Role of ceramides in diabetic foot ulcers (Review)

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Abstract. Diabetes mellitus (DM) is a metabolic disorder, which if not managed properly, can lead to serious health problems over time and impose significant financial burden on the patient, their family and society as a whole. The study of this disease and the underlying biological mechanism is gaining momentum. Multiple pieces of conclusive evidence show that ceramides are involved in the occurrence and development of diabetes. The present review focuses on the function of ceramides, a type of sphingolipid signaling molecule, to provide a brief description of ceramides and their metabolism, discuss the significant roles of ceramides in the healthy skin barrier, and speculate on the potential involvement of ceramides in the pathogenesis and development of diabetic foot ulcers (DFUs). Understanding these aspects of this disease more thoroughly is crucial to establish how ceramides contribute to the etiology of diabetic foot infections and identify possible therapeutic targets for the treatment of DFUs.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by three primary metabolic disorders, namely carbohydrate, lipid and protein metabolism disorders, all of which are caused by inadequate insulin production (1). Patients are usually diagnosed only when serious complications have occurred due to the ignorance of symptoms of diabetes in the early stages. Therefore, early detection, diagnosis and treatment can benefit a patient's health and quality of life, while also reducing the financial burden (2).

The complications of diabetes, including diabetic nephropathy, diabetic retinopathy, diabetic neuropathy (DN), cardiovascular complications, liver fibrosis and other complications, seriously affect the prognosis of diabetic patients (3). Diabetic foot ulcers (DFUs), one of the most prevalent and serious complications of diabetes (4), are common in diabetic patients and can lead to amputation or even death when the condition worsens (5).

Ceramides are a type of sphingolipid, the major lipid component of cell membranes (6). Ceramides not only act as the second messenger molecule in the sphingolipid signaling pathway, but also take part in the formation process of the stratum corneum. The stratum corneum, which is the primary portion of the intercellular matrix containing 40-50% of intercellular lipids, plays a crucial function in maintaining the water balance of the skin (7). Some studies have found that ceramides can inhibit glucose uptake and increase adipose ectopic deposition, while inhibiting the enzymes required for ceramide synthesis can improve the progression of diabetes (8-10). A single study has shown significant differences in the concentration of ceramides in the skin of the feet of diabetic and non-diabetic patients (11). Ceramides can be divided into several types according to their chemical composition. Different chemical structures correspond to different biological functions (12). Ceramides and their metabolites can decrease insulin sensitivity, blood vessel reactivity and pancreatic cell function, as well as maintaining the skin's barrier function, regulating hydration, exerting anti-aging effects and acting as future therapeutic targets for some diseases, such as insulin resistance, obesity, type 2 diabetes, Parkinson's disease, autoimmune rheumatic disease, ventilator-induced lung injury and cancer (especially breast cancer) (9,13-18).

All the aforementioned findings suggest that ceramides may have a close connection with the development of DFUs. The aim of the present review is to assess the role of ceramides in diabetes, the skin and atherosclerosis, and to discuss their

possible mechanisms in DFUs. The hope is that these findings will translate into new screening methods and treatments to alleviate and possibly prevent or cure diabetes and DFUs.

2. Biosynthesis and degradation of ceramides

Bioactive ceramides can be produced in three ways (6). First, the *de novo* pathway produces 3-keto-dihydrosphingosine by condensation of serine and palmitate catalyzed by serine palmityl transferase (SPT) (19). Next, sphinganine is N-acylated by six distinct ceramide synthases (CerSs) to form various dihydroceramides. The dihydroceramides are oxidized by dihydroceramide desaturase into corresponding ceramides. This is the main method of ceramide production (7).

Second, in the sphingomyelinase (SMase) pathway, ceramides are produced by hydrolysis of sphingomyelin (SM) catalyzed by SMases, which are divided into neutral SMases (nSMases 1, 2 and 3), acid SMases (aSMase) and alkaline SMases (20). When cellular stress occurs in a particular compartment, the level of ceramide is quickly increased multiple times through this pathway (21).

The third pathway is the salvage pathway, in which sphingolipids degraded to sphingosine (Sph) are reutilized by reacylation to produce ceramides. Lipid phosphate phosphatases or Sph-1-phosphate (S1P) phosphatase are used to obtain Sph (22). CerSs may acylate Sph to form ceramides. Ceramides can be digested by ceramidases in the opposite direction, resulting in Sph. Sph kinase (SphK) phosphorylates Sph, resulting in S1P, which then reenters sphingolipid metabolism by the SphK and/or one of the six CerSs (23) (Fig. 1).

Each CerS controls the production of endogenous ceramides from scratch. Although certain CerSs are found throughout the body, other isoforms create tissue-specific synthesis of ceramides. CerS1 specifically synthesizes C18 ceramide, and while it is highly expressed in the skeletal muscles, it is nearly undetectable in other tissues (24). CerS2 is mostly expressed in the kidneys and liver, and synthesizes C22-24 ceramides (25). Ceramides with varying acyl chain lengths may trigger diverse cell responses; hence, different CerS isoforms may create different ceramide species (17).

CerS3 is most highly expressed in the skin and synthesizes C28-C32 ceramides (26,27), CerS4 expression is not specific to any tissue and synthesizes C14-C16 ceramides, while CerS5 is primarily expressed in the epithelia of the lungs and CerS6 has been found in almost all tissues in the body, except in the intestines, spleen, lymph nodes and thymus (28). CerS5 and 6 specifically synthesize C14-C16 ceramides. CerS6 is primarily responsible for the production of C16 ceramides, which have been linked to a number of diseases, including insulin resistance and obesity (29).

Ceramides are broken down into free fatty acids (FFAs) and Sph by ceramidases. Five distinct ceramidases have been previously reported in humans, each with a different catalytic pH optimum, namely, acid ceramidase, neutral ceramidase, and alkaline ceramidases 1, 2 and 3, which are encoded by five separate genes (ASAH1, ASAH2, ACER1, ACER2 and ACER3, respectively) (30).

