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Neutral sphingomyelinase-2 and cardiometabolic diseases

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Summary

Sphingolipids, in particular ceramides, play vital role in pathophysiological processes linked to metabolic syndrome, with implications in the development of insulin resistance, pancreatic ß-cell dysfunction, type 2 diabetes, atherosclerosis, inflammation, nonalcoholic steatohepatitis, and cancer. Ceramides are produced by the hydrolysis of sphingomyelin, catalyzed by different sphingomyelinases, including neutral sphingomyelinase 2 (nSMase2), whose dysregulation appears to underlie many of the inflammation-related pathologies. In this review, we discuss the current knowledge on the biochemistry of nSMase2 and ceramide production and its regulation by inflammatory cytokines, with particular reference to cardiometabolic diseases. nSMase2 contribution to pathogenic processes appears to involve cyclical feed-forward interaction with proinflammatory cytokines, such as TNF-α and IL-1ß, which activate nSMase2 and the production of ceramides, that in turn triggers the synthesis and release of inflammatory cytokines. We elaborate these pathogenic interactions at the molecular level and discuss the potential therapeutic benefits of inhibiting nSMase2 against inflammation-driven cardiometabolic diseases.

KEYWORDS
cardiometabolic diseases, ceramide, nSMase2, sphingolipid

Abbreviations: aSMase, acid sphingomyelinase; bSMase, basic sphingomyelinase; C1P, ceramide-1 phosphate; CAT, C-terminal catalytic domain; DES, dihydroceramide desaturase; EED, embryonic ectodermal development; ER, endoplasmic reticulum; FAN, factor associated with nSMase; GSH, glutathione; GSK3β, glycogen synthase kinase; IFN-γ, interferon-γ; IL-1β, interleukin 1β; JNK, c-Jun N-terminal kinase; JX, juxtamembrane; LDL, low-density lipoprotein; MCP-1, Monocyte Chemoattractant Protein-1; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NO, nitric oxide; nSMase2, neutral sphingomyelinase 2; NTD, N-terminal domain; Ox-LDL, oxidized low-density lipoprotein; PBMC, peripheral blood mononuclear cells; PP2A, protein phosphatase-2A; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SMase, sphingomyelinase; sSMase, secretory sphingomyelinase; T2D, type 2 diabetes; TLR, toll-like receptor; TNFR-1, TNF-α receptor; TNF-α, tumor necrosis factor-α; ZDF, Zucker diabetic fatty.

Sardar Sindhu and Yat Hei Leung equally contributed to this work.

1 | INTRODUCTION

Sphingolipids are components of cellular membranes that participate in structural as well as in signaling pathways and regulate many cellular functions. By regulating the pathways of inflammation, apoptosis, and proliferation, sphingolipids serve as bioactive molecules and play a major role in the pathophysiological mechanisms of cardiometabolic diseases such as type-2 diabetes (T2D) and atherosclerosis and also cancer, lung disease, and lysosomal storage disorders.[1–8] The knowledge of the signaling functions of sphingolipids has expanded considerably over the past decade and has expanded the many diverse functions of ceramides in biological processes. Ceramides are synthetized de novo from serine and palmitoyl-CoA, from the hydrolysis of ceramide-1-phosphate, from sphingosine involving ceramide synthase, from the hydrolysis of glucosylceramide, and via the action of sphingomyelinases (SMases), a family of enzymes that hydrolyze the membrane lipid sphingomyelin to generate phosphocholine and ceramide.[9,10] SMase-derived ceramides have been implicated in many signaling (cell growth, differentiation, senescence, and apoptosis) and pathological (inflammation, insulin resistance, diabetes, atherosclerosis, oxidative stress, and neurodegenerative disorders) events.[11–19] SMases are classified into five families based on their pH optima and metal ion requirement for their activity. Their characteristics and roles will be briefly described in a separate section below.

nSMase activity was first reported by Schneider and Kennedy in fibroblasts from patients with Niemann-Pick disease.[20] It was later characterized as a predominant membrane-bound enzyme in the crude extracts of human gray matter.[21] Four nSMases have been described: nSMase1, nSMase2, nSMase3, and mitochondria-associated nSMase.[22] All require a neutral pH and divalent cations (Mg^{++}/Mn^{++}) for the hydrolysis of the phosphocholine-head group of sphingomyelin to generate ceramide.[23] nSMases, except nSMase-3, have a DNase-I type catalytic core, suggesting a common mechanism of these three enzymes for sphingomyelin catalysis.[24] The most investigated isoform, nSMase2, is a 655 amino acid long (71 kDa) protein localized in the plasma membrane and the Golgi apparatus in many cell types.[22,25–27] It has been implicated in diverse functions, in particular cell differentiation and growth arrest[10,28]; postnatal growth and bone development[29,30]; cell cycle[31]; cell adhesion, migration, and proliferation[32]; senescence[33]; cell death and apoptosis[34,35]; autophagy[36]; exosome release[37,38]; and inflammatory immune responses.[28,39–41] nSMase2 also appears to be involved in the pathogenesis of several diseases such as cancer,[42,43] neurological and age-related neurodegenerative diseases (amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease),[44,45] as well as pulmonary,[46,47] circulatory,[47–50] and cardiometabolic diseases.[51–55]

In the present review, we focus on the recent development of our knowledge on the role of nSMase2 in the pathophysiology of metabolic syndrome-related disorders and their complications: obesity, insulin resistance, metabolic inflammation, β-cell dysfunction, T2D, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), atherosclerosis, and heart failure.

2 | SPHINGOLIPIDS, SPHINGOMYELINASES, AND CERAMIDES IN BRIEF

2.1 | Mammalian sphingolipid metabolism

Sphingolipids, a class of lipids that contain a sphingoid base (mainly C_{18} backbone), were first discovered in brain extracts and named after the mythological sphinx for their enigmatic disposition.[56] Sphingolipid metabolism consists of an intertwined network of metabolic pathways that can be grouped into four key modules.[1,57,58] (1) In the endoplasmic reticulum (ER), the central hub of sphingolipid metabolism, where de novo biogenesis of ceramides takes place. In this process palmitoyl-CoA and serine are first condensed to produce 3-keto-dihydrosphingosine, and subsequently, dihydrosphingosine, dihydroceramide, and eventually ceramide are formed. (2) Ceramide is then transported from the ER to the Golgi apparatus, where it is converted into more complex sphingolipids, such as sphingomyelin and glycosphingolipids.[59] (3) In the plasma membrane, ceramide is converted into other bioactive molecules including ceramide-1 phosphate (C1P), sphingomyelin, sphingosine-1-phosphate (S1P), and sphingosine.[57] (4) Ceramide regeneration via hydrolysis of complex sphingolipids like sphingomyelin and glycosphingolipids that helps in the maintenance of sphingolipid homeostasis.[60] Elevated de novo biosynthesis of ceramide in the ER results in its accumulation and ER stress. Reduced hydrolysis of complex sphingolipids offsets homeostasis and leads to excessive sphingolipid storage. Ceramide and sphingosine in excess are pro-apoptotic and can cause cell de-differentiation, senescence, apoptosis, and growth arrest. On the other hand, C1P and S1P are prosurvival molecules involved in angiogenesis, proliferation, migration, transformation, and inflammation.[61–64] Overall, sequestration of these metabolic steps in different subcellular compartments ensures the organized orchestration of the complex roles of the various sphingolipids in membrane biology and cell function, and its dysregulation can promote disease pathogenesis (Figure 1).

