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Ceramides and other sphingolipids as drivers of cardiovascular disease

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Abstract

Increases in calorie consumption and sedentary lifestyles are fuelling a global pandemic of cardiometabolic diseases, including coronary artery disease, diabetes mellitus, cardiomyopathy and heart failure. These lifestyle factors, when combined with genetic predispositions, increase the levels of circulating lipids, which can accumulate in non-adipose tissues, including blood vessel walls and the heart. The metabolism of these lipids produces bioactive intermediates that disrupt cellular function and survival. A compelling body of evidence suggests that sphingolipids, such as ceramides, account for much of the tissue damage in these cardiometabolic diseases. In humans, serum ceramide levels are proving to be accurate biomarkers of adverse cardiovascular disease outcomes. In mice and rats, pharmacological inhibition or depletion of enzymes driving de novo ceramide synthesis prevents the development of diabetes, atherosclerosis, hypertension and heart failure. In cultured cells and isolated tissues, ceramides perturb mitochondrial function, block fuel usage, disrupt vasodilatation and promote apoptosis. In this Review, we discuss the body of literature suggesting that ceramides are drivers — and not merely passengers — on the road to cardiovascular disease. Moreover, we explore the feasibility of therapeutic strategies to lower ceramide levels to improve cardiovascular health.

The prevalence of obesity in the USA and most other locations globally is increasing rapidly, with nearly 70% of adults in the USA classified as being either overweight or obese by the Centers for Disease Control and Prevention[1]. Overnutrition, sedentary behaviour and genetics contribute to the development of obesity and its comorbidities, including type 2 diabetes mellitus, hypertension, coronary artery disease, cardiomyopathy and heart failure (HF). These cardiovascular disorders are major causes of morbidity and mortality in the general population and account for a staggering percentage of medical costs. New therapeutic approaches are needed to combat these costly and deadly diseases.

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Author contributions

The authors contributed substantially to all aspects of the article.

Competing interests

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Excessive lipid deposition in non-adipose organs, such as blood vessel walls and the heart, contributes to the development of these cardiovascular disorders. In healthy tissues, free fatty acids are metabolized via β-oxidation to produce ATP. When caloric consumption exceeds demand, the excess free fatty acids are packaged onto a glycerol backbone to produce inert triglycerides, which can be safely stored in lipid droplets within the cells. Sometimes, however, these pathways become saturated, meaning that deleterious, bioactive lipids start to accumulate. These molecules disrupt heart and blood vessel function to drive the aforementioned diseases. Of the many lipid metabolites that accrue under these conditions, sphingolipids (such as ceramides) are particularly damaging to blood vessels and the heart.

Ceramides are formed by a ubiquitous biosynthetic pathway that starts with the condensation of palmitoyl-CoA and an amino acid (most often serine) to create a sphingoid backbone. This scaffold acquires an additional, variable fatty acid to become a ceramide, which is the base building block for complex sphingolipids. In addition to performing structural roles in cell membranes, ceramides function as intracellular signals of free fatty acid abundance, initiating responses that allow cells to cope with the lipid burden during physiological or nutritional stress. In the long term, these actions contribute to the pathophysiology of type 2 diabetes, fatty liver disease, hypertension, atherosclerosis and HF[2-4]. In humans, circulating ceramide levels have emerged as predictive biomarkers of cardiometabolic complications, including coronary artery disease, diabetes, HF, major adverse cardiac events and death[5-24]. In mice and rats, inhibition of ceramide biosynthesis ameliorates hallmark features of cardiometabolic disease, including insulin resistance, glucose intolerance, diabetes, atherosclerotic plaque formation, hypertension and HF[25-30]. Understanding the molecular mechanisms that control ceramide metabolism and action could reveal new therapeutic strategies for treating cardiometabolic disorders. In this Review, we provide perspectives on how ceramides contribute to cardiovascular dysfunction, focusing on their actions in two organs: the vascular endothelium and the heart.

Ceramide biosynthesis and metabolism

Ceramides are essential precursors of most of the complex sphingolipids, including sphingomyelins, glucosylceramides and sphingosine. These sphingolipids are localized in lipid bilayers, where they perform numerous structural functions[31,32]. Sphingomyelins are the most abundant sphingolipids in cells, whereas the less abundant ceramides and sphingosine 1-phosphate (S1P) function as dynamic signalling molecules. In particular, ceramides are recognized to be central modulators of the cellular stress response[33-35]. The fundamental biosynthetic pathway that produces ceramides and other sphingolipids is operational in every tissue.

Sphingolipid synthesis starts in the endoplasmic reticulum with the condensation of palmitoyl-CoA and serine, a reaction that is catalysed by the multi-subunit and highly regulated enzyme complex serine palmitoyltransferase (SPT)[36] (FIG. 1). Two essential subunits, SPTLC1 and SPTLC2, are required for enzyme function. Other subunits, such as SPTLC3, alter substrate specificity, allowing alternative fatty or amino acids to be incorporated to produce less abundant sphingolipid species[37,38]. In the canonical reaction,

palmitoyl-CoA and serine are used as substrates to produce 3-ketosphinganine, which is rapidly converted into sphinganine by 3-ketosphinganine reductase.

Ceramide synthases, which include six different family members (CERS1–CERS6) encoded by distinct genes, transfer a fatty acid from acyl-CoA to the sphinganine scaffold, forming dihydroceramide. The CERS enzymes differ according to tissue type and substrate specificity and account for much of the diversity in the sphingolipid family[39] (TABLE 1). CERS2, which adds very-long-chain fatty acids (C20–C26) to the sphinganine scaffold, is abundant in many tissues, including the liver, kidneys and heart[40]. CERS4, which adds long-chain fatty acids (C18–C20), is abundant in the heart[41]. CERS5, which produces C14 and C16 ceramides, has also been implicated in the regulation of cardiac function[42]. CERS6, which also produces C14 and C16 ceramides, is upregulated in obesity and is most strongly implicated in the metabolic disturbances observed in adipose tissue and the liver[43-47].

