (18) sphingolipids. In these reports, bariatric surgery consistently decreased circulating sphingolipids, particularly total and very-long-chain ceramides, up to 6 months after surgery. Decreases in very-long-chain ceramides correlated with improvements in measures of glycemic control and insulin sensitivity (62, 71). Accordingly, very-long-chain ceramides positively correlated with HOMA-IR (106) and glycated hemoglobin (40). Thus, lowering circulating ceramides may contribute to the striking metabolic improvements observed with bariatric surgery and extreme weight loss.

5. MOLECULAR MECHANISMS OF CERAMIDE ACTION

Ceramide concentrations are tightly regulated, and disruptions in this organized system lead to disease pathogenesis. We hypothesize that these outcomes stem from an evolutionarily conserved, two-phase mechanism of ceramide action, with the molecule serving as a nutrient sensor that protects cells from acute intracellular elevations in detergent-like free fatty acids. The two mechanisms are delineated below as the (*a*) metabolic program and (*b*) apoptotic/fibrotic program.

5.1. Metabolic Program

Once inside a cell, free fatty acids are rapidly neutralized via esterification to form acyl-CoAs, which are metabolized according to the cellular energy status. Acyl-CoAs may be joined with glycerol to produce triglycerides as an inert energy store and other glycerolipids integral to lipid bilayer formation (134). Alternatively, acyl-CoAs can also be coupled to carnitine and shuttled into mitochondria for beta oxidation (134). A less common fate for acyl-CoAs is their entry into the sphingolipid synthesis pathway. We hypothesize that in states of free fatty acid overload, acyl-CoA flux into the sphingolipid pathway is enriched, increasing cellular sphingolipid concentrations and initiating the metabolic program. This adaptive metabolic program mitigates fatty-acid driven damage by (*a*) altering fuel choice (e.g., decreasing glucose and amino acid uptake), (*b*) promoting fatty acid esterification and storage, and (*c*) decreasing mitochondrial efficiency (Figure 3). When these adaptations are insufficient and ceramides increase beyond a critical threshold, they elicit an apoptotic/fibrotic program to minimize tissue and organismal damage resulting from cell lysis and the release of harmful cellular debris (Figure 3). Ceramide-mediated cellular reprogramming is protective in the short-term but maladaptive with chronic overnutrition. We hypothesize that extended activation of ceramide signaling drives many features of the metabolic syndrome and ultimately drives the pathogenesis of nutrition-related chronic disease.

5.1.1. Decreased uptake and utilization of glucose and amino acids.—Insulin stimulates glucose uptake into muscle and adipose tissues by initiating the translocation of glucose transporter 4 (GLUT4) from the cytoplasm to the plasma membrane (135). Insulin initiates a cascade of signaling events that directly phosphorylates and activates Akt (also known as protein kinase B), which stimulates GLUT4 translocation, allowing glucose to enter the cell (133). Ceramides inhibit Akt phosphorylation downstream of insulin signaling via protein phosphatase 2A (PP2A) and protein kinase C zeta (PKCζ) (135, 161). PP2A dephosphorylates two activating residues, while PKCζ phosphorylates Akt at an inhibitory site (11, 111, 117, 163). Physiologically, this culminates in impaired insulin-stimulated glucose uptake (135). In addition to decreasing uptake and utilization of glucose, ceramides decrease the intracellular amino acid pool and diminish signaling through the mTOR (mammalian target of rapamycin) pathway by sequestering the amino acid transporter, SNAT2, away from the plasma membrane (30, 42, 63). The molecular mechanisms by which ceramides inhibit amino acid uptake are not clearly understood but are also potentially mediated by PP2A (30, 42). By downregulating amino acid transporters, ceramides essentially starve the cell of nonfatty acid energy sources and induce homeostatic autophagy to increase consumption of the overflowing fatty acids (42).

In alignment, genetic and pharmacological inhibition of de novo ceramide synthesis alleviates insulin resistance in multiple rodent models of diabetes (12, 107, 134). Treatment with myriocin, an irreversible, high-affinity inhibitor of SPT, or fenretinide, a synthetic retinoid inhibitor of DES1, prevents or reverses insulin resistance (11, 59, 134). *Sptlc2* deletion in the liver, or whole-body haploinsufficiency, elicits similar glycemic improvements (107, 134). Furthermore, excising the *Degs1* gene (encoding DES1) from whole body, liver, or adipose tissue of adult mice improved glucose tolerance and insulin sensitivity in animals with diet-induced obesity or leptin deficiency (12).

5.1.2. Increased fatty acid uptake, esterification, and storage.—In addition to altering glucose metabolism, ceramides also modify lipid handling. Via PKCζ activation, ceramides increase expression and promote translocation of fatty acid transporter cluster of differentiation 36 (CD36) to the cell membrane, which facilitates passage of fatty acids through lipid bilayers and promotes their esterification (11, 12, 60). Additionally, PKCζ induces sterol regulatory element binding transcription factor 1 (*Srebf1*) and its downstream transcriptional targets, thereby stimulating the transformation of deleterious free fatty acids into inert triglycerides for storage (11, 12). Analogously, ceramides also inhibit lipolysis through PP2A activation, which negatively regulates hormone-sensitive lipase (HSL) (12). En masse, these mechanisms lower cellular free fatty acid levels by enhancing uptake and storage while simultaneously blunting lipolysis, which ultimately protects the cell from free fatty acid overload.

Again, genetic and pharmacological inhibition of de novo ceramide synthesis in mouse models ameliorates symptoms of lipid accumulation. Inducible *Degs1* ablation in mice dramatically lowers hepatic expression of *Srebf1* and its transcriptional targets encoding proteins facilitating triglyceride storage (12). Additionally, *Degs1* ablation inhibits fatty acid uptake into hepatocytes by decreasing activation of PKCζ (12).