Ceramide species produced in the endoplasmic reticulum (ER) of the stratum spinosum inside the epidermis are subsequently acylated by linoleoyl-CoA to generate acyl-ceramides in the skin. In healthy skin, the acyl chain length spans from C16 to C36, with C24 being the most common. Ceramides with distinct molecular architectures have different activities, implying that they play a variety of roles in skin homeostasis (31). Acyl-ceramides are glycosylated in the Golgi apparatus and subsequently transported with other lipids into lamellar bodies. The lamellar bodies, containing glycosylated ceramides, acyl ceramides, cholesterol and very-long-chain FAs, are secreted into the extracellular space of the stratum corneum, where the ceramides are covalently linked to structural skin proteins, such as involucrin, filaggrin and small proline-rich proteins, to form the epidermal permeability barrier (32).

3. Ceramides in the skin

Ceramide levels are significantly higher in insulin-resistant patients than in healthy individuals (33); therefore, the content level of the ceramides may be somehow related to skin disorders in diabetics. Ceramides containing omega carbons from linoleic acid esterified to omega hydroxy fatty acids are found only in the epidermis and help build a multilayered membrane that plays a key role in the skin barrier (34). The primary ceramides generated by the *de novo* pathway play a role in the development of the epidermal permeability barrier. The skin barrier is composed of extracellular ceramides. Intracellular ceramides, on the other hand, signal without O-acylation and have an acyl chain length of 16 to 18 carbon atoms (35).

Epidermal barrier structure. The skin of mammals serves as their body's first line of protection against external threats, making it one of the most important organs of the human body (36).

Both internal and external factors influence the epidermal ceramide expression profile. For example, downregulating CerS3 can reduce the proportion of unsaturated long-chain ceramides (37). Furthermore, AZGP1, an adipokine, has been shown to improve epidermal barrier function in Alzheimer's disease mice by increasing ceramide 3 (NP), ceramide 5 (AS) and ceramide 1 (EOS) (38).

The composition of ceramides in the stratum corneum is changing with age. Children have more ceramide 8 (NH), and less α-hydroxy and esterified ω-hydroxy ceramides than adults. There is less ceramide 1 in older people compared with that in younger people. Moreover, the degree of fatty acid chain saturation of ceramide 1 is associated with the season, being reduced in autumn and winter, which participates in lamellar and lateral lipid organization. Deficiency of this type of ceramide may be associated with seasonal skin dryness (39). Exposure to ultraviolet irradiation can increase plasma ceramide levels and decrease SM levels in mice (40). Therefore, the level of epidermal ceramides is maintained in dynamic equilibrium by control from a number of factors and plays an important role in skin homeostasis (41).

Although ceramides account for the highest proportion of skin lipids, they are no more important than other lipids. Previous studies found that perturbation of the skin with ceramides alone or mixtures of ceramides and FFAs

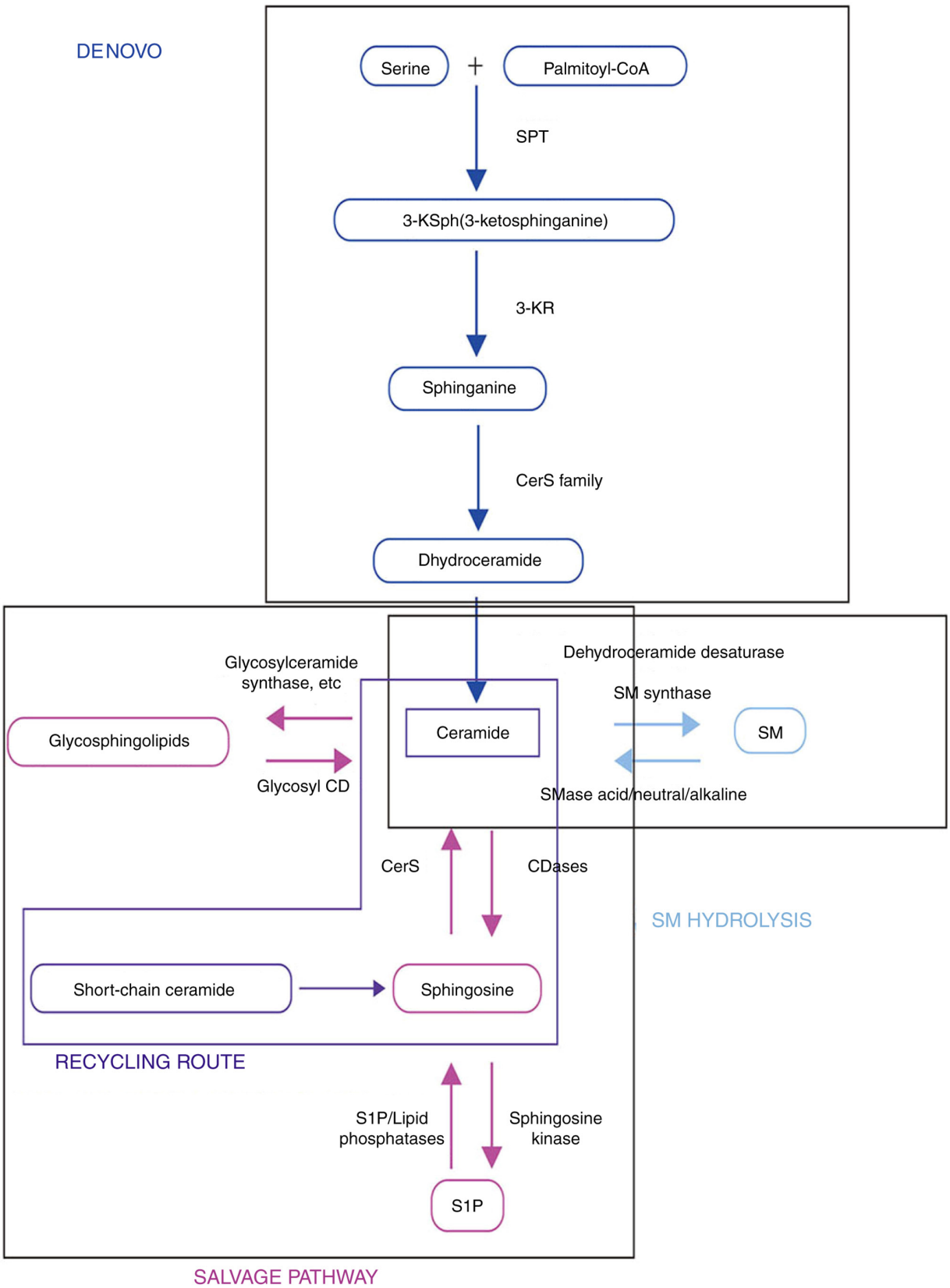

Figure 1. Three pathways for the synthesis of ceramides. There are three classical pathways for the synthesis of ceramides, namely the *de novo* pathway, the hydrolysis of sphingomyelin pathway and the salvage pathway. CerS, ceramide synthase; SM, sphingomyelin; SMase, sphingomyelinase; CD, ceramidase; S1P, sphingosine-1-phosphate; SPT, serine palmitoyltransferase; 3-KR, 3-ketosphinganine reductase.