The final reaction in the ceramide biosynthesis cascade requires dihydroceramide desaturases (DES1 and DES2), which introduce an important double bond into the sphingoid backbone of the dihydroceramides to produce the ceramides[48,49]. DES1 is the major enzyme in most tissues, including those in the cardiovascular system, whereas DES2 is expressed predominantly in the skin, kidneys and intestines[50].

The endoplasmic reticulum-derived ceramides can be transported to the Golgi apparatus, where they are converted into complex sphingolipids, including sphingomyelins, glucosylceramides and gangliosides. This step is facilitated by ceramide transport proteins, such as CERT1, which selectively direct ceramides to the sphingomyelin synthases[51,52].

Ceramides can also be deacylated by a family of ceramidases, which liberate a fatty acid and release the sphingosine backbone. Sphingosine can be further processed by sphingosine kinases to produce S1P, which is mainly synthesized in red blood cells and the vascular endothelium[53]. Sphingosine can be converted back into ceramide by the aforementioned CERS enzymes. The complex sphingolipids (such as sphingomyelin) can be catabolized to regenerate ceramides during stress by sphingomyelinases[54].

Ceramide and S1P have potent but opposing regulatory roles in numerous cell types[55]; Spiegel and colleagues first noted that the balance of ceramide to S1P functions as a cellular rheostat to dynamically regulate apoptosis versus growth, respectively, in human pro-myelocytic cell lines[56]. This dynamic regulation is also apparent in vascular endothelial cells (ECs), where ceramides block vasodilatation and exacerbate the risk of cardiovascular disease[29,30,57,58], whereas S1P promotes vasodilatation and is atheroprotective[59-61]. Activation of adiponectin receptors tunes this rheostat, because these receptors increase the deacylation of ceramide and subsequent generation of S1P in numerous tissues[62]. This outcome is achieved because of the intrinsic ceramidase activity of the adiponectin receptors[63,64]. In total, >1,600 sphingolipids have been curated by the LIPIDMAPS consortium, and >4,800 are predicted to exist on the basis of computational modelling[65].

Ceramides in the vascular endothelium

Blood vessel reactivity

The endothelium that lines the interior walls of arteries, veins and capillaries consists of ECs that attach to the basal lamina, structurally building the intima in blood vessels. The ECs are responsible for maintaining vascular homeostasis by sensing haemodynamic changes, such as shear stress and blood-borne substances (for example, hormones)[66]. In healthy conditions, ECs control the balance between vasorelaxation and vasoconstriction, anti-inflammation and pro-inflammation, and antioxidative and pro-oxidative stress by carefully titrating the synthesis of endothelium-derived relaxing and contracting factors. By far the most studied of these factors is nitric oxide (NO), which is released luminally and abluminally after its generation by endothelial NO synthase (eNOS; also known as NOS3)[67]. In many individuals with metabolic syndrome, ECs produce less NO in response to various stimuli (such as acetylcholine, bradykinin and EC shear stress)[68]. This phenomenon is observed in patients with hypertension or atherosclerosis and is an important feature of metabolic syndrome[69,70].

Elevations in circulating lipid levels lead to the build-up of fatty deposits within the blood vessel lumen and the ectopic formation of ceramides within ECs (FIG. 2). EC-derived ceramides are potent regulators of vascular tone. In small coronary arteries from mice in vitro or in human cultured ECs, administration of ceramide analogues impairs EC-dependent vasorelaxation[71], exacerbates vasoconstriction[72] and decreases NO production[73]. Of note, short-chain ceramide analogues are not natural ceramides, but are rapidly deacylated and recycled into long-chain ceramides[74]. Alternative experimental interventions that also increase endogenous ceramide production in cells or tissues (such as by incubating ECs or isolated vessels with palmitate) recapitulate the effects of C2-ceramide administration by decreasing eNOS phosphorylation, eNOS activity and NO production[29,30]. Convincingly, inhibition of ceramide biosynthesis with the use of pharmacological agents (such as myriocin) or genetic modification (such as *Des1* heterozygous knockout) restores eNOS activity, NO production and EC-dependent vasodilatation in these palmitate-treated samples as well as in mouse and rat models of obesity or hyperlipidaemia[29,30,75]. These studies indicate that endogenous ceramides contribute to the EC-dependent, NO-mediated arterial dysfunction that underlies cardiovascular disease.

The enzymatic activity of eNOS is governed in large part by the phosphorylation of two residues: Thr495 and Ser1177. Phosphorylation of Ser1177 activates the enzyme, whereas phosphorylation of Thr495 is inhibitory[76]. Ceramide activates protein phosphatase 2A (PP2A), which dephosphorylates Ser1177 to diminish eNOS activity[29,30,77]. In vascular ECs, ceramide regulates PP2A by disrupting its interaction with inhibitor 2 of PP2A (I2PP2A), liberating PP2A from this repressive factor and increasing its access to various substrates[58,78]. This sequence of events leads to an increased association between PP2A and eNOS and a concomitant dissociation of eNOS from the activating kinase complex AKT–HSP90 (REFS[29,30]). Blocking PP2A activity ameliorates high-fat-induced or ceramide-induced endothelial dysfunction and hypertension in animal models[29,30].

Reactive oxygen species (ROS) produced by NADPH oxidase also influence eNOS activity[58,79]. NADPH oxidase levels are elevated in animal models of hypertension, diabetes

or atherosclerosis, leading to higher levels of ROS that contribute to EC-dependent vascular dysfunction[80-82]. Early in vitro studies found that the administration of ceramide analogues elevates ROS production, perhaps owing to NADPH oxidase activation, in animal and human ECs[71,73]. Moreover, NADPH oxidase inhibitors attenuated the ceramide-induced impairment of EC-dependent vasodilatation in bovine small coronary arteries in vitro[71]. A similar study found that overexpression of CuZn superoxide dismutase, which scavenges ROS, prevents ceramide-driven impairment of EC-dependent vasodilatation[83]. Curiously, our work has shown that ceramides promote the generation of superoxide anion, but several superoxide scavengers are unable to prevent ceramide actions on eNOS or NO in vitro[29,30].