2.2 | Ceramide metabolism

There are three characteristic pathways of ceramide formation including (1) ceramide release via sphingomyelin hydrolysis in the cell membrane, the pathway of nSMase2 discussed in this review[65,66]; (2) de novo synthesis in the ER, initiated by serine palmitoyltransferase-mediated conversion of serine and palmitoyl-CoA into 3-ketodihydrosphinganine, subsequently yielding dihydroceramide, which is converted by dihydroceramide desaturase (DES) to ceramide[57,60,67]; and (3) the "salvage pathway" in which complex sphingolipids are degraded into ceramide by various enzymes present in acidic lysosomes. Then, acid ceramidase converts ceramide into sphingosine, which re-enters the cytosol and is recycled back into ceramide by the action of ceramide synthase[68,69] (Figure 1).

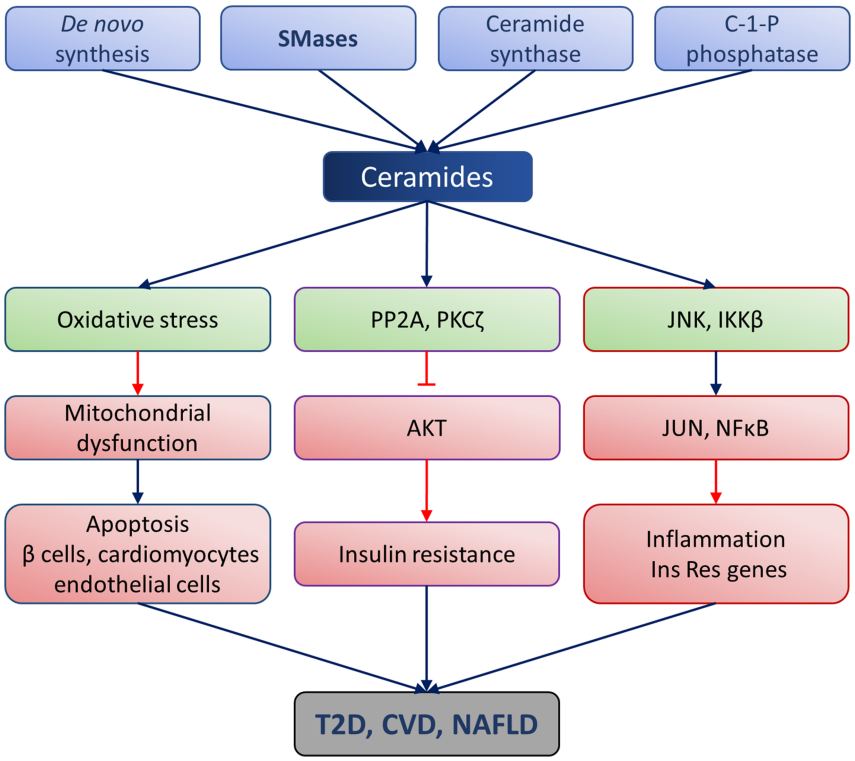

FIGURE 1 Signaling cascade of ceramides from their production to the pathogenesis of metabolic disorders. Ceramides can be synthesized de novo from palmitoyl-CoA, from sphingomyelin through SMases, from sphingosine via ceramide synthase, and from ceramide-1-phosphate (C-1-P) through its phosphatase. Accumulation of ceramides causes oxidative stress and the activation of different protein kinases, which leads to mitochondrial dysfunction, apoptosis, insulin resistance, and inflammation. These processes contribute to pathogenesis of metabolic diseases. CVD: cardiovascular diseases; Ins Res: insulin resistance; NAFLD: nonalcoholic fatty liver disease; T2D: type 2 diabetes

2.3 | Sphingomyelinase family members and their roles

The five classes of sphingomyelinases show these general characteristics. (1) Acid sphingomyelinases (aSMases) show optimum activity at pH 5 and are localized in lysosomes but can also relocate to the outer leaflet of the plasma membrane.[70,71] They play a role in cellular responses to stress, proliferation, differentiation, and cell death.[72,73] (2) Secretory sphingomyelinases (sSMases) share a common protein precursor with aSMases but with a different protein trafficking fate targeted to the Golgi secretory pathway. These enzymes function best at acidic pH and may or may not require Zn^{2+} for their activity.[74–76] (3) Mg^{2+}-dependent, neutral sphingomyelinases (nSMases) are found in the ER and Golgi apparatus,[70,77] as well as in the inner leaflet of the plasma membranes.[78–80] Their optimal pH is 7, requires Mg^{2+}/Mn^{2+}, and they can be activated by several growth factors, tumor necrosis factor-α (TNF-α), and oxidized low-density lipoproteins (Ox-LDL).[81] These enzymes play a role in stress-induced cell death.[82] (4) Mg^{2+}-independent neutral sphingomyelinases, identified in the cytosol of human leukemia cell line HL-60 and rabbit skeletal muscle, show optimal activity at pH 7.5 and are involved in regulation of Ca^{2+} levels in the skeletal muscle.[83,84] (5) Basic or alkaline sphingomyelinases (bSMases), purified and characterized from rat intestine, bile, and liver, are Mg^{2+}-independent but bile salt-dependent and protease-resistant and show optimum activity between pH 9 and 9.5.[85–87]

3 | NSMASE2 BIOCHEMISTRY AND REGULATION

3.1 | Tissue distribution and cellular localization

The nSMases were discovered in 1967 by Schneider and Kennedy.[20] Only after about three decades, purification, cloning, and characterization of mammalian nSMase1 were reported from rat[88] and bovine[89] brain. However, there was still significant sphingomyelinase activity present, even after the removal of nSMase1 activity in brain homogenates, and this soon led to the identification of a second nSMase, named nSMase2.[90] The two nSMase isoforms differ in their tissue distribution, with the expression of nSMase1 being ubiquitous, while nSMase2 is highly expressed in the brain and its expression is low in bone, liver, and spleen.[29,90–92] nSMase2 was originally shown to be localized in a subcompartment of the Golgi apparatus.[91] Then, it was shown that at high cellular density, this enzyme is translocated to the inner leaflet of the plasma membrane in order to be in contact with its lipid substrate[93,94] (Figure 2).