delayed skin barrier recovery, and normal recovery was only possible with equimolar mixtures of all key lipids, as determined by transepidermal water loss (42,43). These findings demonstrate that FFAs, cholesterol and ceramides are hydrophilic extracellular lipid matrices that are indispensable for skin permeability (44).

Despite the lack of direct evidence, ceramides have been hypothesized to regulate keratinocyte proliferation and differentiation in the skin. Both *in vitro* and *in vivo*, keratinocyte differentiation is reported to be accompanied by an increase in ceramide production (45).

Skin immune responses. Ceramides and their derivatives play a crucial role in controlling inflammation and the immune system. The activation of dendritic cells (DCs) with proinflammatory agents like lipopolysaccharide, TNF or interleukin 1 (IL1A and IL1B) has been shown to increase the intracellular concentrations of ceramides by promoting the stability of Toll-like receptor 4 (TLR4) (46). Changes in the transcriptional levels of ceramide synthetase and ceramidase in the lipid skeleton of TLR9-stimulated wild-type DCs were found to regulate the ceramide ratio (47). Ceramides are intracellular modulators of the NLR family, pyrin domain containing 3 (NLRP3) inflammasome assembly, and studies in microglia have shown that they can promote the release of IL1B (48). A single study has found that in mouse models, the presence of *Staphylococcus epidermidis* increases the content of ceramides in the epidermis by secreting SMase, which interferes with the growth of other pathogenic microorganisms and affects the immune pathway (49). Ceramides are therefore considered to be bioactive signaling molecules active in a number of inflammatory signaling pathways.

4. Ceramides and DFUs

One of the major chronic complications of diabetes, and the main cause of disability and mortality, is DFUs (50). Approximately 15-25% of diabetic patients will develop DFUs. Severe DFUs progress to gangrene and infection, leading to amputation (4). Despite the fact that DFUs can be treated, they can return up to 40% in the first year and almost 100% in the next decade (51). Numerous risk factors affect DFUs, but they are not independent causes of foot ulcers. Age, blood pressure, blood sugar levels, smoking, various types of nerve injury, impaired vascular circulation and diabetes over an extended period of time are all risk factors (52). DFUs are caused by peripheral neuropathy, peripheral artery disease, and repetitive external and minor trauma (Fig. 2) (53).

Ceramides, a crucial part of the keratinocyte membrane and a factor in the incidence and progression of several skin illnesses, are associated with the skin's barrier and permeability functions (35). Ceramides are also closely associated with insulin resistance, diabetes, and the microvascular and macrovascular side effects of diabetes, including atherosclerosis and DN (10). These findings raise the question of whether ceramides are crucial lipid molecules in diabetic patients who develop vascular disease, neuropathy and foot ulcers.

Pathophysiology of DFUs. The etiology of DFUs involves peripheral artery occlusive disease, neuropathy and trauma with subsequent infection (54).

DN included motor, peripheral and autonomic neuropathies (55). The manner in which diabetes causes DN is not clear. However, after the occurrence of DN, the muscles will atrophy, resulting in changes in the anatomical structure of the foot, and even the formation of deformities. Due to the abnormal mechanical action, a foot hematoma occurs, which is mainly caused by motor neuropathy. Autonomic neuropathy causes blood vessels to dilate and the temperature of the foot to rise, drying out the skin and making it more vulnerable to damage. A lesion of the peripheral nerve makes the situation worse by depriving the patient of the protective sensation in the foot (56,57). Abnormal glucose metabolism occurs through the polyol and hexosamine pathways, leading to reactive oxygen species (ROS) production and inflammation through mitochondrial damage. In addition, abnormal glucose metabolism, resulting in glycosylation of proteins, the production of advanced glycation end products (AGEs), interaction with age-specific receptors and their own accumulation, can also lead to ROS production and the release of inflammatory factors. Similarly, excess free fat can also generate ROS through β-oxidative catabolism, causing local inflammation. The increase in ROS initiates the oxidative stress mechanism and causes tissue damage (58). Sphingolipid metabolism has been found to be associated with DN (59). The formation of abnormal deoxysphingolipids is harmful to pancreatic β-cells and nerve cells. Elevated levels of deoxysphingolipids have been found in patients with type 1 DM (T1DM) and T2DM with inherited sensory and autonomic neuropathy (60). Furthermore, these studies highlight the potential of plasma ceramide and deoxyceramide species as diagnostic and prognostic biomarkers for DN.

DM is now clearly associated with the development of cardiovascular disease (61). The vascular disease associated with DFUs is peripheral arterial disease of the lower extremity, which refers to an atherosclerotic occlusion from the main iliac artery to the foot artery (62). Hyperglycemia-induced epithelial dysfunction reduces pro-angiogenic signaling and nitric oxide (NO) production. NO prevents smooth muscle relaxation and leads to foot hypoperfusion, which can lead to an impaired skin barrier (63). Patients with diabetes are prone to atherosclerosis, resulting in poor blood perfusion in the foot and neuropathy making patients feel numb, coupled with repeated trauma and unreasonable pressure in daily life, so that the healing of the foot wound is delayed. This is followed by an infection of the foot, which can lead to gangrene and even amputation (64).

In addition, foot deformities, trauma and secondary infections accompanied by weakened resistance further aggravate DFUs, forming a vicious cycle (65).