5.1.3. Decreased mitochondrial efficiency.—Cardiometabolic diseases are frequently associated with impaired mitochondrial function and increased oxidative stress, which may be partially explained by ceramide actions. Ceramides decrease electron-transport chain activity, increase membrane permeability, and promote mitochondrial fission (11). Elevated Cer(d18:1/16:0), in particular, is demonstrated to inhibit electron-transport chain complex II and IV activity and increase reactive oxygen species production (162). Specifically, Cer(d18:1/16:0) derived from CERS6 promotes mitochondrial fragmentation by interacting with mitochondrial fission factor, leading to a change in mitochondrial morphology and a decrease in respiratory capacity (46). Ultimately, these actions make mitochondria less efficient, which we presume allows the cell to consume more fatty acid substrate at the expense of increasing reactive oxygen species generation.

Several studies show that inhibiting ceramide synthesis leads to improved oxidative phosphorylation. Firstly, *Cers6* knockout mice were protected from high fat diet–induced mitochondrial dysfunction and displayed significantly increased oxygen consumption rates and extracellular acidification rates compared with controls (46). Additionally, *Degs1* depleted mice showed enhanced mitochondrial complex activity in white adipose tissue compared with controls (12).

5.2. Apoptotic and Fibrotic Program

Ceramide induction of apoptosis allows for controlled cell death, preventing the release of cytosolic content into the extracellular space, which would otherwise occur following uncontrolled cell lysis. Similarly, the induction of fibrosis allows the organism to minimize widespread damage related to tissue inflammation and necrosis. Thus, ceramide-mediated apoptosis and fibrosis protect the organism from damage resulting from uncontrolled injury.

As ceramides accumulate, they increase mitochondrial outer membrane permeability, which stimulates cytochrome c release and apoptosis initiation (125). Blocking ceramide production reverses the proapoptotic cascade (110). In addition to apoptosis, ceramides are suspected to activate TGF-β signaling, which is a key regulator of collagen expression (110). More specifically, ceramides have a synergistic effect on the intensity of TGF-β signaling cascade by inducing mothers against decapentaplegic homolog 3 (SMAD3) phosphorylation and increasing collagen promoter activity (118). Correspondingly, myriocin treatment of rats fed a high-fat diet attenuated hepatic ceramide accumulation, fibrosis, and cleaved caspase 3 levels (68).

Collectively, the aforementioned studies suggest that ceramides are influential regulators of glucose homeostasis, lipid metabolism, and programmed cell death. Ceramides could therefore be a therapeutic target to ameliorate the pathological mechanisms and clinical endpoints of nutrition-linked chronic disease.

6. SUMMARY AND CONCLUSION

A rapidly growing and clinically translatable body of evidence implicates ceramides as lipotoxic drivers and potent biomarkers of nutrition-linked chronic diseases. Further technical refinement and study in globally representative populations are required to facilitate widespread clinical application. Harmonization of sphingolipid measurement methods and development of reference populations is a critical technical bottleneck that demands attention. Furthermore, effective ceramide-lowering recommendations merit development in order for patients to reduce their hyperceramidemia-related cardiometabolic disease risk. A detailed understanding of dietary ingestion of sphingolipids, gut microbiota sphingolipid synthesis, and metabolic disease state influence on ceramides—as well as a map of the downstream mechanisms of action—is necessary to effectively reap the therapeutic and prognostic potential of ceramides in nutrition-linked chronic disease. Understanding the genetic basis of hyperceramidemia, as well as the response to lifestyle changes including exercise, metabolic surgery, and dietary interventions on ceramide concentrations, will all contribute to the effective implementation of ceramide-based clinical algorithms and successful ceramide-associated cardiometabolic disease risk mitigation.

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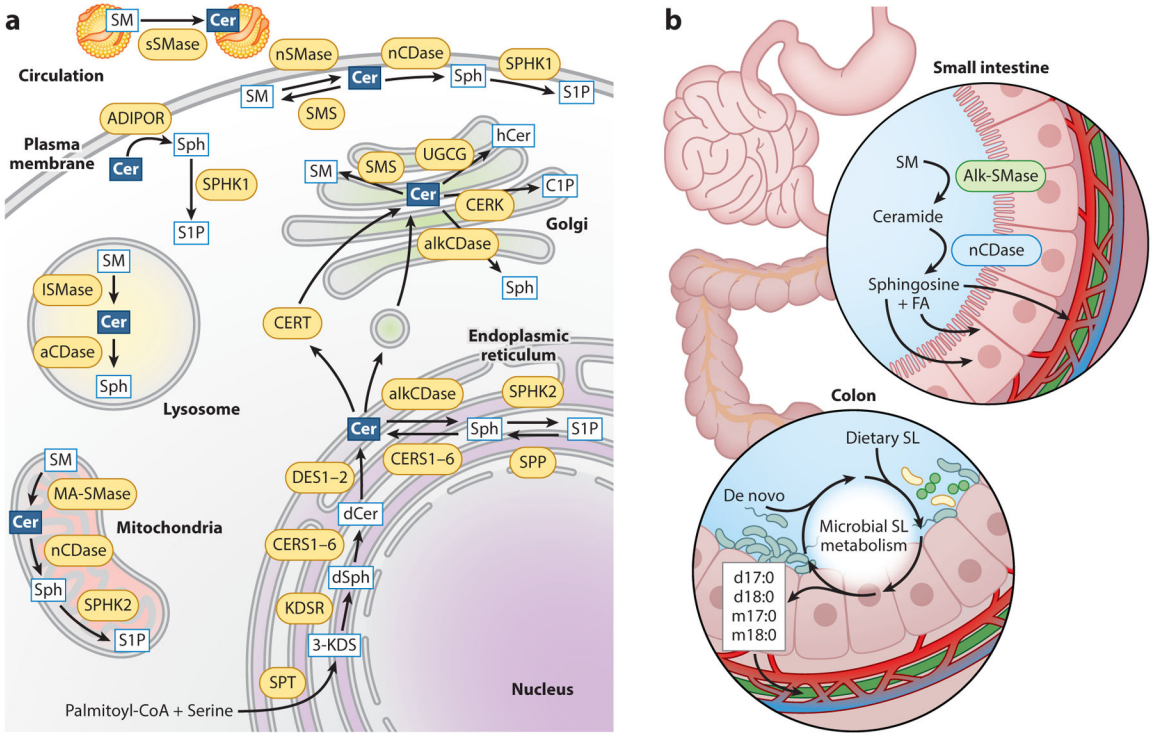

Figure 1.