3.2 | Gene expression and regulation

3.2.1 | Transcriptional regulation

nSMase2, encoded by the *SMPD3* gene, is the most studied enzyme within the sphingomyelinase family. Chemotherapeutics, such as

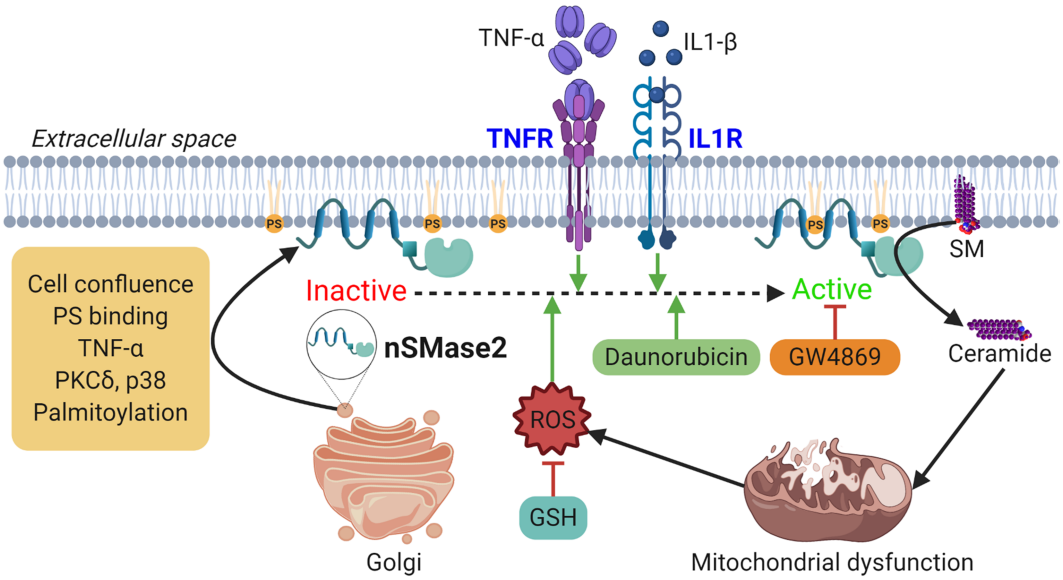

FIGURE 2 Schematic representation of nSMase2 localization and regulation. nSMase2 can translocate from the Golgi apparatus to the plasma membrane, and this process is influenced by various factors. The enzymatic activity of nSMase2 can be modulated by oxidative stress, inflammatory cytokines, glutathione, chemotherapeutic agents (daunorubicin), and the pharmacological inhibitor GW4869. GSH: glutathione; PKCδ: protein kinase C isoform-δ; PS: phosphatidylserine; ROS: reactive oxygen specie; SM: sphingomyelin; TNF-α: tumor necrosis factor-α. Created with BioRender.com

daunorubicin and camptothecin, and also all-trans retinoic acid, were shown to induce the transcription from *SMPD3* gene in cancer cells by recruiting the transcription factors Sp1 and Sp3 that bind to a segment of nucleotides 147 bp upstream of exon 1 in the *SMPD3* gene.[95] In addition, it was found that in the *ob/ob* mouse model of T2D associated with obesity and hyperinsulinemia, elevated TNF-α might lead to high levels of adipose tissue nSMase2 mRNA and plasma ceramide.[96] Similarly, in subcutaneous adipose tissue samples from individuals with obesity and diabetes, mRNA levels of nSMase were found to be elevated compared to nondiabetic obese and lean subjects.[97] Transcription of *SMPD3* gene was shown to be elevated fivefold in growth-arrested confluent breast cancer MCF7 cells compared to nonconfluent cells, with resultant increase in total nSMase activity and ceramide levels. The increase in nSMase2 mRNA levels in MCF7 cells was correlated with the G0/G1 cell cycle arrest.[93] Thus, the nSMase2/*SMPD3* gene activity appears to be positively controlled under conditions of obesity, T2D, and inflammation but turned down in proliferating cancer cells.

3.2.2 | Regulation of nSMase2 protein levels and its localization

The expression of nSMase2 protein and its activity in HAE lung epithelial cell line were shown to be enhanced by oxidative stress induced by hydrogen peroxide (H_2O_2), whereas treatment with the antioxidant reduced glutathione (GSH) led to a decline in the protein expression.[98] With respect to nSMase2 localization, protein kinases p38 and PKCδ were shown to regulate nSMase2 translocation to the plasma membrane in response to cytokines such as TNF-α. The role of PKCδ in this translocation appears to be independent of TNF-α effects on nSMase2 activation.[99,100] Confluence-related G0/G1 cell cycle arrest in MCF7 cells was also found to be associated with the translocation of nSMase2 protein to the plasma membrane from the cytoplasm.[93] Interestingly, in the HAE cells, while H_2O_2 promoted the translocation of nSMase2 to plasma membrane where it can generate ceramide and cause apoptosis, treatment with reduced GSH led to nSMase2 translocation to nucleus where this enzyme cannot produce ceramide.[98] Thus, nSMAse2 activity and localization appear to be controlled by the cellular redox state.

3.2.3 | Modulation of nSMase2 activity

The importance of nSMase2 in the ceramide-dependent signaling pathways[22,101] is highlighted by the observations that its enzymatic activity is upregulated by phosphatidylserine and various stress factors such as cytokines (IL-1β, TNF-α, interferon-γ [IFN-γ]), oxidative stress (reactive oxygen species [ROS] and oxidized lipids), UV radiation, chemotherapeutics, amyloid-β peptides, and nitric oxide (NO).[22,23,102–107] The proinflammatory cytokine TNF-α via direct coupling with its receptor has been shown to acutely increase, in cell free system, the hydrolytic production and accumulation of ceramide by direct nSMase activation.[108,109] It was also suggested that nSMase2 is activated by superoxide (O_2^-) produced by plasma membrane-associated NADPH oxidase-1 via the signaling cascade involving membrane-associated estrogen receptors.[110] Interestingly, amyloid β-peptide was shown to directly interact with nSMase2 and activate the enzyme.[111] A study identified five specific serine residues that can be phosphorylated in nSMase2 and reported that oxidative stress

promotes nSMase2 phosphorylation which positively controls its activity as well as stability.[112]

By contrast, some antioxidant agents and conditions are known to negatively regulate nSMase activity. Thus, the antioxidant function of plasma membrane redox system (CytB5 reductase-CoQ-NQO1-α-Tocopherol), which contains the antioxidant ubiquinol, has been shown to negatively regulate the activity of the plasma membrane-bound nSMase2.[22] nSMase2 activity has been shown to be reduced by calcineurin-mediated dephosphorylation.[113] GSH, with its antioxidant properties, has also been shown to decrease nSMase activity by acting on TNF-α activation pathways.[114] Finally, an inhibitor, GW4869 (IC_{50} of 1 μM), of the enzyme has been identified through high throughput screening. It is a cell membrane permeable, non-competitive inhibitor and is widely used to study nSMase2 functions in cell and animal models.[23,103] Altogether, the evidence indicates that nSMase2 cellular localization, activity, and protein degradation are controlled by cytokines, ROS, and the cellular redox state, as well as via its phosphorylation state.