5. Ceramides and vessels

Ceramides and the endothelium. Regulation of vascular tone requires the production of vasoactive substances, such as NO, by endothelial cells (66). According to the current evidence, ceramides have a detrimental effect on endothelial function. Endothelial dysfunction is caused by decreased NO synthesis or increased NO breakdown due to the generation of ROS (67). Ceramides can activate NADPH oxidase, increasing ROS, leading to increased oxidative stress, which degrades ceramidase (68). Ceramides have been shown to inhibit the *de novo* pathway of ceramides in diet-induced obese C57Bl/6 mice, which produces normalization of endothelial dysfunction and systemic hypertension (69). However, endothelium-dependent vasodilation is impaired when the

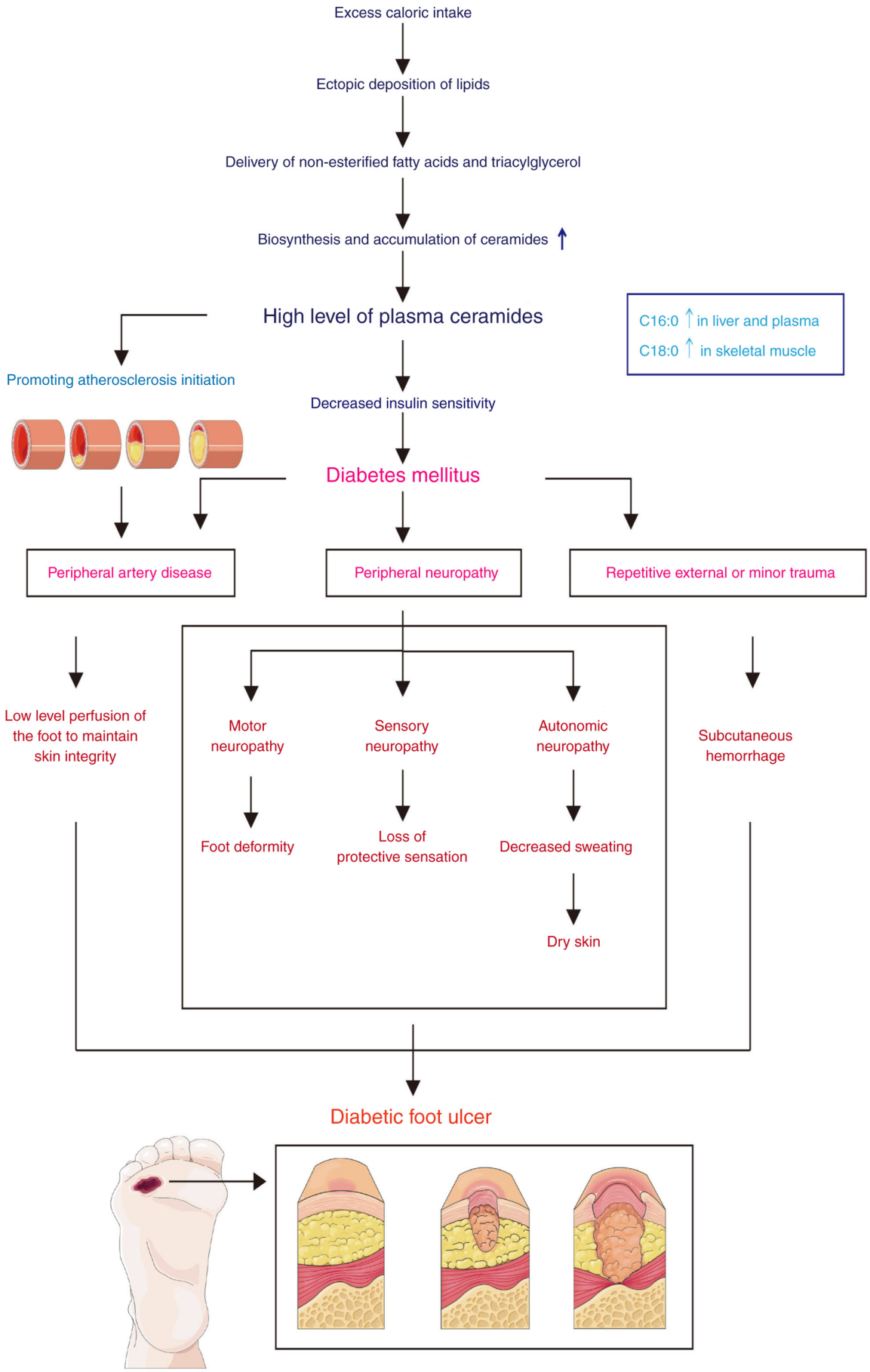

Figure 2. Mechanism of DFUs. Due to changes in lifestyle and increased calorie intake, plasma ceramide levels increase, especially those of C16:0, but mainly the levels of C18:0 in skeletal muscle, which in turn leads to diabetes and atherosclerosis. DFUs result from peripheral neuropathy, peripheral artery disease, and repetitive external and minor trauma in diabetic patients. DFU, diabetic foot ulcer.

endothelium is briefly exposed to exogenous ceramides. This finding indicates that ceramides are crucial in the impairment of the endothelium caused by inflammation or obesity. Exogenous C16 ceramide-mediated endothelial NO synthase (NOS) uncoupling and dysregulated protein phosphatase 2 (PP2A) were found to damage human aortic endothelial cells in a study of atherosclerotic patients (70). Another study in patients with coronary arteries also found that ceramides were associated with coronary endothelial dysfunction (71). However, more mechanistic studies are needed to confirm the findings. Karakashian *et al* (72) demonstrated that nSMase activity persistently increases with aging, resulting in greater ceramide and endothelial NOS (eNOS) inactivation, as well as a reduction in NO production. In addition, nsMase-derived ceramides participate in the conversion of NO to H_2O_2, which leads to the reduction of NO and the formation of coronary artery disease (73). Ceramides have been shown to decrease eNOS3 (NOS3; also known as eNOS) activity, either under baseline conditions or after stimulation (74). On the other hand, in high-fat diet mice, inhibiting the synthesis of ceramides by the *de novo* pathway may indirectly improve endothelial dysfunction (75). Moreover, a research study has demonstrated that ceramides can activate NADPH oxidase (76), but other studies contend that ceramides can directly influence the mitochondrial electron transport chain to increase ROS in various cell types, including endothelial cells (77,78). Following a harmful self-amplifying cascade of events, the resultant O_2 can ultimately cause the decoupling of NOS3 and the generation of ROS in endothelial cells. Last but not least, ceramides have the ability to activate the NLPR3 inflammasomes and release inflammatory cytokines, thereby contributing to atherosclerotic lesions and vascular dysfunction (48).