Intracellular and intestinal ceramide metabolism. (*a*) Sphingolipids are metabolized in various subcellular compartments. Ceramides are produced via a de novo biosynthetic pathway in the endoplasmic reticulum (ER) and are transported to the Golgi apparatus for synthesis of complex sphingolipids. Ceramide can be regenerated from SM hydrolysis in the mitochondria, lysosome, plasma membrane, and circulating lipoproteins. Deacylation of ceramides generates sphingosine, which can be phosphorylated to form S1P. (*b*) Dietary SM and ceramide are digested in the small intestine by alk-SMase and nCDase, respectively, to form sphingosine, which is absorbed by enterocytes. In the large intestine, dietary and de novo sphingolipids are metabolized by gut microbiota to generate unique odd-chain and deoxysphingolipid species. Abbreviations: 3-KDS, 3-ketosphinganine; aCDase, acid ceramidase; ADIPOR, adiponectin receptors; alkCDase, alkaline ceramidase; alk-SMase, alkaline sphingomyelinase; C1P, ceramide-1-phosphate; cer, ceramide; CERK, ceramide kinase; CERS, (dihydro)ceramide synthase; CERT, ceramide transport protein; dCer, dihydroceramide; DES, dihydroceramide desaturase; dSph, dihydrosphingosine; FA, fatty acid; hCer, hexosylceramide; KDSR, 3-ketodihydrosphingosine reductase; lSMase, lysosomal ceramidase; MA-SMase, mitochondria-associated sphingomyelinase; nCDase, neutral ceramidase; nSMase, neutral sphingomyelinase; S1P, sphingosine-1-phosphate; SL, sphingolipid; SM, sphingomyelin; SMS, sphingomyelin synthase; sph, sphingosine; SPHK1, sphingosine kinase 1; SPHK2, sphingosine kinase 2; SPP, sphingosine-1-phosphate phosphatase; SPT, serine palmitoyltransferase; sSMase, secretory sphingomyelinase; UGCG, glucosylceramide synthase.

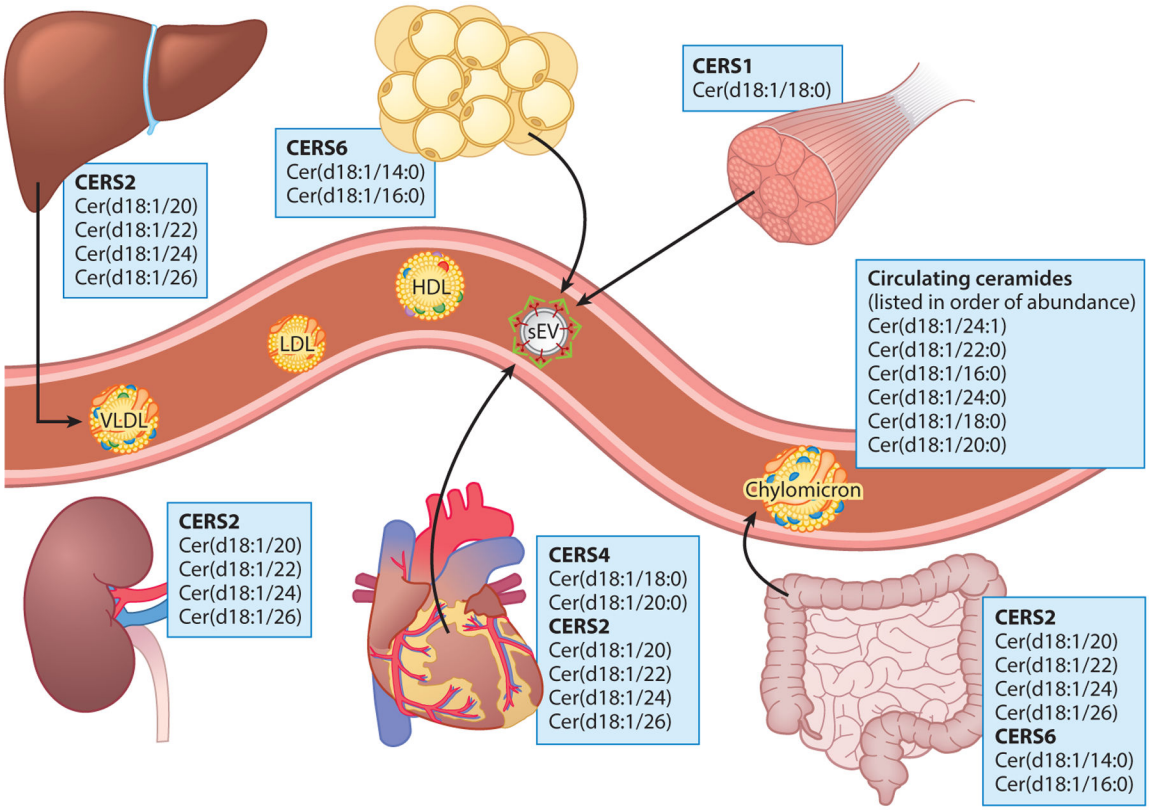

Figure 2.