Multiple studies have also demonstrated that ceramides induce the production of ROS by disrupting the mitochondrial electron transport chain or induce apoptosis by altering the permeability of the mitochondrial outer membrane[84]. These mitochondrial effects of ceramides might also be relevant to EC survival, particularly in response to the inflammatory cytokine tumour necrosis factor (TNF). TNF increases the endogenous ceramide content of bovine and human ECs in vitro by activating sphingomyelinase or by stimulating de novo synthesis[85,86]. Inhibition of ceramide biosynthesis with the use of fumonisin B1, an inhibitor of ceramide synthases, protects bovine cultured cerebral artery ECs from TNF-induced or cycloheximide-induced cell death[86]. A summary of these cellular mechanisms is shown in TABLE 2.

Atherosclerosis

Atherosclerosis is a leading cause of death worldwide and the pathological basis of many cardiovascular disorders. The mechanisms of atherosclerosis progression have not been fully elucidated, but initially involve lipoprotein retention in the vascular intima. Entry of lipoproteins into the intima is associated with various factors, including lipoprotein size, charge and composition. The accumulated lipoproteins mainly consist of LDLs, which have a high affinity for proteoglycans, leading to increasing residence time in the intima[87]. When lipoproteins aggregate in the intima, ECs recruit monocytes, resulting in monocyte differentiation into macrophages and the formation of foam cells[88]. During atherosclerosis progression, ECs generate a large amount of ROS and limit NO bioavailability, which can exacerbate inflammatory responses and reduce EC-dependent vasodilatation.

Studies with large clinical cohorts reveal that serum ceramide levels are strong predictors of coronary artery disease[7,11,15,17,89,90]. Serum ceramide levels also predict atherosclerotic plaque instability[16] and detrimental outcomes of coronary artery disease, including death[15,91]. Ceramides also accumulate in atherosclerotic plaques, where they have been implicated in the onset of lipoprotein aggregation[92,93]. Several studies have shown that ceramides have causal roles in atherosclerotic plaque formation, because inhibiting de novo ceramide synthesis alleviates lipid-induced atherogenic processes. For example, administration of the SPT inhibitor myriocin decreased plasma sphingolipid concentrations, including those of ceramide, and atherosclerotic lesion size in the aorta of atherosclerosis-prone *Apoe*$^{-/-}$ mice[94-97]. Similar protective effects are observed in *Sptlc1* haplo-insufficient mice[98]. Treatment with myriocin not only is beneficial in reducing atherosclerosis but also improves insulin sensitivity and resolves hepatic steatosis in animal models[25,99,100].

However, regulating SPT activity by knocking out the gene or administering myriocin influences the production of nearly all sphingolipids; therefore, further work is required to differentiate between the specific effects of ceramide and those of complex sphingolipids (such as glucosylceramides or sphingomyelins).

Another approach to lowering ceramide levels is to target the enzyme DES1, which converts dihydroceramide to ceramide (FIG. 1). Inhibition of DES1 with a chemical inhibitor (fenretinide) or genetic modification protects mice from insulin resistance, hepatic steatosis and/or vascular dysfunction[29,101-103]. Fenretinide also prevents dyslipidaemia and hepatic steatosis in mice[102,104]. Surprisingly, treatment with fenretinide worsens atherosclerosis in mice, although it has been suggested that this effect is driven by the retinoid actions of the drug and is, therefore, independent of its effects on DES1 (REF.[105]). Consistent with this interpretation, whole-body deletion of *Des1* in mice does not increase inflammation or promote splenomegaly, which were major features observed in fenretinide-treated animals[101]. Additional pharmacological and genetic studies are needed to determine the effects of DES1 inhibition on atherosclerotic plaque formation.

Jiang and colleagues investigated whether sphingomyelins, rather than ceramides, drive atherosclerosis[106]. The sphingomyelins are also independent markers of cardiovascular disease[106]. Jiang and co-workers explored whether inhibition of sphingomyelin production by sphingomyelin synthases (SMS1 and SMS2) might be a therapeutic approach to treat atherosclerosis. In mouse models, adenoviral-mediated overexpression of *Sms1* and *Sms2* increased plasma sphingomyelin levels and worsened atherosclerosis[107-109]. By contrast, SMS2 deficiency in mice attenuated atherosclerotic lesion formation and inflammatory responses[110-112]. Interestingly, inhibiting SMS1 elicited abnormalities, including metabolic dysfunction and inflammation[113,114].

The studies described above demonstrate that lowering ceramide synthesis using pharmacological inhibitors or genetic engineering alleviates vascular dysfunction and atherosclerosis in animal models. Although many mechanisms have been identified, the full spectrum of events by which ceramides promote atherosclerotic plaque formation remains elusive. Nonetheless, the studies present exciting possibilities that lowering plasma ceramide levels could be an effective means of improving vascular health.

Role of ceramides in the heart

Heart failure

Despite the successes of certain glucose-lowering therapies for the management of diabetes and delaying or preventing cardiac complications, HF remains the major cause of death in patients with diabetes[115]. HF includes two major subtypes: HF with reduced ejection fraction (HFrEF), which encompasses ischaemic and non-ischaemic HF and is characterized by dilated ventricles and systolic dysfunction, and HF with preserved ejection fraction (HFpEF), which is characterized by stiffened ventricles and diastolic dysfunction (FIG. 3). Through the 1990s, HFrEF accounted for 75% of all HF diagnoses but now accounts for only about 50%[116]. This change is because the incidence of HFpEF is increasing at alarming and accelerating rates[117,118].

Although HFrEF and HFpEF have shared risk factors (such as diabetes), these subtypes of HF are distinguished by subtle cellular and molecular differences. HFrEF is often a result of ischaemic injury or chronic β-adrenergic signalling and is followed by cardiomyocyte apoptosis, inflammation and subsequent fibrotic repair; these cellular processes disrupt contractility of the left ventricle[119]. By contrast, HFpEF is preceded by chronic morbidities such as obesity, dyslipidaemia and hypertension and is caused by cardiac fibrosis in the absence of severe apoptosis, resulting in cardiac hypertrophy and left ventricular diastolic dysfunction[119]. Ceramides seem to be relevant to both of these conditions.