Vasoactivity of ceramides. Ceramides have been found to be connected with vasoactivity. Ceramides can not only contract the blood vessels, but also vasodilate them (74). However, the mechanisms by which ceramides induce vasoactivity remain unclear.

A single study has shown that ceramide-induced vasodilation is partially endothelium-dependent via activation of NOS3 and subsequent NO generation, as aforementioned, by impairing endothelium function (79). Angiotensin II (ANGII) may indirectly increase ceramide production, thereby activating SMases in the peripheral vasculature to control vascular function (80).

However, a considerable amount of research has found that ceramides can promote vasoconstriction (74,81,82). Total ceramide levels in the aortic tissue of hypertensive rats and humans were much higher than those in normotensive mice and humans, with the main changes being increases in the levels of ceramides C24:1 and C24:0 (83). However, a correlation between increased blood pressure and increased ceramides in plasma does not indicate a causal relationship (84). Notably, it has been suggested that nSMase-derived ceramides promote the vasoconstriction caused by changes in the levels of thromboxane A2, ANG II and oxygen tension (85,86).

In short, the vascular effects of ceramides appear to be complicated and remain unclear. This vasoactivity may be influenced by a variety of variables. Numerous exogenous and endogenous processes can produce ceramides, and can also transform them into other active molecules. The different vascular effects may result from the different types of vessels and their various diameters, as well as the cell types they act on. Notably, different lengths of ceramides have different pathological functions (87).

6. Ceramides and diabetes

Diabetes and cardiovascular disease are mostly caused by lipotoxicity, an abnormal accumulation of lipids in non-adipose tissue. The most dangerous sphingolipids are those that promote cell death, insulin resistance and decreased insulin gene expression (88). A key component of the metabolism of sphingolipids is ceramides (29,30). Furthermore, previous studies have provided clear evidence that sphingolipids, particularly ceramides, have an important role in T1DM and the complications of T2DM (89,90). Indeed, several metabolic diseases, including DM, cardiomyopathy, insulin resistance and atherosclerosis, can be treated by preventing ceramide production or accelerating ceramide breakdown (87).

Ceramides and pancreatic β-cell apoptosis. TNF, IL1B and interferon γ are examples of inflammatory agents producing cytotoxicity to pancreatic β-cells by increasing the levels of sphingolipids, particularly ceramides, which are either exogenously delivered or produced endogenously (91). By contrast, several studies have shown that reducing ceramide production, such as inhibition of SPT or inhibition of CerS inhibitors, reduces cytotoxicity to rodent (92) and human (93) β-cells. In fact, there is evidence of a very small increase in ceramides following FFA therapy in trials involving cell apoptosis (94). However, some studies have shown that inhibiting ceramide synthesis reduces β-cell apoptosis (95-98). There may be three reasons to explain this result. First, the increased ceramide level induces apoptosis only at the designated site, namely the mitochondria, without changing the total ceramide mass (93). Second, upon triggering apoptosis, ceramides are changed into another sphingolipid metabolite (glucosylceramide) (99). Third, distinct ceramide isoforms may have different apoptotic potentials. In order to promote apoptosis, hyperglycemia increases the levels of the harmful isoforms of ceramides, namely C22:0, C24:1 and C18:0 (100).

Ceramides and insulin resistance. There is increasing evidence that ceramides play an important role in insulin resistance. Insulin resistance is a pathophysiological state characterized by hyperinsulinemia, high blood glucose levels and decreased responsiveness of peripheral tissues to insulin. Numerous studies have shown that excessive consumption of FFAs, the use of glucocorticoids, corpulence and decreased physical activity are some of the major contributors to insulin resistance, in which ceramides serve as a key intermediary (101). Increased ceramide accumulation from palmitate exposure occurs in several cells, including muscle cells (102), adipocytes (103) and cardiomyocytes (104). Simultaneous Akt inhibition results in decreased insulin sensitivity (105). Sphingolipid recycling or the salvage pathway can cause a buildup of ceramides in response to an excessive supply of saturated or unsaturated FAs. Ceramides are an obligatory intermediate in saturated FA-induced insulin resistance (101). Zalewska *et al* (106)

showed that mice administered a high-fat diet expressed higher levels of CerS than mice fed a regular diet. However, Holland *et al* (107) found that infusion of both highly saturated and highly unsaturated FAs reduced glucose uptake and Akt activation in rats, but infusion of highly saturated FAs alone increased ceramide levels. Both saturated fats and unsaturated fats can promote insulin resistance through different mechanisms, but ceramides only participate in saturated fat-induced insulin resistance (107). However, unsaturated FAs were not associated with increased levels of ceramides, which are not the only lipids responsible for insulin resistance. In line with this, overexpression of acid ceramidase can lower ceramide levels, prevent the buildup of ceramides caused by palmitate and enhance insulin signaling (108).

Glucocorticoids can increase the levels of ceramides, which may be the mechanism for inducing insulin resistance (107,109). By activating enzymes such as SPT, SMase and CerS, the commonly used glucocorticoid dexamethasone increases ceramide levels in a number of cell types and animal species, such as 3T3-L1 cells, wild-type mice and Sprague-Dawley rats (107,110,111). Pretreating C57Bl6/J mice with myriocin, a potent inhibitor of SPT, was shown to avoid some of these effects (112). However, the study involved a low dose of myriocin, so the alterations cannot be ruled out as a result of subsequent changes in the gut microbiota. Peroxisome proliferator-activated receptor α (PPARA) is activated by ceramide accumulation, and thus genetic disruption of PPARA, or other damage causing decreased hepatic PPARA expression, has been reported to inhibit insulin resistance in some conditions (113).

Obesity is known to contribute to T2DM by influencing glucose homeostasis and insulin sensitivity (114). However, the levels of ceramides in the skeletal muscles of rats, mice and diabetic patients have been reduced with long-term aerobic training (115,116). In other studies, for some unknown reason, even after exercise training in rats and people, the level of muscle ceramides does not significantly decrease (115,117,118).