3.3 | Structure, function, and properties

Considering the significance of the cellular localization of nSMase2 and its reaction product ceramide for cellular signaling, it is important to understand the topology and the distribution and function of different domains of this protein in the plasma membrane. nSMase2 is predicted to contain two transmembrane segments, N-terminal domains (NTD), and a C-terminal catalytic domain (CAT). In addition, between the two transmembrane segments and the catalytic region, there is a collagenous domain, but its function is unclear. There are two palmitoylation sites that are necessary for anchoring nSMase2 to the membrane.[115] Recent crystallographic studies on the structure of human nSMase2 showed that there is an interaction between the two hydrophobic, membrane bound, NTD, and the soluble cytosolic CAT domains.[26,94] The juxtamembrane (JX) NTD region was found to act as an allosteric activator, stimulating sphingomyelin hydrolysis, through its association with phospholipids, such as cardiolipin and phosphatidylserine.[14,22] It was further elaborated that phosphatidylserine binding at the NTD and juxtamembrane linker region in nSMase2 acts as a conformational switch, leading to interdomain, intramolecular interactions that are critical for nSMase2 activation.[116] It was also revealed that the asparagine residue, ASN130, coordinates the binding of Mg^{2+} ion in the active site which contributes to the catalytic activity of nSMase2 by stabilizing the phosphodiester bond of the sphingomyelin in the substrate binding site before its cleavage into phosphocholine and ceramide.[26,117]

In addition, the region separating the NTD and CAT domain of nSMase2 harbors five serine residues that can be phosphorylated[115] and also a calcineurin-binding site[113] that are relevant for nSMase2 activation as well as stability as discussed above. Oxidative stress was shown to enhance the nSMase2 activity in human airway epithelial cells and this activation associated with the phosphorylation of serine residues S173, S208 near the calcineurin binding site and S289, S292, and S299 close to the catalytic domain.[112] Phosphorylation is a major mechanism by which nSMase2 is activated by cytokines such as TNF-α which is elevated in conditions of inflammation, obesity, and insulin resistance. In the plasma membrane, nSMase2 interacts with the TNF-α receptor (TNFR)-1, which facilitates PKCδ and p38-MAPK mediated nSMase2 phosphorylation and regulation. Binding with a phosphatase-like calcineurin leads to nSMase2 dephosphorylation and thereby decreases its activation.[115] Figure 2 illustrates the factors influencing nSMase2 cellular localization and activity and how this enzyme is regulated.

3.4 | nSMase2, sphingolipids, and ceramides in metabolic disorders

In different cell types and animal models, decreased sphingomyelin levels in association with elevated ceramides have been implicated in many biological processes such as the control of cell differentiation, cell cycle arrest, and programmed cell death.[106] This topic has already been covered by excellent reviews[1,115,118] and will not be discussed here. Because nSMase2 is the most studied neutral SMase and has been much implicated in inflammation, the production of cytokines and many additional processes involved in cardiometabolic disorders, nSMase2, is an interesting therapeutic target.[96,119] Below, we cover our recent understanding of the role of nSMase2 in diseases related to nutrient excess and the metabolic syndrome.

3.5 | Insulin signaling and insulin resistance

Dysregulated interactions between metabolic and inflammatory pathways contribute to the pathogenesis of insulin resistance and associated metabolic complications in obesity. Metabolic inflammation is considered as the main driver of insulin resistance and associated metabolic complications.[120] Obesity induces a state of chronic low-grade inflammation which is a hallmark of the infiltration of immune cells in fat tissue and liver and the production of proinflammatory cytokines and chemokines. Among the proinflammatory cytokines, TNF-α and IL-1β produced by adipocytes as well as immune cells can increase nSMase2 activity and the production of ceramide, a mediator of insulin resistance (Figure 3).[1,15,121,122]

In vitro studies in L6 rat skeletal muscle cells showed that TNF-α and ceramide caused attenuation of insulin action by the activation of protein phosphatase-1 and subsequent dephosphorylation and inactivation of AKT, SRp40 (required for insulin-stimulated glucose uptake) and glycogen synthase kinase (GSK3β).[123] It was shown that ceramide directly activates PKCζ, which phosphorylates and inhibits the translocation of AKT/PKB.[124] In addition, ceramide stimulates the activity of cytosolic protein phosphatase-2A (PP2A), which dephosphorylates and inactivates AKT/PKB.[125,126] Thus, elevated ceramide levels in conditions of metabolic syndrome, such as fatty liver disease and obesity, appear to contribute to insulin resistance.[127,128] In addition, ceramide analogs have been shown to inhibit insulin-stimulated glucose

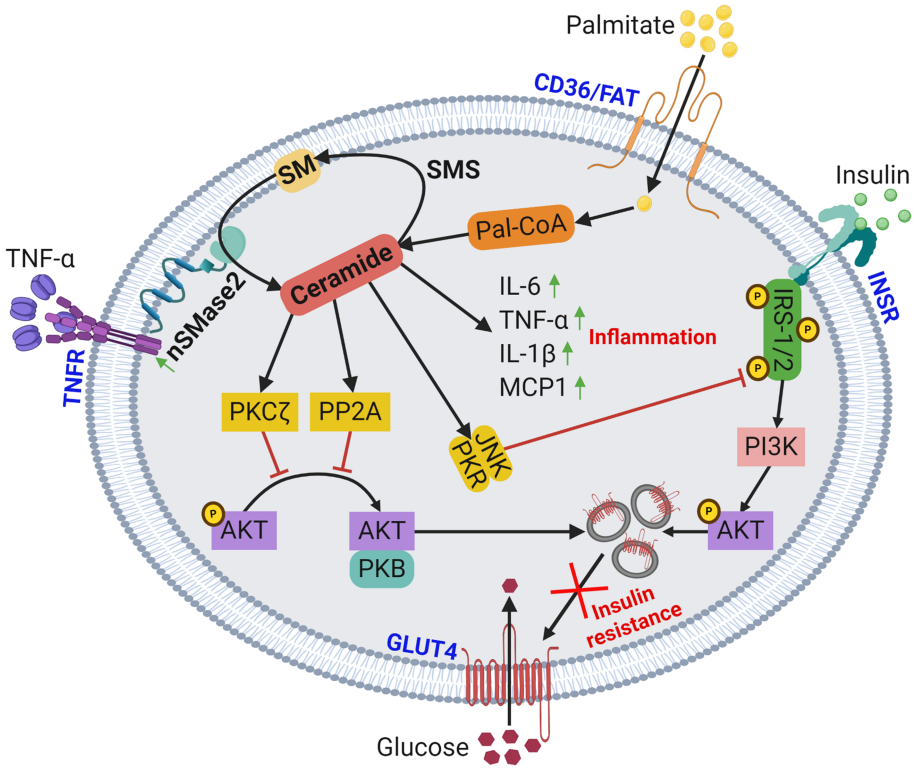

FIGURE 3 Pathways of insulin signaling inhibition by nSMase2 activation and ceramides. Ceramides produced from sphingomyelin by nSMase2 can activate PKCζ, PP2A, and PKR/JNK, all of which can impair insulin signaling by blocking the translocation of glucose transporter (GLUT)-4 to plasma membrane. Accumulation of ceramides increases proinflammatory cytokine levels, which can also lead to insulin resistance. In addition, secreted TNF-α and IL-1β can in turn activate nSMase2, accelerating ceramide production. INSR: insulin receptor; IRS-1/2: insulin receptor substrates 1 and 2; Pal-CoA: palmitoyl-CoA; SM: sphingomyelin; SMS: sphingomyelin synthase; TNFR: TNF-α receptor. Created with BioRender.com