Species-level ceramide sources based on CERS enzyme expression profile and substrate specificity. Ceramide synthases, the step in the de novo synthesis pathway that adds a variable acyl chain to the sphingoid backbone, result in much of the diversity of the sphingolipid pool. Varied tissue expression and substrate selectivity result in unique tissue distributions of ceramide species. The circulating ceramide pool reflects the tissue distribution of the CERS enzymes and their substrate preferences. Abbreviations: CERS1–6, ceramide synthases 1–6; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; sEV, small extracellular vesicle).

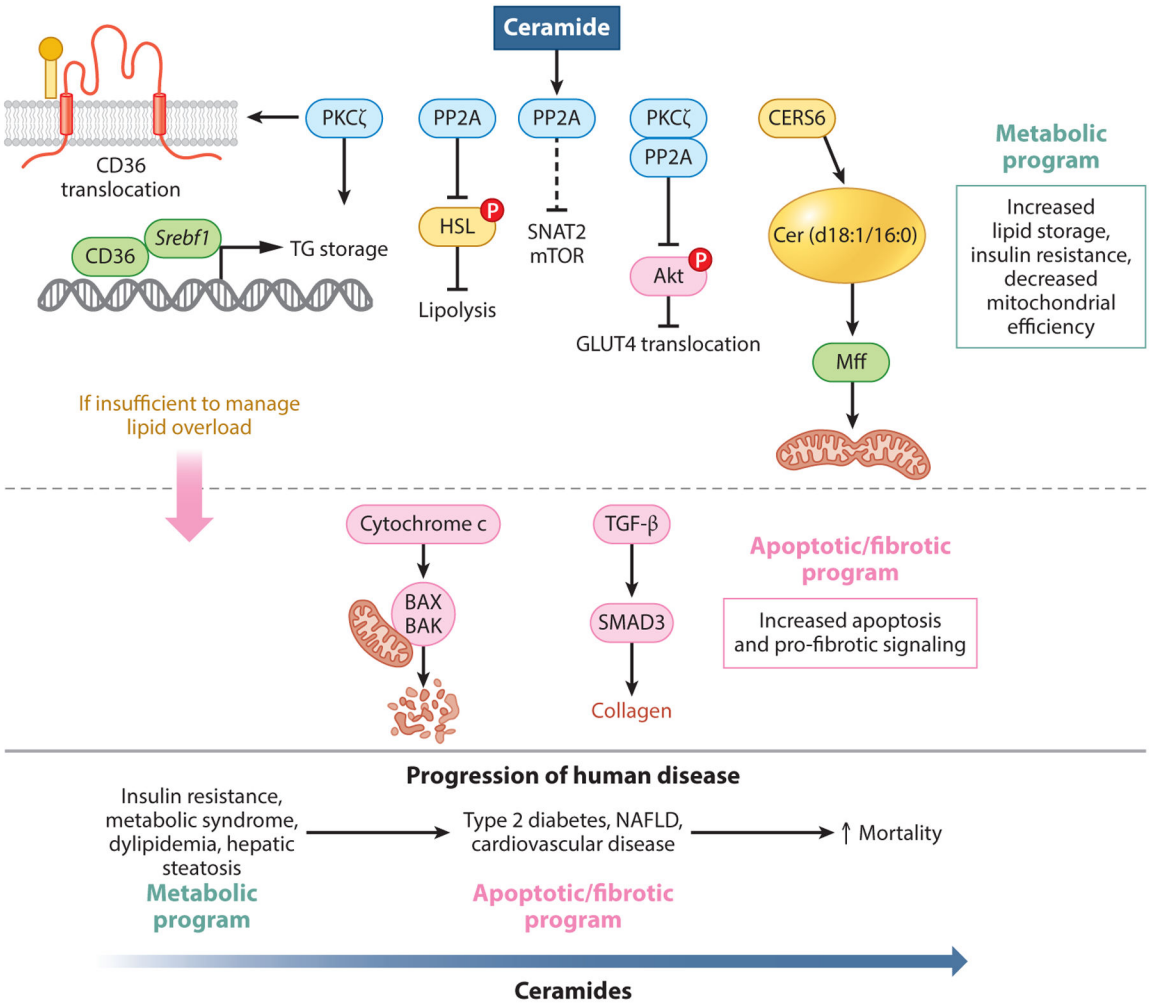

Figure 3.

Ceramide-mediated alterations of cellular metabolism in states of fatty acid overload. In the protective metabolic program, ceramides activate PP2A, which enacts downstream mechanisms promoting fatty acid esterification and storage, inhibiting lipolysis via inhibitory phosphorylation of HSL and reducing glucose and amino acid metabolism. Ceramides also activate PKCζ, which inhibits glucose uptake by preventing phosphorylation of Akt. Additionally, CERS6 derived Cer(d18:1/16:0) species interact directly with mff, promoting mitochondrial fragmentation and reduced efficiency. If this metabolic program is unable to quench incoming fatty acids, ceramides trigger an apoptotic/fibrotic program to minimize lipid-mediated cellular damage. This program includes programmed cell death and collagen deposition. While mechanisms of ceramide are protective in the acute setting, chronic overnutrition leads to maladaptive ceramide signaling driving disease pathogenesis. Abbreviations: Akt, protein kinase B; BAK, BCL2-antagnist/killer 1; BAX, BCL2 associated X protein; CERS6, (dihydro) ceramide synthase 6; CD36, cluster of differentiation 36; cytochrome c, cytochrome complex; GLUT4, glucose transporter 4; HSL, hormone sensitive lipase; mff, mitochondrial fission factor; mTOR, mechanistic target of rapamycin; NAFLD, nonalcoholic fatty liver disease; P, phosphorylation; PKc,

protein kinase C; PP2A, protein phosphatase 2A; SMAD3, mothers against decapentaplegic homolog 3; SNAT2, sodium-coupled neutral amino acid transporter 2; *Srebf1*, sterol regulatory element binding transcription factor 1; TG storage, triglyceride storage; TGF-β, transforming growth factor beta.