7. Ceramides and atherosclerosis

Sphingolipids are bioactive lipids found in atherosclerotic plaques, which have been linked to both the development and progression of atherosclerosis. Although their specific effects in human atherosclerotic plaques are still unclear, ceramides are an important component in sphingolipids that are also associated with atherosclerosis (71). By promoting their aggregation through ceramide-ceramide interactions, SM may be converted to ceramides by aSMase on low-density lipoprotein (LDL) surfaces, thus accelerating the onset of atherosclerosis. Additionally, the pro-atherogenic pathways may be stimulated by the S1P produced from ceramides (119).

Ceramides from human plaques can cause plaque inflammation and cell death. Ceramide levels were higher in plaques connected to the inflammatory response, as determined by examining the histology and measuring cytokine levels of the plaques (120). Similar findings were obtained in mouse models (121).

Inhibiting SPT in the ceramide biosynthesis pathway with myriocin has been shown to decrease the progress of atherosclerosis in rodent models (122). Ceramides have been shown to have more precise roles in the pathophysiology of cardiovascular disease than other existing lipid biomarkers, making them a promising biomarker for prediction purposes. Also, in mouse models, the inhibition of enzymes involved in ceramide synthesis can reduce cardiovascular complications. However, it is not easy to understand the possible mechanism of atherosclerosis without more research. On study found that ACER2 is a target gene for HIF-2a, which reduces ceramide levels in fat cells and improves atherosclerosis (123). However, the study cannot rule out other mechanisms involved in protecting atherosclerosis.

8. Mechanism of ceramides in diabetes

Mechanism of ceramide-induced β-cell apoptosis. Due to its preservation of the integrity of the internal environment, apoptosis is described as genetically programmed cell death. The mechanism of apoptosis is complex. There are three known apoptotic signaling mechanisms: The intrinsic mitochondrial, intrinsic ER and extrinsic death receptor pathways. A number of studies have shown that ceramides are associated with the induction of β-cell apoptosis (124). Zhang *et al* (125) found that amyloid peptides induce β-cell apoptosis, partly through the production of ceramides by activating aSMase through activation of K^+ channels on the cell membrane, which is a marker of cell apoptosis.

Ceramides have also been shown to cause apoptosis through their effect on the mitochondria, which controls regional levels of certain lipids like sphingolipids to detect cellular stress (126). BAX and ceramides work together to synergistically induce mitochondrial outer membrane permeabilization (MOMP). According to Ganesan *et al* (127), when ceramides are present, they are the key molecules in mitochondrial permeabilization, suggesting that the inhibition of ceramides without activating BAX-induced mitochondrial permeabilization, blocks the induction of MOMP, resulting in the death of yeast cells. According to the study by James *et al* (128), ceramides may be suggested in a new pathway leading to cell apoptosis by inducing the generation of Creola bodies, a marker of apoptotic lung epithelial cells. However, this was found in mouse lung tissues, and the mechanisms in other tissues require more investigation. Meanwhile, ROS also increased as a result of the increased ceramide level, which can contribute to apoptosis. Studies have found that ceramide-mediated insulin resistance-induced apoptosis was associated with the DNA-damage response and mitogen-activated protein kinase pathways, and that ceramides could mediate mitochondrial dysfunction (129,130). ROS are primarily produced by the mitochondria, and impairment of the mitochondria is generally considered to be associated with increased ROS. Apoptosis-inducing factors are generated when ceramides are being synthesized. Ceramides may inhibit the activation of mitochondrial NADPH oxidase, thus blocking electron transport at complex I and complex III of the respiratory chain, and inducing apoptosis by increasing ROS production (131,132).

Ceramides are a mediator of palmitate-induced cell toxicity, according to certain studies (133-135). One hypothesis that has been postulated is that ceramides can induce β-cell death by

activating protein kinase C δ-type (PKCD), which is necessary for apoptosis in numerous cell types (136).

Numerous studies have indicated that ER stress is crucial for cell apoptosis (137-139). During diabetes, insulin is produced in large amounts to counteract high blood sugar. In this situation, there is an increased burden on the ER, which serves as the location for the synthesis and folding of released proteins. The unfolded protein response (UPR), which aids in re-establishing normal ER function, is activated by ER stress (140). In addition, miR-204 has been found to activate the apoptotic signaling pathway in β-cells (141).

Another possible way to induce β-cell apoptosis is to inhibit the function of Akt, which is a serine/threonine kinase. First, it was shown that Akt could increase cellular proliferation, but that its inhibition could induce apoptosis (142). Second, Akt upregulates CDKN1B to trigger apoptosis while adversely regulating the transcriptional activity of FOXO1 (143). Third, Akt promotes mTOR/p70S6K signaling and directly phosphorylates and inactivates BCL2 members, including BAD, BAX and BID, to cause apoptosis (144). In addition, AKT induce apoptosis by activating cell signaling such as that of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (98).

Mechanism of ceramide-induced insulin resistance. The phosphorylation of insulin receptor substrate-1 (IRS1), a mediator protein that links the binding of insulin and insulin growth factor 1 to related intracellular receptors of the insulin pathways, can activate ceramides to lead to impaired islet signaling (145). The increased IRS1 level induces serine 307 phosphorylation to inhibit insulin signaling (146). Ceramides can also suppress IRS1 expression by triggering the PKR/JNK/Prep1/p160 axis and/or the c-Jun amino-terminal kinase/PBX regulatory protein 1 axis (89). In addition, glucosylceramides have been demonstrated to impair insulin signaling by inhibiting insulin receptors (101,147).

As aforementioned, ceramides can inhibit AKT to cause β-cell apoptosis, and other studies have shown that they can also mediate the secretion of insulin (98,148,149). Ceramides first catalyze the dephosphorylation of AKT by activating PP2A (150). Ceramide secondly prevent the translocation of AKT to the PIP3-PDK1 complex in the plasma membrane. Ceramides can activate PKCZ (151), and then ceramide-induced phosphorylation inhibits this at the serine 473 or threonine 308 residue (24,102). This can form a stable AKT-PKCZ complex to prevent its interactions with PIP3. In further studies, PKC inhibitors were shown to improve insulin sensitivity and block the ceramide-induced loss of AKT activity (152-154). Ceramides were also elevated in other animal models such as Zucker diabetic fatty (ZDF) rats (155) and ob/ob mice (156), as well as in obese humans (157). If ceramides cause insulin resistance by inhibiting AKT, the proximal insulin signaling should be intact; however, there are also abnormalities in this proximal insulin signaling. In addition, the presence of the HIF-2α-neuraminidase 3-ceramide pathway was also observed in mice administered a high-fat diet, and insulin sensitivity was improved in mice ablated with enteric-specific HIF-2α. However, this only occurred in the absence of oxygen (158).