uptake, GLUT4 translocation, and glycogen synthesis in isolated brown adipocytes and L6 skeletal muscle cells.[129,130] Besides, it has been shown that ceramide produced by nSMase2 accumulates in the ER and contributes to ER stress which, in turn, causes the activation of c-Jun N-terminal kinase (JNK), resulting in inhibition of insulin receptor signaling.[131] The importance of nSMases in insulin resistance was also demonstrated by studies showing that inhibition of nSMase by GW4869 is protective against insulin resistance, metabolic dysfunction, cellular stress, and inflammation induced by saturated fatty acid in C2C12 skeletal muscle myotubes.[132] Also, nSMase2 inhibition by GW4869 abrogated the TNFα-mediated inflammatory response (IL-1β and MCP-1 expression) in monocytic cells and macrophages.[133]

In vivo studies in high-fat diet fed rodents, *ob/ob* mice, and Zucker diabetic fatty (ZDF) rats revealed that inhibition of ceramide synthesis by administration of myriocin, an inhibitor of serine palmitoyltransferase, led to improved insulin sensitivity, showing the importance of ceramides as mediators of insulin resistance.[134] Furthermore, the antidiabetic drug pioglitazone, which is known to protect against insulin resistance[135] through the activation of peroxisome proliferator-activated receptor gamma (PPARγ) and its target genes,[136,137] was also found to suppress skeletal muscle TNFα levels with associated decrease in muscle nSMase2 activity in Zucker fatty rats,[138] suggesting that pioglitazone-mediated suppression of nSMase2 is one of multiple mechanisms that could improve insulin sensitivity in the skeletal muscle.[138] Thus, there is ample in vitro and in vivo evidence indicating that enhanced inflammation-induced nSMase activity leads to elevated production of ceramide which promotes insulin resistance and that inhibiting ceramide production by reducing nSMase activity improves insulin sensitivity (Figure 3). Either whole body or liver-specific ablation of dihydroceramide desaturase-1 (DES1), which conducts the final step in de novo synthesis of ceramide from dihydroceramide, was shown to decrease ceramide/dihydroceramide ratios in tissues and serum and to prevent diet-induced insulin resistance and hepatic steatosis in mice.[139] To the contrary, a recent study involving 7 lean healthy volunteers and 21 individuals afflicted with obesity and NAFLD reported a decreased ceramide/dihydroceramide ratio in liver and plasma and leads to insulin resistance and fatty liver disease, questioning the relevance of the mouse studies for human pathology.[140] Nevertheless, total hepatic dihydroceramides and lactosylceramide were found to be elevated in NASH patients in comparison to patients with normal or nonalcoholic fatty liver, without progression to NASH.[141] These and other studies[139–143] suggest that more mechanistic studies are required to clearly define the role of ceramide metabolism in human disease context and the players (ceramide vs. dihydroceramide) involved in insulin resistance, NAFLD/NASH, and adipose inflammation.

3.6 | Pancreatic ß-cell dysfunction and death

Glucolipotoxicity-mediated β-cell dysfunction in conditions of obesity and T2D is thought to be due, at least in part, to the increased esterification of fatty acyl-CoA and accumulation of diacylglycerols and ceramides.[144,145] In the β-cell, even though ceramide synthesis is thought to be predominantly via the de novo pathway, starting from serine and palmitoyl-CoA,[146] nSMase2 has also been shown to contribute to ceramide synthesis under conditions of ER stress.[147] nSMase2-mediated production of ceramide increases in ß-cells exposed to TNF-α and IL-1β and contributes to their damage and dysfunction.[147] Ceramides can accumulate in mitochondria and alter

mitochondrial membrane integrity and were proposed to induce apoptosis and necrosis in β-cells.[148–150] In terms of toxicity mechanisms, nSMase2 has been shown to synergize with BH3-only pro-apoptotic proteins by promoting translocation of the proapoptotic protein Bax to mitochondrial membrane, triggering the opening of mitochondrial permeability transition pore and the release of cytochrome *c* into the cytosol.[151] On the other hand, ceramide has been reported to lower the mRNA expression of the anti-apoptotic protein Bcl-2 in INS-1 β-cells and activate caspases 3/7.[152] It has also been shown in human islets that fatty acid-associated toxic effects are mediated by ceramides via Bcl-2 downregulation and activation of caspases.[153] In the presence of cytokines, such as IL-1β and IFN-γ, human islets were found to undergo ER stress with subsequent β-cell apoptosis caused by ceramide that was produced through the action of Ca^{2+}-independent phospholipase-A2β and nSMase2.[148] Thus, it appears that elevated level of ceramide caused by enhanced activity of nSMase2 induced by cytokines or glucolipotoxicity can trigger the mitochondria/cytochrome c/caspases-3/7 mediated apoptosis in β-cells and that this mechanism may in part underlie the ß-cell failure in both types 1 and 2 diabetes, since both forms of diabetes are characterized by islet inflammation and elevated levels of cytokines.

It has also been suggested that nSMase2 regulates ß-cell excitability and insulin granule exocytosis as this enzyme localizes to plasma membrane, where it regulates the levels of sphingomyelin.[147,152] Consistent with this view, mice with genetic deletion of sphingomyelin synthase-1, which converts ceramide to sphingomyelin, display increased ceramide accumulation, mitochondrial degeneration, and increased ROS production in the β-cells, with accompanied defective glucose-stimulated insulin secretion.[154] Surprisingly, it has been shown in a recent study in mice that ceramide levels are increased by cigarette smoking and cause oxidative and ER stress in the β-cells, leading to reduced insulin production, processing, and secretion and also decreased viability and proliferation of β-cells.[155] Also, it has been shown that inhibiting the formation of ceramide protects β-cells from destruction in T2D rodent models.[149,155]

Thus, mounting evidence supports a role for nSMase2 and ceramides in the development of β-cell dysfunction and failure in T2D. However, in order to better understand the precise role of nSMase2 in β-cell function in health and disease, further studies employing animal models with β-cell specific deletion of the nSMase2/*SMPD3* gene are needed. Whole body nSMase2 knockout mice were found to display very significant growth retardation with a form of dwarfism, hypothalamus-induced combined pituitary hormone deficiency as well as abnormalities in skeletal development. Glucose and insulin homeostasis were not studied in these mice.[29]