As noted previously, circulating ceramide levels are strong biomarkers of long-term cardiovascular outcomes[13-16]. Studies with heart biopsy samples indicate that ceramides, and the ceramide biosynthesis enzymes, also accumulate in the failing myocardium[28]. Patients with severe HFrEF often undergo surgery to receive a left ventricular assist device, which improves cardiac function and metabolism[120-122]. Placement of a left ventricular assist device reduces the levels of ceramides of nearly all chain lengths in the myocardium[28]. Curiously, women with HFpEF who underwent gastric bypass surgery had improved plasma ceramide profiles and cardiac function but no changes in myocardial ceramide content[20]. Therefore, ceramides are likely to influence cardiac function by mechanisms that are intrinsic (that is, intramyocardial) and extrinsic (such as via blood pressure regulation or dyslipidaemia) to the heart.

Targeting ectopic lipid accumulation might be a powerful approach to combating heart disease. In the CORONA trial[123], statin therapy was associated with a 15–20% reduction in the risk of hospitalization for HF. Statins lower the circulating levels of many lipoprotein-bound lipids, including ceramides[17]. Therefore, the question arises as to whether ceramide-focused interventions might have additional protective effects. To specifically assess the role of lipids in the heart, Goldberg and colleagues studied genetically engineered mouse models of dilated lipotoxic cardiomyopathy[27]. The researchers generated mice with cardiac-specific overexpression of the gene encoding glycosylphosphatidylinositol-anchored human lipoprotein lipase (*Lpl*^{GPI}), which liberates fatty acids from circulating lipoproteins and facilitates their incorporation into neighbouring tissue. *Lpl*^{GPI} overexpression resulted in elevated cardiac ceramide levels and impaired function of the heart[27]. The intervention also downregulated the levels of glucose transporter 4 and upregulated markers of HF, such as atrial natriuretic peptide, B-type natriuretic peptide and pyruvate dehydrogenase kinase isoform 4. Administration of the SPT inhibitor myriocin to *Lpl*^{GPI}-overexpressing mice improved cardiac systolic function and increased survival rates[27].

Schulze and colleagues further investigated the role of ceramides in a mouse model of ischaemia-induced HF[28]. The researchers induced a myocardial infarction in mice by ligating the left anterior descending coronary artery, producing left ventricular dysfunction and progressive cardiac remodelling and dilatation[28]. Myriocin administration reduced ceramide levels in the heart and reduced ventricular remodelling and fibrosis in this model of HFrEF[28]. Similar results were found in *Sptlc2*$^{+/-}$ mice, which are deficient in the SPTLC2 subunit[28]. These data identify ceramides as a cardiotoxin that impairs heart function and suggest that ceramide-lowering interventions could have cardioprotective actions.

An alternative approach to lowering ceramide levels in the heart and plasma is to promote ceramide degradation (for example, via ceramidases). Adiponectin is a fat-derived hormone that promotes weight loss, increases insulin sensitivity, decreases inflammation and inhibits apoptosis[124]. Therefore, this adipokine has antidiabetic and cardioprotective actions. Adiponectin increases the ceramidase activity that is intrinsic to its two receptors, AdipoR1 and AdipoR2 (REFS[62,64]). Inhibiting acid ceramidase activity in mice after myocardial infarction worsened cardiac function, whereas increasing acid ceramidase activity improved cardiac function[125], indicating the important role that ceramidase reactions have in HFrEF.

In addition to degrading ceramides, activating the ceramidase step leads to increased production of S1P, which might have cardioprotective actions. S1P signals through one of five receptor family members[126]. Studies using knockout mice implicate the $S1P_2$ and $S1P_3$ receptors as being potent mediators of cardioprotection after ischaemia–reperfusion injury, with a similar effect reported in human myocardial tissue[126-130]. An adiponectin–S1P axis is protective against cardiomyocyte cell death[131,132].

Ceramide mechanisms that contribute to HF

One mechanism linking ceramides to impaired cardiomyocyte function relates to their actions in mitochondria, where they can impair energetics and ultimately induce apoptosis[84] (FIG. 4). Accumulation of ceramides in the inner mitochondrial membrane disrupts electron transport chain activity, impairing respiratory capacity[43_47_133-135]. When ceramides accumulate in the outer mitochondrial membrane, they increase permeability to cytochrome *c* and initiate intrinsic apoptosis pathways[136]. Although many of the studies to dissect the mechanisms of these actions have been performed in other cell types, the actions seems to be highly relevant to cardiomyocytes[137-141]. For example, ceramide analogues are sufficient to induce apoptosis in cardiomyocytes[138]. Researchers have also confirmed that endogenous ceramides, induced by palmitate exposure, can drive apoptosis in this cell type[139-141]. Moreover, overexpressing *SPTLC1* and/or *SPTLC2* in AC16 human cardiomyocytes induces ceramide accumulation, impairs mitochondrial respiration and induces apoptosis[28]. Similarly, overexpression of *Cers2* in mice elicited a similar spectrum of effects in cardiomyocytes[142]. Overexpression of *Cers2* also increased ROS production, an effect that was exacerbated by delivering an excess of mitochondrial substrates and inhibited by blocking sphingolipid synthesis[142]. By contrast, inhibiting sphingolipid synthesis had no effect on superoxide-induced ROS, placing ceramide as an obligatory upstream mediator of ROS generation[142].

Although intramyocardial levels of ceramides are high in animal models of HFpEF[143], this form of HF occurs in the absence of severe apoptosis[144]. This observation might be explained by increased expression of anti-apoptotic proteins, such as B cell lymphoma 2 (BCL-2). Indeed, Dong and colleagues created a model of HFpEF that was associated with an increased expression of *Bcl2* (REF.[145]). Moreover, studies in other cell and tissue types have shown that BCL-2 family members prevent ceramide-dependent apoptosis[146-148]. Additional work is warranted to understand the protection from apoptosis that occurs in the HFpEF syndrome.

An alternative mechanism linking sphingolipids to HF involves their regulation of autophagy, a highly coordinated process that prevents the accumulation of damaged molecules within the cell[149]. In an in vitro model of diabetic cardiomyopathy, myristate (C14:0) but not palmitate (C16:0) induced CERS6-dependent increases in autophagy that contributed to cardiomyocyte hypertrophy[42].