Table 1

Ceramides as biomarkers of nutrition-linked chronic disease

Participants/region	Study design	Disease outcome	Measurement	Sphingolipid associations
FINRISK 2002 (*n* = 8,101 adults), Finland (51)	Prospective cohort (13 years follow-up)	Incident MACE, recurrent MACE, fatal incident MACE	4 ceramides (in serum), targeted LC-MS/MS	Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1) were positively associated with incident MACE, fatal incident MACE, and recurrent MACE.
Corogene (*n* = 160), Finland; Special Program University Medicine-Inflammation in Acute Coronary Syndrome (SPUM-ACS) (*n* = 1,637), Switzerland; Bergen Coronary Angiography Cohort (BECAC) (*n* = 1,587), Norway (78)	Prospective cohort studies	Cardiovascular death	4 ceramides (in serum), targeted LC-MS/MS	Cer(d18:1/16:0), Cer(d18:1/24:1) were positively associated with cardiovascular death in Corogene and BECAC; Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1) were positively associated with cardiovascular death in SPUM-ACS; Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1) relative to Cer(d18:1/24:0) were significantly associated with cardiovascular death in all 3 cohorts.
Utah CAD Study (*n* = 674), United States (109)	Case control study	CAD prevalence	6 ceramides, 6 dihydroceramides, 6 glucosylceramides, 6 dihydro-sphingomyelins, 6 sphingomyelins, sphinganine, sphingosine (in serum), targeted LC-MS/MS	All ceramides were significantly positively associated with CAD; study generated the sphingolipid-inclusive CAD risk score.
Ludwigshafen Risk and Cardiovascular Health (LURIC) study, patients with CAD (*n* = 445); separate randomized parallel arm treated with simvastatin (*n* = 24) or ezetimibe (*n* = 24) or both (*n* = 24); participants with LOF (R46L) mutation in PCSK9 gene (*n* = 19), Germany (136)	Case control derived from prospective cohort study with parallel randomized control study arm	CAD death; ceramide concentration in response to lipid lowering medication	8 ceramides (in plasma), UPLC-MS	Simvastatin decreased ceramides by 25% but ezetimibe had no effect on ceramide concentrations; patients harboring the R46L PCSK9 mutation had decreased ceramide concentrations.
Study including all patients admitted to the Department of Emergency of the First Affiliated Hospital of Xinxiang Medical University with first-ever ischemic stroke (*n* = 404), China (43)	Case control study	Acute ischemic stroke	3 ceramides (in plasma), targeted LC-MS/MS	Plasma ceramides are significantly increased in patients with ischemic stroke compared with age- and sex-matched controls; increased ceramides are associated with moderate to high risk of stroke.
Western Norway Coronary Angiography Cohort (WECAC) (*n* = 3,789), Norway; Long-Term Intervention with Pravastatin in Ischemic Disease trial (LIPID) (*n* = 5,991); Langzeiterfolge der KARdiOLogischen Anschlussheilbehandlung (KAROLA) (*n* = 1,023), Germany (54)	Prospective cohort studies	Cardiovascular death	4 ceramides, 3 phospholipids, targeted LC-MS/MS	Study used ceramide and phospholipid data to develop the CERT2 score, which is highly significant in predicting cardiovascular death in all 3 cohorts.
Cardiovascular Health Study (*n* = 4,249), United States (85)	Prospective cohort study	Incident heart failure	4 ceramides, 4 sphingomyelins (in plasma), targeted LC-MS/MS	Cer(d18:1/16:0) was significantly associated with incident heart failure.
Stabilization of Atherosclerotic Plaque by Inhibition of Darapladib Therapy (STABILTY) (*n* = 11,222), multiple geographical locations (57)	Case control study	MACE, MI, stroke, cardiovascular death,	3 ceramides, 3 phospholipids, targeted LC-MS/MS	CERT2 score and its components were significantly associated with MACE, MI, stroke,