3.7 | Obesity, T2D, and associated inflammatory conditions

Excess energy intake leads to the accumulation of fat in adipose tissues and also ectopically in other tissues such as liver, skeletal muscle, and the myocardium, leading to obesity and the associated inflammation and cardiometabolic diseases. Lipotoxicity due to lipid overload contributes to cellular and organ dysfunction and cell death. Ceramide accumulation was proposed to mediate cardiomyocyte apoptosis induced by ischemia–reperfusion, TNF-α, and palmitate in several rodent models of obesity and diabetes, including the ZDF rats, *db/db*, and *ob/ob* mice.[96,156] It has also been shown that cardiomyocyte apoptosis in individuals with obesity and diabetes, as compared to lean individuals, is accompanied by elevated expression of neutral sphingomyelinase and serine palmitoyltransferase, emphasizing the potential role of altered ceramide metabolism in cardiac failure in patients with obesity, with and without T2D (Figure 4).[55]

What is the mechanism that underlies these alterations in sphingolipid metabolism? The saturated fatty acids that accumulate in adipocytes can activate the toll-like receptor (TLR)-4 which contributes to inflammation.[121,149] Adipose tissue inflammation caused by the increased macrophage recruitment and activation leads to elevated production of proinflammatory cytokines (e.g. TNF-α, IL-1β). We recently showed that the disruption of nSMase2 activity, but not of aSMase, in monocytes and macrophages prevents the TNF-α-driven inflammatory responses, including the expression of the inflammatory (M1) macrophage marker CD11c and the secretion of prototypic inflammatory mediators IL-1β and MCP-1.[133] The evidence indicated that the inhibition of the TNF-α-driven inflammatory responses involves the NF-κB/MAPK signaling pathway. In agreement with these data, peripheral blood mononuclear cells (PBMC) from individuals with obesity were shown to display increased levels of nSMase2, which is known to associate with TNF-α expression in these cells. These findings collectively support the view that nSMase2 acts, at least in part, as a master switch in TNF-α-mediated inflammatory responses in monocytes and macrophages in the obesity setting.[133]

As detailed in the above section, ceramides and nSMase2 appear to contribute to the development of β-cell failure in T2D. Many studies have shown that blocking or lowering the ceramide accumulation in various tissues improves the diabetic condition.[16,134,139,148,149,157] High levels of dietary fiber are known to lower the risk of coronary heart disease and associated mortality, and a recent study revealed that dietary fiber, such as inulin, reduces the expression of liver nSMase2 and plasma ceramide levels which is a major risk factor for cardiometabolic diseases.[158] Overall, significant evidence suggests that inhibition of ceramide generation by nSMase2 and other pathways can help prevent adipose inflammation and monocyte/macrophage activation as well as retard the development of T2D and other metabolic syndrome-related disorders.

3.8 | NAFLD/NASH

NAFLD represents a group of pathologies that include hepatic steatosis, NASH, fibrosis, and cirrhosis,[159] and the underlying pathogenic mechanisms are multifactorial and complex. Even though NAFLD is highly prevalent in the general population, most affected patients suffer mainly from nonlife threatening simple steatosis, while

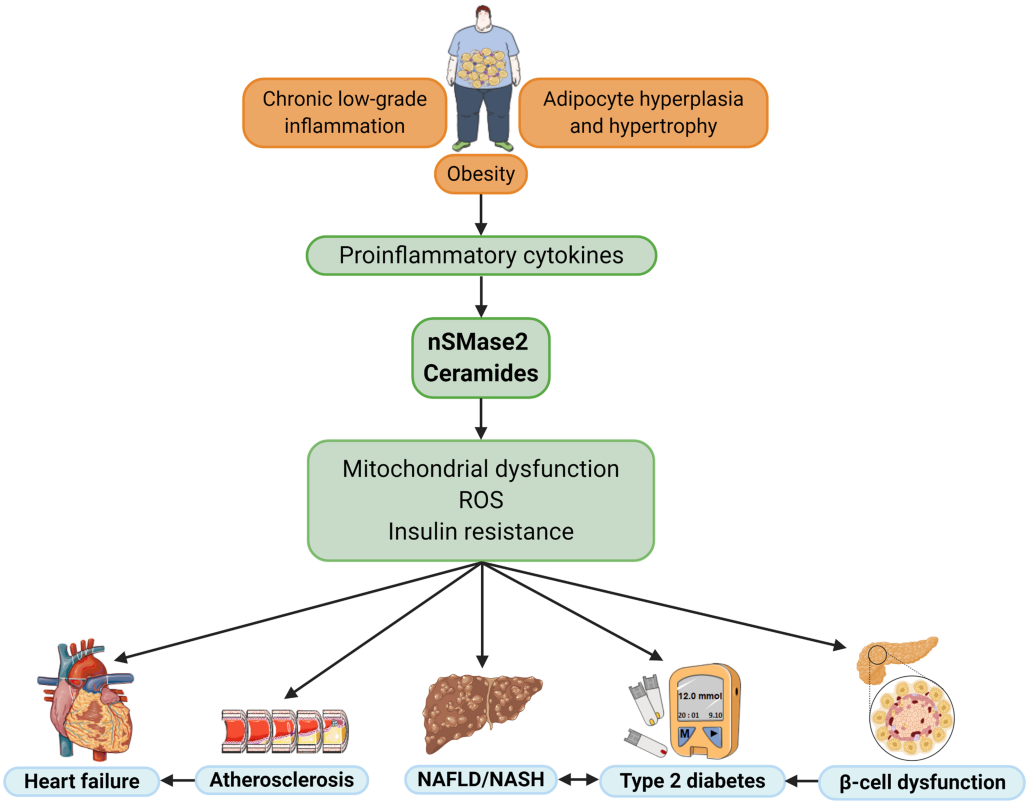

FIGURE 4 Proposed role of nSMase2 and ceramides in the pathogenesis of different metabolic diseases. The chronic low-grade inflammation in individuals with obesity drives the production of proinflammatory cytokines such as TNF-α and IL-1β, which can lead to the upregulation of nSMase2 expression and activity. This, in turn, increases the production of ceramides leading to mitochondrial dysfunction, production of reactive oxygen species (ROS) and insulin resistance. Accumulation of ceramides in different organs seems to be responsible for the onset of different metabolic diseases. Created with BioRender.com