Ceramides might also affect the heart through actions in other cell types. ECs make up the majority of the non-cardiomyocyte cells in the myocardium, and ECs are dysfunctional in both HFrEF and HFpEF. In particular, NO bioavailability is greatly attenuated in HFpEF[150,151]. Our studies suggest that, in patients with HFrEF, the placement of a left ventricular assist device improves coronary artery EC function[152] and reduces serum ceramide levels (S.A.S., W.L.H., unpublished observations). As described previously, ceramides have important roles in ECs that might explain the attenuation of NO production in HFpEF.

HF is also accompanied by an inflammatory response that is characterized by the infiltration of macrophages and neutrophils into the myocardium[151,153]. The inflammatory cascade is another important step in the onset and progression of diastolic dysfunction. Ceramides contribute to these inflammatory processes, both as upstream regulators of cytokine production and as downstream effectors that mediate cytokine-induced stress responses[154]. Lowering ceramide levels in the heart (such as by overexpressing *Asah1*, encoding acid ceramidase) reduces macrophage infiltration in mouse models of myocardial infarction[125].

Lastly, ceramides have emerged as potential inducers of fibrosis[4], independent of their actions on preceding apoptosis events. For example, ceramides stimulate proteolytic processing of cAMP-responsive element-binding protein 3-like protein 1 (CREB3L1)[155,156], a transcription factor that induces collagen production. An intricate mechanism has been described in which ceramides invert the topology of the inhibitory protein transmembrane 4 L6 family member 20 (TM4SF20); this inversion removes the inhibitory signal and allows CREB3L1 exposure to site-1 proteases[155,156]. Although these actions have not been explored in the heart, these and other mechanisms might explain how ceramides contribute to the fibrotic response that drives HFpEF.

Importance of ceramide chain length in cardiac dysfunction.—In tissues such as the liver and adipose tissue, long-chain C16:0 ceramide is deleterious, whereas very-long-chain ceramides (such as C24:0) are considered to be benign[43,45-47,157-159]. The C16-ceramides are thought to form unique platforms in membranes that initiate stress responses (for example, impaired mitochondrial respiration and apoptosis), whereas the very-long-chain ceramides lack these attributes. Consistent with this hypothesis, in the clinical CERT1 score, high levels of C16:0 ceramides increase the score (indicating a heightened risk of cardiac events), whereas elevated C24:0 levels decrease the score (indicating a diminished risk)[5]. Moreover, in humans with severe HFrEF, who have increased ceramide levels in the myocardium and plasma[28], unloading the heart with the use of a left ventricular assist device improves heart function and restores physiological healthy C24:0 to C16:0 ratios[21,160].

Notwithstanding the data above, studies in mice and cells have produced ambiguous results on which ceramide species are damaging to the heart. To test the idea that C16-ceramides are pathogenic in the heart, mice lacking CERS5, which together with CERS6 makes the C16-ceramides, were studied[42]. The researchers induced cardiomyopathy by feeding the mice a milk-fat-based diet and found that ablation of *Cers5* negated certain elements of the condition, including cardiac autophagy and hypertrophy[42]. Surprisingly, however, the investigators concluded that C14-ceramides derived from myristate and produced by CERS5 — rather than the canonical C16-ceramides that are produced from palmitate and have been implicated in tissue dysfunction — were the damaging species. In a later study, the researchers evaluated the consequences of overexpressing *Cers2* (encoding CERS2, which produces very-long-chain ceramides) in cardiomyocytes[142]. In these cell culture experiments, *Cers2* overexpression selectively increased the levels of certain very-long-chain ceramides and induced mitochondrial dysfunction, oxidative stress and cell death[142]. This result was surprising because studies in other tissues and cell types have indicated that these ceramide actions require CERS6 and the C16 acyl chain, whereas longer-chain ceramides are unable to alter mitochondrial function[43,45-47,159].

Additional evidence suggests that sphingolipids with unusual sphingoid bases, such as those derived from the condensation of myristate (rather than palmitate) with serine, are important drivers of heart dysfunction[161]. The capacity of the SPT complex to use myristate as a substrate is influenced by the presence of the SPTLC3 subunit, which is abundant in rat cultured cardiomyocytes and mouse hearts[161]. Moreover, the d16-base sphingolipids that are derived from myristate comprised 30% of cardiomyocyte sphingolipids[161]. Lastly, these d16 sphingolipids were found to promote cell death[161]. These findings raise the interesting possibility that alternative sphingolipids might contribute to cardiomyocyte dysfunction.

Ceramide-related therapeutic targets

Since the early 1900s, plasma LDL-cholesterol levels have been considered to be the best biomarker for predicting cardiovascular events. This discovery triggered the development of LDL-cholesterol-lowering therapeutics, including statins, ezetimibe, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and fenofibrates. Studies with these drugs have demonstrated that reductions in LDL-cholesterol levels effectively decrease the risk of cardiovascular events[162,163]. Although plasma ceramide levels have been identified as a strong predictor of cardiovascular disease, the effects of existing lipid-modifying drugs on sphingolipids, especially ceramides, have not been studied extensively. Treatment with either simvastatin or rosuvastatin can lower plasma ceramide levels by 20–30% compared with patients treated with ezetimibe or placebo, respectively[17,164]. Treatment with a PCSK9 inhibitor for 2–12 months was shown to reduce the plasma levels of several ceramides (~30% decrease), together with the expected lowering of plasma LDL-cholesterol levels[165]. Further studies are required to determine whether reductions in plasma ceramide levels make an important contribution to the effects of these existing drugs.

As noted previously, circulating ceramide levels are elevated in individuals with obesity, diabetes, cancer, hepatic steatosis, hypertension, HF or atherosclerosis. The diagnostic power of plasma ceramide levels seems to be similar to that of plasma LDL-cholesterol

levels, to the extent that clinics have introduced diagnostic tests measuring plasma ceramide levels, owing to their utility in predicting insulin resistance, the severity of coronary artery disease, the incidence of major adverse cardiac events and death. In addition, the aforementioned studies in mice further indicate that ceramides have causal roles in these pathologies, suggesting that therapeutic strategies that lower plasma ceramide levels could be a good approach to combating these obesity-related disorders.