Participants/region	Study design	Disease outcome	Measurement	Sphingolipid associations
		hospitalization due to heart failure		cardiovascular death, and hospitalization due to heart failure.
Framingham Heart Study (*n* = 2,642), United States; Study of Health in Pomerania (SHIP) (*n* = 3,134), Germany (108)	Prospective cohort studies	Coronary heart disease and mortality	3 ceramides, LC-MS	Cer(d19:1/24:0)/Cer(d18:1/16:0) ratio was inversely associated with cardiovascular disease risk; Cer(d19:1/24:0)/Cer(d18:1/16:0) and Cer(d181/22:0)/Cer(d18:1/16:0) ratios were inversely associated with all-cause mortality.
Cohorte LausannoiseCohort (CoLaus) (*n* = 150), Switzerland; Devenir des Spondyloarthrites Indifferenciees Recentes (DESIR) cohort (*n* = 160), France (152)	Longitudinal cohort studies	Prevalent and incidence diabetes	5 dihydroceramides, 9 ceramides, 7 globotriaosyl ceramides, 9 glucosyl/galactosyl ceramides, 7 lactosyl ceramides, targeted LC-MS/MS	Cer(d18:0/16:0), Cer(d18:1/22:0), Cer(d18:0/23:0), Cer(d18:0/24:0), Cer(d18:0/24:1), Cer(d18:0/23:0), Cer(d18:1/18:0), and Cer(d18:1/22:0) were elevated 5 years before diabetes diagnosis; Cer(d18:0/24:0), Cer(d18:0/24:1), Cer(d18:0/22:0), and Cer(D18:0/23:0) were elevated 9 years before diabetes diagnosis.
European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis (ATHEROREMO) (*n* = 581), Europe (3)	Prospective cohort study	Incident MACE in patients with CAD	5 cholesterol esters, 4 ceramides (in serum), targeted LC-MS/MS	Cer(d18:1/16:0) was associated with MACE; Cer(d18:1/16:0), Cer(d18:1/20:0), and Cer(d18:1/24:1) relative to Cer(d18:1/24:0) were associated with death and nonfatal acute coronary syndrome.
Cardiovascular Health Study (*n* = 3,645), United States (32)	Prospective cohort study	Incident diabetes	5 ceramides, 6 sphingomyelins, 3 hexosyl-ceramides (in plasma), targeted LC-MS/MS	Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20), and Cer(d18:1/22:0) were associated with higher risk of diabetes.
Strong Heart Study (*n* = 435), Strong Heart Family Study (*n* = 1,902), United States (American Indian population) (31)	Nested case control stud, parallel prospective cohort	Incident diabetes	6 ceramides, 6 sphingomyelin species, 3 glucosyl ceramides, 1 lactosyl ceramide (in plasma), sequential LC-MS (in plasma)	Cer(d18:1/18:0), Cer(d18:1/20:0), and Cer(d18:1/22:0) were associated with a higher risk of diabetes.
Strong Heart Family Study (*n* = 2,086), United States (American Indian population) (86)	Prospective cohort study	Baseline and follow-up HOMA-IR and insulin; associations with BMI	5 ceramides, 6 sphingomyelins, 3 glucosyl ceramides, 1 lactosyl ceramide, targeted reverse phase chromatography	Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0) were associated with higher plasma insulin and HOMA-IR at baseline; SM(d18:1/18:0), SM(d18:1.20:0), SM(d18:1//22:0), and SM (d18:1/24:0) were inversely associated with insulin and HOMA-IR in individuals with a normal BMI.
FINRISK 2002 (*n* = 8,045 adults), Finland; Western Norway Coronary Angiography Cohort (WECAC) (*n* = 3,344), Norway; Prevent Metabolic Syndrome Cohort (PrevMetSyn) (*n* = 371) (55)	Prospective cohort studies, interventional trial	Incident diabetes	4 ceramides, targeted LC-MS/MS	Cer(d18:1/18:0)/Cer(d18:1/16:0) ceramide ratio was predictive of incident diabetes; ceramide ratio decreased in individuals with 5% or more weight loss.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; Cer, ceramide; CERT2, Cardiac Event Risk Test 2; HOMA-IR, homeostatic model assessment of insulin resistance; LC-MS, liquid chromatography tandem mass spectrometry; LOF, loss of function; MACE, major adverse cardiovascular event; MI, myocardial infarctions; MS, mass spectrometry; SM, sphingomyelin; UPLC-MS, ultra performance liquid chromatography tandem mass spectrometry.

Table 2

Circulating ceramides with dietary pattern interventions

Participants/region	Study design	Experimental design	Measurement/class	Sphingolipid outcomes
980 participants from the PREDIMED Trial, 25% with incident cases of CVD, Spain (148)	Case-cohort	1 year of Mediterranean diet supplemented with EVOO or nuts, with low-fat diet control	Untargeted LC-MS/MS metabolomics; plasma Cer(d18:1/16:0), Cer(d18:1/22:0), Cer(d18:1/24:0), and Cer(d18:1/24:1)	No change was detected in plasma ceramides.
5,124 adults from the Framingham Offspring Study, United States (147)	Cross-sectional	Dietary pattern scores (DGAI, MDS) assessed from Harvard FFQ	Targeted LC-MS/MS; plasma Cer(d18:1/16:0), Cer(d18:1/22:0), and Cer(d18:1/24:0)	DGAI score was associated with lower Cer(d18:1/16:0), Cer(d18:1/22:0), Cer(d18:1/24:0), and Cer(d18:1/22:0)/Cer(d18:1/16:0) ratio; SFA intake was associated with Cer(d18:1/16:0); fat and sugar intake were inversely associated with Cer(d18:1/22:0)/Cer(d18:1/16:0) ratio; MDS was associated with lower Cer(d18:1/16:0) and Cer(d18:1/22:0) and higher Cer(d18:1/24:0) and Cer(d18:1/24:0)/Cer(d18:1/16:0) ratio; Cer(d18:1/24:0) was associated with nut intake and MUFA/SFA intake ratio and negatively associated with fruit intake; and Cer(d18:1/24:0)/Cer(d18:1/16:0) ratio was associated with nut intake, MUFA/SFA intake ratio, and vegetable intake.
106 adults with metabolic syndrome, Finland (81)	Randomized controlled trial	12-week parallel study of healthy diet with whole grain products, fatty fish (3 servings/week) and bilberries (300 g/day); whole grain enriched diet with whole grain products; or control diet with refined grain products	Targeted UPLC-MS; plasma ceramides	No change was present in plasma ceramides.
200 adults with metabolic syndrome from the Systems Biology in Controlled Dietary Interventions and Cohort studies (SYSDIET) study, Finland, Denmark, Sweden, Iceland (80)	Randomized controlled trial	18–24 week parallel study of healthy Nordic diet (whole grains, fruits, vegetables, berries, vegetable oils and margarines, fish, low-fat dairy, low-fat meat) and average Nordic diet control	Targeted UPLC-MS; plasma ceramides	Cer(d18:1/22:0), Cer(d18:1/23:0), and Cer(d18:1/24:0) decreased after 8 weeks in the Healthy Nordic diet group; differences normalized by 18 and 24 weeks.
96 middle-aged adults, United States (26)	Cross-sectional	HEI-2015 assessed from 24-hour dietary recall	Targeted LC-MS; plasma Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/22:0), and Cer(d18:1/24:0)	Higher HEI-2015 score and total vegetable and whole grain intake were inversely associated with Cer(d18:1/22:0); saturated fat and sugar intake were positively associated with Cer(d18:1/22:0).
36 overweight young adults, United States (93)	Non-randomized, repeated measures	8-week high-fruit, high-vegetable diet (FRUVED) according to USDA MyPlate, alone or with low-refined-carbohydrate (FRUVED+LRC) or low-fat (FRUVED+LF) guidelines	Targeted LC-MS/MS; serum ceramides, glucosylceramides, and lactosylceramides	Total ceramide, Cer(d18:1/22:0), Cer(d18:1/24:1), and Cer(d18:1/26:0) were significantly lower at 5 weeks in FRUVED+LRC and FRUVED+LF groups compared with baseline. GluCer(d18:1/22:0), GluCer(d18:1/24:0), GluCer(d18:1/24:1), LacCer(d18:1/22:0), and LacCer(d18:1/24:0) levels decreased with time in FRUVED+LRC and FRUVED+LF groups. Cer(d18:1/16:0) was higher in all study groups at 8 weeks compared with baseline.