5%–10% of patients with NAFLD actually go on to develop NASH, with one third of these patients progressing to cirrhosis.[160] The two-hit hypothesis of NAFLD pathogenesis states that the life style-induced lipid accumulation in the liver acts as the first hit which sensitizes the liver to second hit(s), such as ER/oxidative stress,[161,162] proinflammatory cytokines,[163,164] and lipotoxicity,[165] leading to steatohepatitis and fibrosis. However, this simplistic view is now replaced by the "multiple hit" hypothesis which proposes that multiple hits are implicated in NAFLD pathogenesis, such as dietary factors, gut microbiota, insulin resistance, adipokines, genetic, and epigenetic factors acting in combination on the genetically predisposed subjects to eventually lead to NAFLD.[160,166] Sphingolipids have been implicated in the pathogenesis and progression of steatosis and NAFLD, mostly based on the evidence from studies on different murine[163,167–174] and rat models[175–178] of NAFLD suggesting that reduction of certain sphingolipids like ceramide is likely beneficial against NAFLD/NASH.[159,167–178] However, the importance of ceramide in human NAFLD/NASH has been questioned recently. Thus, the prevention of hepatic steatosis observed in a model of either whole body or liver-specific DES1 deletion in mice[139] by decreasing tissue and plasma ceramide/dihydroceramide ratios was found to be nontranslatable to humans as the reverse was noticed in a study comparing lean healthy individuals with patients with obesity and NAFLD.[140] Similarly, a recent clinical study involving 288 patients revealed no association between NAFLD (and its spectrum) and plasma levels of typical sphingolipids, such as the long chain sphingoid base containing sphingosine and sphinganine, even though elevated plasma deoxy-sphingolipid levels were noticed in patients with steatosis.[179] These deoxy-sphingolipids are generated by serine palmitoyl transferase (SPT), by the condensation of palmitoyl-CoA with L-alanine or L-glycine, when these amino acids are present in excess, instead of serine.[180] In addition, four missense mutations (C133W, C133Y, V144D, and G387A) in the SPT1LC subunit of SPT enzyme were found to shift the substrate specificity of the mutated SPT from serine to alanine and glycine, leading to the accumulation of unmetabolizable deoxy-sphingolipids, resulting in the hereditary disease, hereditary sensory, and autonomic neuropathy type 1 (HSNA1).[181] Because of the lack of C1-hydroxyl group, the deoxy-sphingolipids cannot be metabolized via sphingolipid catabolism pathways but are partly metabolized by enzymes of the cytochrome P450 subfamily.[180,182] The plasma levels of deoxy-sphingolipids are also found to be elevated in patients with metabolic syndrome and T2D,[183,184] nonalcoholic steatohepatitis,[185] and diabetic sensory polyneuropathy.[186] Thus, the precise pathogenic role of elevated sphingolipids in the onset of NAFLD is still unclear, though these lipids appear to be associated with steatosis and more mechanistic clinical

studies are required to clearly define the role of ceramide metabolism in NAFLD/NASH pathogenesis.

There is significant evidence for a role of acid SMase which is highly expressed in the liver in NASH whereas, a role for nSMase2, which is relatively less expressed in liver, is not yet established.[187] Indeed, none of the studies assessing liver gene expression changes in fatty liver disease in humans reported link with nSMase2.[188,189] A role for nSMase2 in the hepatic steatosis emerges only from studies on mice, and so far, there is no strong human disease relevance.[190] Inasmuch as the elevated ceramides, which positively regulate the expression and release of proinflammatory cytokines, are known to play an important role in steatohepatitis as well as dysregulation of nSMase2 has been shown to contribute to inflammation, further studies are needed to ascertain the role of nSMase2 in human NAFLD/NASH pathogenesis.

3.9 | Atherosclerosis

Atherosclerosis is marked by the accumulation of plaque or atheroma in the vascular endothelium. Atherosclerotic plaque formation involves the aggregation of atherogenic lipoproteins which play an important role in the initiation and progression of atherosclerosis.[191] Lipoprotein aggregation may occur during the process of sphingomyelin hydrolysis by SMases into ceramide and phosphocholine.[192,193] Low-density lipoproteins (LDL), oxidized LDL, and groups of lipids such as sphingolipids, ceramides (produced via aSMases and nSMases), and glycosphingolipids (such as glucosylceramide and lactosylceramide) have been proposed to contribute to the atherosclerotic process and thrombotic diseases.[194,195] Ceramide and lactosylceramide were found to be involved in apoptosis of wild-type human osteosarcoma cell line MG-63 via the action of nSMases,[196] whereas lactosylceramide might cause human aortic smooth muscle cell proliferation and led to plaque formation.[194,197] Ceramides can induce systemic proinflammatory responses, and the accumulation of glycosphingolipids may lead to the induction of adhesion molecules on vascular endothelial cells, thereby activating neutrophils and triggering plaque inflammation.[198] Likewise, Lallemand et al. reported that deficiency of nSMase2 in mice or its inhibition by GW4869 attenuated inflammation and atherosclerosis in Apoe$^{-/-}$ mice.[199] Also, a recent study in LDL receptor-deficient mouse model of atherosclerosis revealed that dietary inulin reduced the liver expression of nSMase2 and plasma ceramides and prevented the risk of atherosclerosis in these mice.[158] Thus, an interplay between nSMase2, inflammation and lipoproteins appears to be involved in the pathogenesis of atherosclerosis.

3.10 | Heart failure

nSMases appear to be involved in cardiovascular pathophysiological processes, such as cardiomyocyte proliferation and death, vascular smooth muscle cell contraction, lipotoxic cardiomyopathy, formation of atherogenic plaques, and heart failure[195,200] (Figure 4). The temporal profiling of regulatory enzymes involved in myocardial ceramide metabolism revealed that elevated synthesis and accumulation of ceramides is associated with cardiovascular disease mortality in patients with coronary artery disease.[201,202] In vascular cells, nSMase2 is activated by a number of stress stimuli including proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IFN-γ), unsaturated fatty acids, oxidized LDL, anionic phospholipids, and oxidative stress, whereas antioxidants such as GSH inhibit this enzyme.[119,203] The induction and activation of nSMase2 involve the activation of TNF-α receptor and related cofactors/adapter proteins such as factor associated with nSMase (FAN), cardiomyocyte-associated Polycomb protein embryonic ectodermal development (EED), as well as a protease cascade consisting of furin and matrix metalloprotease-2.[204,205] It has been shown in a model of postmyocardial infarction failing rat hearts that activation of nSMase2 leads to apoptosis of left ventricular cardiomyocytes via caspase-3 activation.[206] Indeed, nSMase2 inhibition and the reduction of circulating ceramide levels with N-acetylcysteine and lipoic acid have been shown to be beneficial for reducing rat endothelial dysfunction and improving postmyocardial infarction failing hearts in rats.[206,207] There is evidence from animal model studies implicating nSMase2 in cardiac failure, but more mechanistic studies in animals are needed together with clinical studies to ascertain if nSMase2 plays a role in human heart failure and could be a target for therapy.