SPT, the initial enzyme in the de novo sphingolipid synthesis pathway, was the first enzyme to be seriously considered as a therapeutic target to lower plasma ceramide levels and treat cardiometabolic disease. Inhibiting the SPT enzyme decreases the levels of all sphingolipid species, including ceramides, sphinganine, dihydroceramides and sphingomyelins, in a wide variety of tissues. In mice, treatment with the irreversible SPT inhibitor myriocin prevents insulin resistance and diabetes, atherosclerosis, hypertension and HF[25-29,166-168]. Heterozygous *Sptlc1*-knockout mice have been shown to be protected from dyslipidaemia, atherosclerosis and HFrEF[28,98]. Unfortunately, attempts by pharmaceutical companies to generate safe SPT inhibitors have so far failed, largely owing to toxicity to the gut[169]. This toxicity is likely to be an on-target drug effect, because knockout mice lacking SPTLC2 subunits die shortly after gene ablation owing to disruption of the gut architecture[170,171].

Other targets in the de novo ceramide biosynthesis pathway have also received attention as potential therapeutic targets. CERS6 has received considerable attention, largely because its ablation confers protection from insulin resistance and dyslipidaemia and improves mitochondrial function[43,45,159]. Studies on this target are ongoing.

DES1 catalyses the last step in the de novo ceramide biosynthesis pathway by inserting a crucial double bond into the backbone of dihydroceramides to produce the deleterious ceramides. Fenretinide, a retinoid that additionally has inhibitory actions against DES1, is insulin-sensitizing in humans and in obese mice[102,104,172-174]. Fenretinide also reduces blood pressure in hypertensive rats by attenuating inflammation[175]. However, fenretinide administration has been shown to worsen atherosclerosis, although it has been suggested that this effect is driven by the retinoid actions of the drug and is therefore independent of its effects on DES1 (REF.[105]). Future work is needed to determine the effects of DES1 inhibition on atherosclerotic plaque formation. Studies in mice further support the efficacy of this therapeutic strategy. Heterozygous *Des1*-knockout mice are protected from diet-induced vascular dysfunction[29]. Inducible depletion of DES1 from mice protects against glucose intolerance, insulin resistance and hepatic steatosis[101]. Therefore, ablation of *Des1* ameliorates many of the metabolic disorders that increase the risk of heart disease[101], but this approach has not yet been tested as a means of improving heart function.

Lastly, one might consider approaches to activate adiponectin receptors to catalyse ceramide deacylation. Indeed, we have previously found that adiponectin, by reducing cardiac ceramide levels, ameliorates HF driven by caspase activation in so-called HEART-ATTAC mice[62]. AdipoRon is a selective, orally active agonist of adiponectin receptors. Intraperitoneal delivery of AdipoRon in mice can rapidly lower ceramide levels in adiponectin-responsive tissues[63]. Subsequent in vitro studies in immortalized H9C2 rat cardiomyocytes, mouse primary cardiomyocytes and human vascular smooth muscle cells

supported the utility of AdipoRon as a tool to combat ceramide-induced lipotoxicity and improve cardiometabolic health[176,177].

Conclusions

Lipid-induced vascular and cardiac dysfunction are important components of the major comorbidities of diabetes, obesity and dyslipidaemia that reduce the quality and length of life. Among the various lipid species that accumulate in these diseased tissues, ceramides are highly pathogenic, because they elicit many of the tissue defects that underlie cardiovascular pathologies. Therapeutic strategies to lower plasma, vascular and cardiac ceramide levels hold enormous promise for treating a wide variety of cardiometabolic disorders, including hypertension, atherosclerosis, diabetes and HF. More work is needed in this promising area to understand the precise mechanisms controlling ceramide production and action and to identify therapeutic means of safely lowering ceramide levels to improve health.

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Key points

- Ceramides have been shown to accumulate in many tissues, including blood vessels and the heart, in individuals with cardiovascular disease (such as hypertension, heart failure and atherosclerosis).
- Serum ceramide levels are measured clinically as prognostic indicators of major adverse cardiovascular events.
- Inhibiting ceramide biosynthesis in mice and rats prevents the development of hypertension, atherosclerosis, diabetes mellitus and heart failure.
- Ceramides have pleiotropic actions that are relevant to metabolic disease, including inhibiting nitric oxide synthase, decreasing insulin sensitivity, altering mitochondrial bioenergetics, and inducing apoptosis and fibrosis.
- Several enzymes that control ceramide production or metabolism have emerged as attractive therapeutic targets for treating a wide range of cardiometabolic pathologies.