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Participants/region	Study design	Experimental design	Measurement/class	Sphingolipid outcomes
31 CAD patients previously treated with PCI, Sweden (24)	Cross-over randomized controlled trial	4-week lacto-ovo-vegetarian diet (VD) or isocaloric diet with daily meat consumption (MD) separated by 4-week washout period	Targeted UPLC-MS/MS; plasma ceramides, hexosylceramides, lactosylceramides, and sphingomyelins	Cer(d18:1/16:0) and a WGCNA lipid cluster dominated by SM and ceramide species were lower in VD than MD. Cer(d18:1/16:0) and Cer(d18:1/22:0), HexCer(d18:1/22:0), and LacCer(d18:1/16:0) decreased in VD from baseline.
5,124 adults from the Framingham Offspring Study, United States (147)	Cross-sectional	SSB intake assessed from Harvard FFQ	Targeted LC-MS/MS; plasma Cer(d18:1/16:0), Cer(d18:1/22:0), and Cer(d18:1/24:0)	SSB intake was positively associated with Cer(d18:1/16:0) and Cer(d18:1/22:0). SSB intake was positively associated with Cer(d18:1/24:0) only in prediabetic or diabetic individuals.
30 adolescent and overweight male habitual SSB consumers, United States (14)	Cross-over randomized controlled trial	3 weeks of daily consumption of 24 oz soda or 22 oz 2% milk separated by a 2-week washout period	Targeted UPLC-MS/MS; plasma sphingoid bases, ceramides, hexosylceramides, and spingomyelins	GluCer(d18:1/16:0), LacCer(d18:1/16:0), and LacCer(d18:1/18:0) significantly decreased during the milk consumption period.
1,099 participants in the Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-up Study, United States (92)	Cross-sectional	Frequency of nut intake assessed from FFQs	Untargeted LC-MS; plasma sphingolipids	Nut consumption was positively correlated with Cer(d18:1/24:0), SM(d18:1/22:0), and SM(d18:1/24:0) and negatively associated with SM(d18:1/18:0).
10 obese adults, United States (140)	Cross-over, double-blinded, randomized controlled trial	5-day inpatient study with daily ingestion of 48 g walnuts or placebo with a 1-month washout period	Untargeted LC-MS; plasma sphingolipids	Walnut feeding was inversely associated with total ceramide, SM, and mono- and di-hexosylceramide.
20 healthy adults, United States (139)	Cross-over, double-blinded, randomized controlled trial	2 weeks of daily consumption of chocolate spread enriched with EVOO or palm oil	Targeted LC-MS/MS; 25 plasma sphingolipids	Cer(d18:1/16:0), Cer(d18:1/16:0)/Cer(d18:1/22:0)+Cer(d18:1/24:0) ratio, and SM(d18:1/18:0) were higher with palm oil feeding than EVOO.
61 overweight adults, Sweden (115)	Double-blinded, randomized controlled trial	8-week parallel feeding study of overconsumption with muffins enriched in sunflower oil (PUFA) or palm oil (SFA) followed by a 4-week caloric restriction period	Targeted UPLC-MS; serum and adipose dihydroceramides, ceramides, hexosylceramides, sphingoid bases, and sphingomyelin	SFA overfeeding led to an increase in the majority of serum sphingolipids, whereas PUFA overfeeding was associated with a decrease in all serum sphingolipids excepting Cer(d18:1/24:0) and Cer(d18:0/24:0). Serum sphingolipids normalized following caloric restriction period. Differences in adipose sphingolipids were not specified.
18 healthy adults, United States (73)	Cross-over, randomized controlled trial	3-week high-palmitate (HPA) or low-PA, high-oleic acid (HOA) diet separated by a 1-week washout period	Targeted LC-MS/MS; serum ceramides	Serum ceramides were significantly higher with HPA versus HOA feeding.
2,860 Chinese Singaporeans from the Singapore Prospective Study Program, Singapore (120)	Cross-sectional	Dietary fat and protein intake estimated from FFQs	Targeted UPLC-MS; plasma ceramides, hexosylceramides, sphingoid bases, and sphingomyelins	SFA intake was associated with total SM; long chain SM; and ceramides, hexosylceramides, SM, and phosphorylated sphingoid bases with a 16:1 backbone. PUFA intake was negatively associated with Cer(d18:1/16:0), HexCer(d18:1/16:0), Cer(d18:1/18:0), and HexCer(d18:1/18:0). No associations were present between MUFA intake and plasma sphingolipids. Protein intake was negatively associated with all sphingolipid classes and individual species with 18:1 and 18:2 backbones, excepting Cer(d18:1/16:0) and Cer(d18:1/18:0) ceramides; protein intake was positively associated with 16:1 backbone SMs.