Exosome secretion and/or biogenesis may be impacted by ceramides. The protein adiponectin, which is released by adipocytes, stimulates the synthesis of exosomes in skeletal muscle and endothelial cells, and induces the expression of cadherin 13 (159). Given that research indicates that the microRNA profiles of exosomes were changed in patients with T2DM, Santovito *et al* (160) reported that exosomes, a metabolic mediator, produced by adipose tissue macrophages, impact insulin sensitivity in mice. Lean mice developed glucose intolerance and insulin resistance after exposure to exosomes from obese mice. By contrast, exosomes from adipose tissue macrophages in lean mice reduced insulin resistance and glucose intolerance in obese animals (161). Exosomes may also convert monocytes into macrophages, lead to inflammation and disrupt insulin signaling to cause T2DM (162).

9. Mechanism of ceramides in atherosclerosis

The most frequent cardiovascular consequence of T2DM is atherosclerosis, which develops in big and medium-sized arteries (163); it is characterized by inflammation, lipid and macrophage buildup, cell death and fibrosis (164). In addition, sphingolipids, including ceramides, are elevated in human atherosclerotic lesions, and it is widely recognized that chronic inflammation is the constant sign of T2DM and atherosclerosis (101). Additionally, sphingolipid metabolites are important in inflammatory signaling. Sphingolipids, particularly ceramides (the center of sphingolipid metabolism), have been proved to have an association with cell death, insulin resistance, inflammation and lipotoxicity, as previously described (6). The most prevalent and harmful metabolites in mammalian tissue are C16- and C18-ceramides (165). It was reported that ceramides cause inflammation in smooth muscle cells of the human coronary arteries (10). However, myriocin could suppress SPT by preventing ceramide *de novo* synthesis, which reduced atherosclerosis in apolipoprotein E-knockout mice (122). Atherosclerosis is also associated with lipoprotein aggregation. However, hydrolysis of SM to ceramides, especially nSMaes2, has been found to cause lipoprotein aggregation and participate in atherogenesis (166).

ER stress and the NLRP3 inflammasome have a close association with the development of atherosclerosis (167). As aforementioned, patients with T2DM may have defects in the downstream insulin signaling pathway that affect glucose transport due to activation of the IRS1 tyrosine phosphorylation/phosphoinositide 3 (PI-3) kinase axis (168). In addition, since the same PI-3 kinase pathway also activates NOS3, less NO is produced, which impairs endothelial function and accelerates atherosclerosis (169).

NFKBIB and NFKB are connected in the cytoplasm. Increased NFKBIB/NFKB signaling activity may be a significant factor in T2DM inflammation and insulin resistance (170). Fatty acyl-CoAs are an example of an inflammatory factor that activates NFKBIB kinase, phosphorylates NFKBIB and then translocates to the nucleus to bind to target genes, thus increasing the production of inflammatory cytokines that are involved in atherosclerosis (TNF, IL1B, IL6 and PKC) (171,172). Ceramides have the ability to activate certain plasma membrane receptors, including