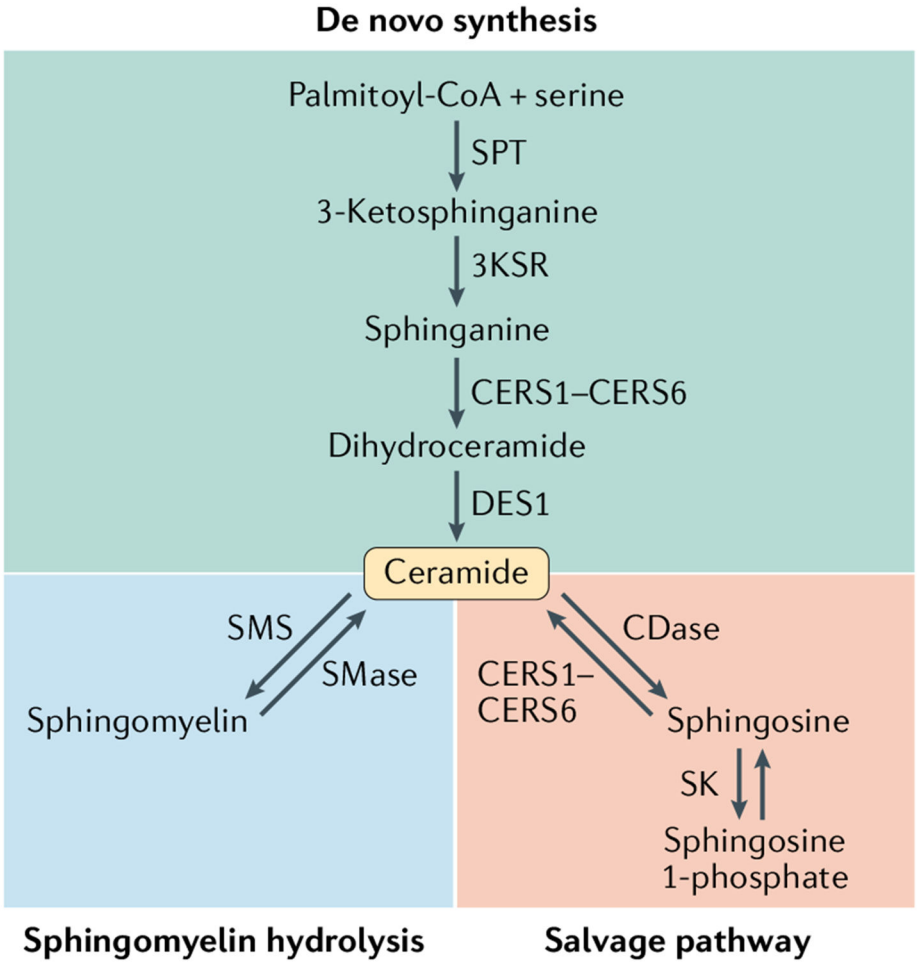

Fig. 1 | Pathways controlling ceramide levels in the cardiovascular system.
Schematic depiction of the major pathways controlling ceramide levels in the heart and vascular endothelium: de novo synthesis, hydrolysis of complex sphingolipids (for example, sphingomyelin hydrolysis) and the salvage pathway. 3KSR, 3-ketosphinganine reductase; CDase, ceramidase; CERS, ceramide synthase; DES1, dihydroceramide desaturase 1; SK, sphingosine kinase; SMase, sphingomyelinase; SMS, sphingomyelin synthase; SPT, serine palmitoyltransferase.

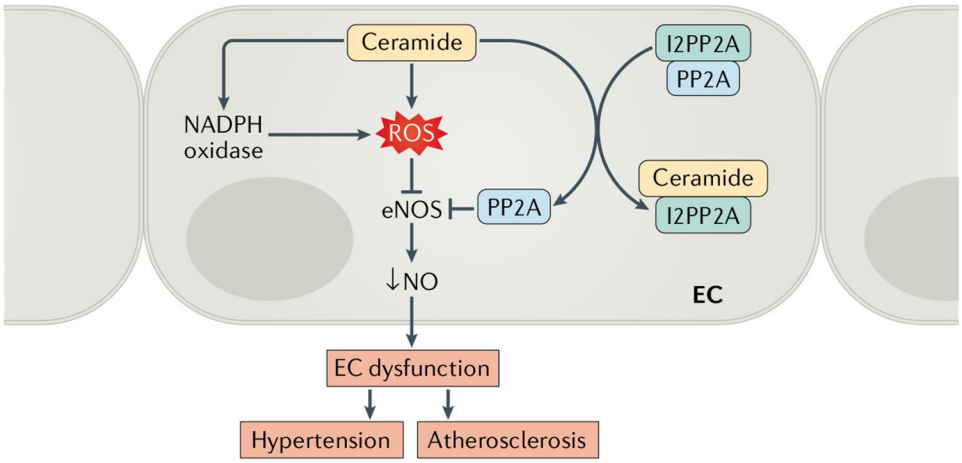

Fig. 2 |. Ceramide-induced endothelial cell dysfunction.

Endothelial cell (EC) dysfunction is an impairment of the vascular endothelium to regulate vascular homeostasis, mainly owing to the loss of nitric oxide (NO) bioavailability. Ceramides have been shown to decrease NO production by increasing the production of reactive oxygen species (ROS) and by activating protein phosphatase 2A (PP2A). The latter effect is as a result of the capacity of ceramides to dissociate the inhibitor 2 of PP2A (I2PP2A) from the PP2A, liberating the enzyme to act on cellular substrates, including endothelial nitric oxide synthase (eNOS).

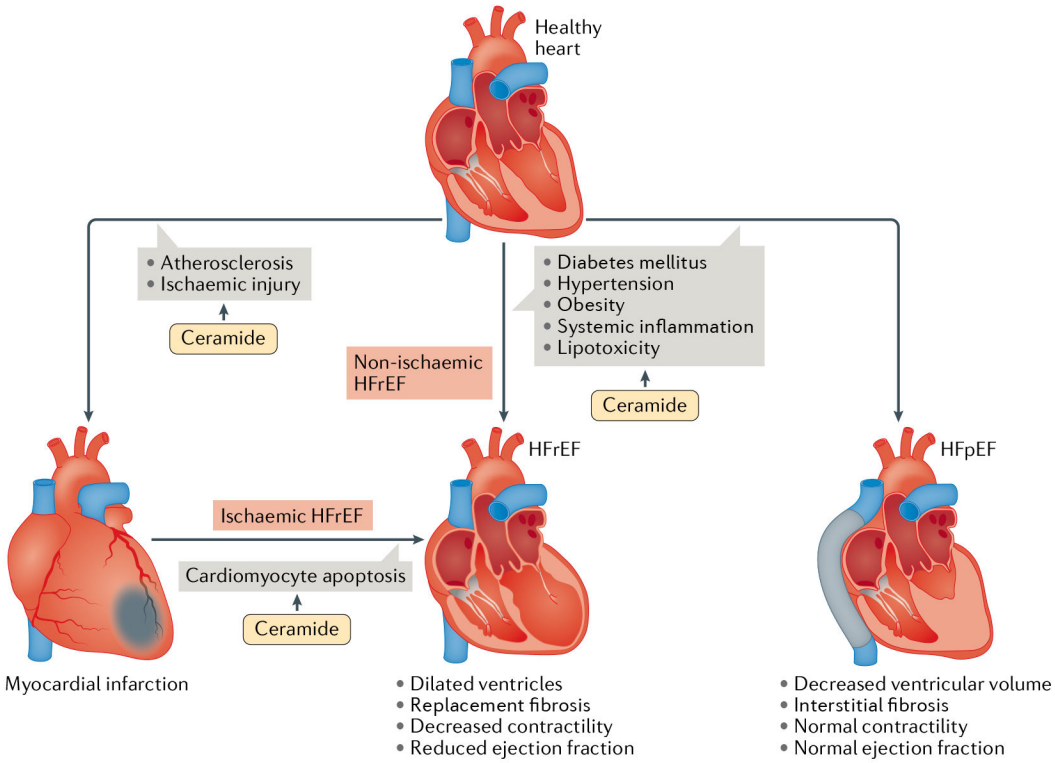

Fig. 3 |. Types of heart failure and the contribution of ceramides.