Participants/region	Study design	Experimental design	Measurement/class	Sphingolipid outcomes
33 subjects with recent acute myocardial infarction or unstable ischemic attack, Finland (79)	Randomized controlled trial	8-week parallel study of fatty fish, lean fish, or control diet	Targeted UPLC-MS; plasma sphingolipids	Total ceramides decreased from the baseline in the fatty fish group.
33 healthy adults, Norway (105)	Double-blinded, randomized controlled trial	7-week parallel study of supplementation with 8 g/day fish oil or sunflower oil	Targeted UPLC-QTOFMS; plasma ceramides and sphingomyelins	Very-long-chain SM species significantly increased in the fish oil group. No change was reported in ceramides.
58 postmenopausal women in the Milk Polar Lipids Consumption, Lipid Metabolism, and Inflammation in Menopausal Women cohort (VALOBAB-C) and 4 ileostomy patients (VALOBAB-D), France (83)	Double-blinded, randomized controlled trial	VALOBAB-C: 4-week parallel study of daily supplementation with milk polar lipid (PL)-enriched cream cheese (0, 3, or 5 g PL); VALOBAB-D: Cross-over study with acute milk PL-enriched cream cheese intake (0, 3, or 5 g PL) separated by 4–6 week washout periods	Targeted HPLC-MS/MS; serum, chylomicron, ileal efflux, and fecal ceramides and SM	Milk PL intake decreased serum SM(d18:1/16:1), SM(d18:1/18:1), SM(d18:1/20:1), and Cer(d18:1/24:1); serum Cer(d18:1/20:0), SM(d18:1/20:0), and SM(d18:1/22:1) increased with PL feeding. Milk PL intake decreased intestinally derived chylomicron ceramide and SM content. Milk PL intake increased ceramide and SM levels in ileal efflux and feces.
105 healthy adults from the DESIR cohort, France (36)	Observational	Cheese and noncheese dairy consumption was assessed with FFQs	Shotgun lipidomics; plasma dihydroceramides and ceramides	Noncheese dairy intake was inversely correlated with total dihydroceramide, Cer(d18:0/16:0), Cer(d18:0/22:0), Cer(d18:0/23:0), Cer(d18:0/24:0), Cer(d18:0/24:1), Cer(d18:1/26:0), and Cer(d18:1/26:1) levels in women.
16 healthy adult men, Australia (94)	Cross-over, randomized controlled trial	Breakfast meals consisting of dairy fat or soy oil were consumed 4–6 weeks apart	Targeted ESI-MS/MS; plasma dihydroceramide, ceramide, hexosylceramide, and sphingomyelin	Total dihydroceramide and sphingomyelin levels increased postprandially 4 h following the dairy fat meal. Total dihydroceramide, ceramide, GM3, and sphingomyelin decreased postprandially 1 h following the soy oil meal.
29 adult men, United States (19)	Prospective cohort	8-week period of overfeeding: 140% of kcal to maintain body weight, 44% of kcal from fat	Targeted LC-MS/MS; muscle ceramides	Muscle Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/18:1), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0), Cer(d18:1/24:1), and total ceramides increased post-overfeeding compared with baseline.
40 healthy adults, Australia (52)	Prospective cohort	4-week period of overfeeding: 1,250 kcal above baseline, 45% kcal from fat	Targeted HPLC-MS; serum sphingosine, dihydroceramides, ceramides, hexosylceramides, GM3, and sphingomyelins	Total ceramides increased with overfeeding. Cer(d18:1/22:0), Cer(d18:0/22:0), HexCer(d18:1/22:0), GM3(d18:1/22:0), Cer(d18:1/24:0), Cer(d18:0/24:0), HexCer(d18:1/24:0), and GM3(d18:1/24:0) increased with overfeeding. Cer(d18:1/18:0), Cer(d18:0/18:0), GM3(18:1/18:0), Cer(d18:1/24:1), HexCer(d18:1/24:1), and GM3(d18:1/24:1) decreased with overfeeding.
38 overweight adults, Finland (90)	Randomized controlled trial	3-week parallel study of 1,000 kcal/day overfeeding with saturated fat (SAT), unsaturated fat (UNSAT), or simple sugars (CARB)	UPLC-QTOFMS; plasma ceramides, and dihydroceramides	Total ceramide, total dihydroceramide, Cer(d18:1/24:0), Cer(d18:1/24:1), Cer(d18:0/24:0), and Cer(d18:2/23:0) increased in the SAT overfeeding group. No changes were reported in sphingolipids in the UNSAT or CARB groups.
50 adults with active rheumatoid arthritis, Sweden (88)	Cross-over, randomized controlled trial	10-week Mediterranean-style diet intervention or Western-style diet control separated by a 2–5 month washout	Targeted LC-MS/MS; serum ceramides, lactosylceramide, glucosyl/galactosylceramide, globoceramide, and sphingomyelin	CERT2 risk score and total serum ceramides increased during control diet feeding period but did not decrease after Mediterranean diet intervention.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; Cer, ceramide; CERT2, Cardiac Event Risk Test 2; CVD, cardiovascular disease; DGAI, Dietary Guidelines for American Adherence Index; EVOO, extra-virgin olive oil; ESI-MS, electrospray ionization mass spectrometry; FFQ, food frequency questionnaire; GluCer, glucosylceramide; GM3, monosialodihexosylganglioside; HEI-2015, healthy eating index-2015; HexCer, hexosylceramide; LacCer, lactosylceramide; LC-MS, liquid chromatography tandem mass spectrometry; MDS, Mediterranean Diet Score; MUFA, mono-unsaturated fatty acid; PA, palmitic acid; PCI, percutaneous coronary intervention; PUFA, poly-unsaturated fatty acid; SFA, saturated fatty acid; SM, sphingomyelin; SSB, sugar sweetened beverages; UPLC-MS, ultra-performance liquid chromatography tandem mass spectrometry; UPLC-QTOFMS, ultra-performance liquid chromatography quadrupole time of flight mass spectrometry; USDA, United States Department of Agriculture; WGCNA, weighted correlation network analysis.