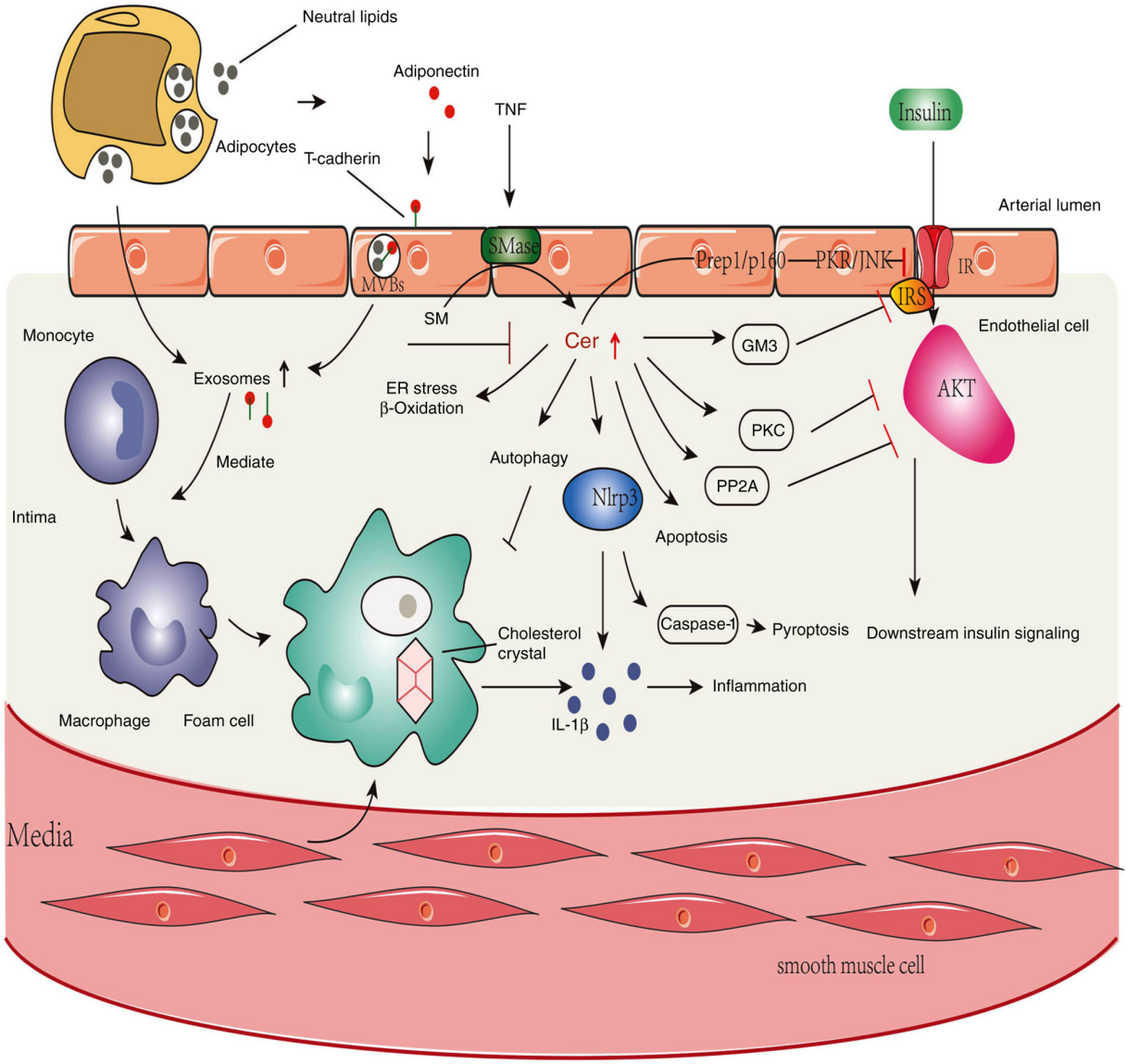

Figure 3. Molecular mechanisms mediating DFUs in diabetes and atherosclerosis. Possible mechanisms of ceramides causing DFUs: i) Ceramides are associated with the induction of β-cell apoptosis by the activation of PKCD or by the inhibition of the function of AKT. ii) Ceramides inhibit insulin signaling via IRS1 by activating the PKR/JNK axis and/or Prep1/p160 axis or by GM3. iii) Ceramides activate PP2A and PKCZ to catalyze the dephosphorylation of AKT and then decrease the secretion of insulin. iv) Ceramides can affect exosome biogenesis and/or secretion to interfere with the downstream insulin signaling and inflammation through lipotoxicity. SMase, sphingomyelinase; Cer, ceramides; ER, endoplasmic reticulum; SM, sphingomyelin; IL, interleukin; PPA2, protein phosphatase 2; PKCD, protein kinase C δ-type; PKCZ, protein kinase C ζ-type; MVB, multivesicular body; Nlrp3, nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3; Prep1, PBX regulatory protein 1; PKR, dsRNA-activated protein kinase; JNK, c-Jun amino-terminal kinase; IR, insulin receptor; IRS, insulin receptor substrate; GM3, ganglioside GM3.

TLR4, which might lead to inflammation and insulin resistance (173). The TLR4 mRNA/protein levels are also elevated in the muscle of obese patients and those with T2DM, and they closely correspond with NFKBIB/NFKB activation, which is another mechanism for promoting atherosclerosis. Therefore, it is likely that ceramides are closely related to this development (174).

Since it has been shown that sphingolipid production is inhibited, atherosclerosis is reduced by the decreased expression of sterol-regulatory element binding transcription factors (SREBFs), which include SREBF1A, SREBF1C and SREBF2 (175). However, in animals orally treated with myriocin (an inhibitor of SPT and a necessary enzyme for the synthesis of ceramides), SREBF1C levels decreased, which was potentially due to decreased very-LDL particle size. Myriocin was found to be anti-atherosclerotic. These effects may be partly correlated with SREBF (176,177).

In conclusion, ceramides are closely associated with diabetes and atherosclerosis, and the mechanism of action may involve the induction of DFUs (Fig. 3). Additional mechanisms of action of ceramides need to be found to support ceramides as the underlying molecule involved in the advent of DFUs.

10. Shared mechanisms between diabetic complications and DFUs

A certain degree of common pathogenesis is shared between diabetic complications and DFUs.

Diabetic nephropathy and diabetic retinopathy have the same pathogenesis, which is caused by the dysfunction of microvascular endothelial function (178). Consistent with DFUs, hyperglycemia through various pathways such as the polyol pathway, the AGE/receptor for AGE axis and the PKC pathway increases ROS production, causes oxidative stress and a series of inflammatory responses, and eventually leads to the accumulation of AGEs and endothelial dysfunction. At the same time, recent studies have found that renal cells can produce exosomes, which promote the development of inflammation and lead to endothelial cell damage (179-181). This is supported by the finding that the transplantation of new endothelial cells can prevent the development of a diabetic foot (182).

Diabetic peripheral neuropathy is involved in the development of DFUs, as aforementioned. The same diabetes leads to increased ROS levels and mitochondrial dysfunction, leading to impairment of axonal transport function in peripheral nerves, especially Schwann cells. However, the mechanism of diabetic peripheral neuropathy is still unclear (65).

Cardiac autonomic neuropathy and DFUs have also been recently linked, although the mechanism of cardiac autonomic neuropathy is still unclear. At present, ROS and AGEs are suspected to be associated with the occurrence of inflammation and microvascular lesions, which is also consistent with the occurrence of DFUs (183).

There is no evidence for a common mechanism of liver fibrosis and DFUs. In only one case report, excluding those on hepatitis and autoimmune liver diseases, was there a record of a patient with liver fibrosis that was associated with microangiopathy of the liver, as well as diabetic nephropathy and DFUs (184). This still needs more research and discussion.

11. Conclusions

Research has shown that ceramides are important in diabetes and cardiovascular disease. Ceramides are precursors of complex sphingolipids that form the epidermal barrier structure and are involved in maintaining skin homeostasis. Direct experimental evidence suggests that the plasma levels of ceramides are higher in diabetics, that ceramides antagonize insulin signaling, and that the inhibition or elimination of every ceramide biosynthesis-related enzyme is consistent with insulin sensitization, anti-atherosclerosis and heart protection. DFUs are a chronic complication of diabetes and an important cause of disability and death from diabetes, mainly caused by peripheral artery disease, peripheral neuropathy and recurrent external or mild trauma. Most of the existing reviews describe the association between ceramides and DM. The present review attempts to identify the function of ceramides in the occurrence and development of DFUs by elaborating on the association between ceramides and DM. The hope is that these findings will translate into new screening methods and treatments to alleviate and possibly prevent or cure diabetes and DFUs.

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Authors' contributions

YW conceived the topic and wrote the first draft. ZS, GZ, LZ and ZW revised the manuscript and figures. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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