Heart failure (HF) with reduced ejection fraction (HFrEF) can be caused by ischaemic injury, such as myocardial infarction, and is characterized by dilated ventricles, apoptosis and replacement fibrosis. Ceramides can contribute to the development of atherosclerosis and ischaemic injury and also to cardiomyocyte apoptosis after myocardial infarction has occurred. HF with preserved ejection fraction (HFpEF) is caused by chronic systemic inflammation, often in the context of diabetes mellitus, hypertension, obesity and lipotoxicity, and is characterized by constricted ventricles and interstitial fibrosis. Ceramides are also associated with risk factors for the development of HFpEF and non-ischaemic HFrEF.

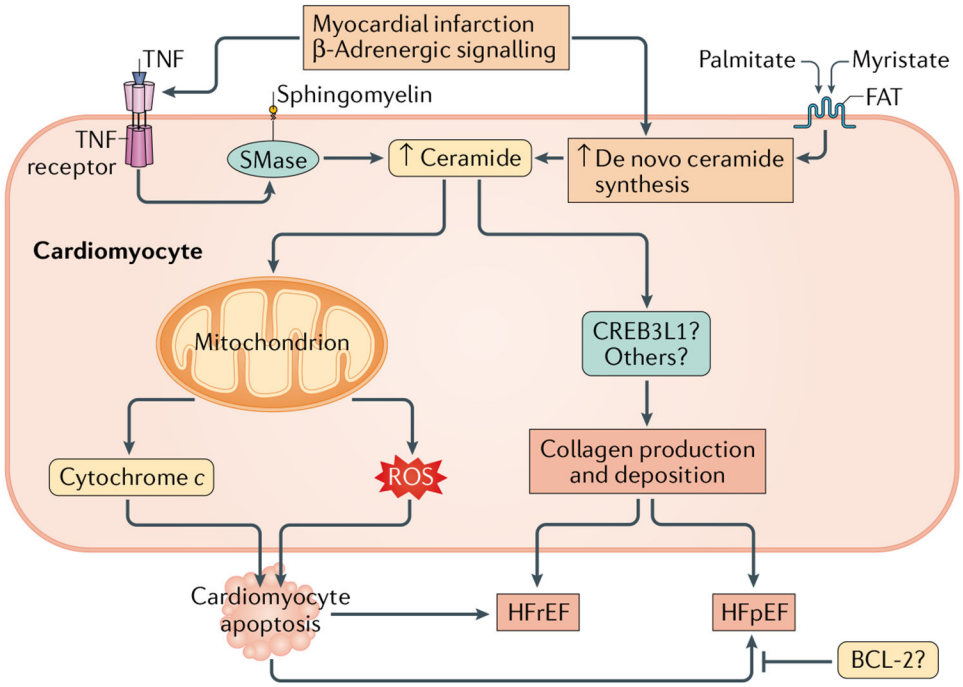

Fig. 4 |. Mechanisms linking ceramides to heart failure.

Ceramides produced either de novo or by sphingomyelin (SM) hydrolysis by sphingomyelinase (SMase) have been implicated in several actions in cardiomyocytes that could contribute to heart failure. Through actions in mitochondrial membranes, ceramides alter cellular energetics, induce reactive oxygen species (ROS) formation and promote cytochrome c release to initiate apoptosis. Some researchers have speculated that upregulation of anti-apoptotic proteins, such as B cell lymphoma 2 (BCL-2), could minimize this action in patients with heart failure with preserved ejection fraction (HFpEF). Ceramides have also been implicated in profibrotic pathways, such as the activation of cAMP-responsive elementbinding protein 3-like protein 1 (CREB3L1) to promote collagen deposition. FAT, fatty acid translocase (also known as platelet glycoprotein 4); HFrEF, heart failure with reduced ejection fraction; TNF, tumour necrosis factor.

Table 1 |

Comparison of ceramide synthases

Ceramide synthase	mRNA tissue expression	Fatty acid substrate	Pathologies associated with increased ceramide synthase activity
CERS1	Brain, skeletal muscle	C18:0	Skeletal muscle insulin resistance
CERS2	Heart, liver, lungs, ubiquitous	C20:0	Unknown
		C22:0	Cardiac mitochondrial dysfunction
		C24:0	Neutral/benign
		C24:1	Cardiac mitochondrial dysfunction
		C26:0	Unknown
CERS3	Skin, testes	≥C26:0	Farber disease biomarker
CERS4	Heart, lungs	C18:0	Unknown
		C20:0	Lung cancer, heart failure
CERS5	Heart, lungs, kidneys, ubiquitous	C14:0	Unknown
		C16:0	Heart failure, apoptosis
CERS6	Brain, adipose, liver, ubiquitous	C14:0	Unknown
		C16:0	Heart failure, apoptosis, adipose tissue dysfunction, liver insulin resistance and liver fibrosis

Table 2 |

Potential contributors to ceramide-induced vascular dysfunction

Factor	Influence of ceramides	Effects	Refs
NADPH oxidase	Activation	↑ROS, ↓eNOS, ↓NO	71,73,79
ROS	Increase	↓eNOS, ↓NO	81,82,178
I2PP2A	Inhibition	↑PP2A, ↓AKT–HSP90 complex, ↓eNOS, ↓NO	29,73,77

AKT, RACα serine/threonine-protein kinase; eNOS, endothelial nitric oxide synthase; HSP90, heat shock protein 90; I2PP2A, inhibitor 2 of protein phosphatase 2A; NO, nitric oxide; PP2A, protein phosphatase 2A; ROS, reactive oxygen species.