3.11 | Exosomes and nSMase2

Many types of cells release lipid bilayer membrane vesicles extracellularly,[208] and these vesicles contain several cellular components including cytosolic proteins, microRNAs, and mRNAs and have the same membrane topology with transmembrane proteins as the cells.[209] Extracellular vesicles originating from intraluminal vesicles are called exosomes, and these are released by the fusion of endosomes with plasma membrane subsequent to the accumulation of exosomes in endosomes.[210] Several studies have implicated exosomes in cell–cell communication and in the transport of large molecules from cell to cell.[209,210] Involvement of exosomes in the development of many pathological conditions such as diabetes and its complications, Alzheimer's disease, cancer metastasis, immune suppression, and also in obesity related complications including inflammation is now recognized.[211] Lipids play an important role in the biogenesis of exosomes and particularly nSMase2, and its product ceramide has been shown to contribute to the exosome production by intraluminal vesicle budding.[212,213] Thus, exosomes have been shown to be enriched in ceramide and sphingomyelin compared to the parent cells.[212,214] An important role for nSMase2 and ceramide in the exosome-mediated miRNA secretion has been suggested in the studies using HEK293 cells and metastatic MDA-MB231 breast cancer cells, by the suppression of nSMase2 activity by its inhibitor GW4869 or by RNAi knockdown and by nSMase2 overexpression.[215,216] Ceramide synthesis by nSMase2 was shown to facilitate the exosomes-mediated transfer of

high molecular weight aggregates of α-synuclein, tau, and prion proteins and to contribute to the spread of neurodegenerative diseases.[217–219] Importance of exosomes in cardiometabolic diseases is emphasized in a recent study showing that visceral adipose exosomes in pregnant obese mice contribute to fetal cardiac dysfunction.[220] Interestingly, exosomes secreted from adipocytes in response to ER stress induce steatosis, inflammation, and fibrosis in the liver and lead to NASH through the alteration of hepatic lipid dynamics.[221] Also, it has recently been shown that intestinal exosomes released from high-fat diet fed mice or T2D patients can induce insulin resistance in normal mice.[222] Pancreatic β-cells, when exposed to increased fatty acid levels both in vitro and in vivo in mice, have been shown to release exosomes packed with miR-29, which is involved in antagonizing insulin signaling in liver.[223] Additionally, INS1E β-cells overexpressing human islet amyloid polypeptide (hIAPP) have been shown to secrete exosomes packed with hIAPP as a detoxification process, but the released hIAPP loaded exosomes can lead to neurodegenerative diseases.[224] On the other hand, miR-690 was found to be in the exosomes from M2-polarized bone marrow-derived macrophages and these exosomes with miR-690 were shown to improve insulin sensitivity and glucose tolerance in mice both in vitro and in vivo.[225] Inasmuch as nSMase2 and ceramide likely play an important role in exosome release, it is likely that nSMase2 and ceramide-mediated mechanisms are involved in the various pathological conditions and in cell to cell communications facilitated by exosomes. However, more convincing studies are needed using animal models with genetic alteration of nSMase2 expression as most of the earlier studies have been done using nSMase2 inhibitor or RNAi knockdown.

4 | CONCLUSIONS AND PERSPECTIVE

Sphingolipids are important membrane structural components, implicated in various cellular signaling pathways and in the pathogenesis of inflammation, cardiometabolic diseases, and cancer. Dysregulation of nSMase2, encoded by the *SMPD3* gene, appears to underlie many of the inflammation-related pathologies, through the elevated synthesis of ceramides. Ample evidence supports the view that nSMase2 is activated by increased oxidized state, ROS, and proinflammatory cytokines, all of which are known causative factors for inflammation, β-cell dysfunction, insulin resistance, T2D, NAFLD/NASH, and atherosclerosis. Indeed, a feed-forward interaction likely exists between enhanced ceramide formation by nSMase2 and production of proinflammatory cytokines, such as TNF-α and IL-1β. Through such cyclical interaction, proinflammatory cytokines enhance the activity of nSMase2, and ceramide thus produced increases in the synthesis and release of these cytokines. Considering that such an interaction between nSMase2 and inflammatory cytokines may trigger elevated cellular ROS levels and inflammation which contribute to the pathogenesis of cardiometabolic diseases, a specific blockade of nSMase2 can have therapeutic effects. However, considering that the constitutive whole body deletion of nSMase2 causes growth retardation in mice, there is a need for tissue-specific conditional knockout mice for a better understanding of the pathophysiological roles of this enzyme in different organs and for assessing the therapeutic value of nSMase2 inhibition. Such animal models are needed to answer many uncertainties with regard to the in vivo role of nSMase2 in diseases like NAFLD, atherosclerosis, and heart failure. Such animal models are also needed to verify the involvement of nSMase2 and ceramide, in vivo, in the secretion of exosomes that mediate intercellular communications and lead to several pathological conditions. In addition, further studies using human tissues/cells, both under normal and pathological conditions, are needed to better define the relevance of nSMase2 in human physiology and pathology. Also, as the currently available pharmacological agents do not appear to be entirely specific for nSMase2, further studies are needed to develop specific nSMase2 inhibitors for pharmacological evaluation of nSMase2 as a therapeutic target.

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CONFLICT OF INTEREST

No conflict of interest was declared.

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REFERENCES

1. Pralhada Rao R, Vaidyanathan N, Rengasamy M, Mammen Oommen A, Somaiya N, Jagannath MR. Sphingolipid metabolic pathway: an overview of major roles played in human diseases. *J Lipids*. 2013;2013:178910.
2. Russo SB, Ross JS, Cowart LA. Sphingolipids in obesity, type 2 diabetes, and metabolic disease. *Handb Exp Pharmacol*. 2013;216:373-401.
3. Ng ML, Wadham C, Sukocheva OA. The role of sphingolipid signalling in diabetes-associated pathologies (Review). *Int J Mol Med*. 2017;39(2):243-252.
4. Sui J, He M, Wang Y, Zhao X, He Y, Shi B. Sphingolipid metabolism in type 2 diabetes and associated cardiovascular complications. *Exp Ther Med*. 2019;18(5):3603-3614.
5. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. *Nat Rev Cancer*. 2018;18(1):33-50.
6. Ghidoni R, Caretti A, Signorelli P. Role of sphingolipids in the pathobiology of lung inflammation. *Mediators Inflamm*. 2015;2015:487508.
7. Chakinala RC, Khatri A, Gupta K, Koike K, Epelbaum O. Sphingolipids in COPD. *Eur Respir Rev*. 2019;28(154):190047.
8. Platt FM. Sphingolipid lysosomal storage disorders. *Nature*. 2014;510(7503):68-75.
9. Bielawska A, Linardic CM, Hannun YA. Ceramide-mediated biology. Determination of structural and stereospecific requirements through the use of N-acyl-phenylaminoalcohol analogs. *J Biol Chem*. 1992;267(26):18493-18497.
10. Clarke CJ, Mediwala K, Jenkins RW, Sutton CA, Tholanikunnel BG, Hannun YA. Neutral sphingomyelinase-2 mediates growth arrest by

retinoic acid through modulation of ribosomal S6 kinase. *J Biol Chem*. 2011;286(24):21565-21576.
11. Sharma K, Shi Y. The yins and yangs of ceramide. *Cell Res*. 1999;9(1):1-10.
12. Arana L, Gangoiti P, Ouro A, Trueba M, Gómez-Muñoz A. Ceramide and ceramide 1-phosphate in health and disease. *Lipids Health Dis*. 2010;9(1):15.
13. Venable ME, Lee JY, Smyth MJ, Bielawska A, Obeid LM. Role of ceramide in cellular senescence. *J Biol Chem*. 1995;270(51):30701-30708.
14. Kuzmenko DI, Klimentyeva TK. Role of ceramide in apoptosis and development of insulin resistance. *Biochemistry (Mosc)*. 2016;81(9):913-927.
15. Sokolowska E, Blachnio-Zabielska A. The role of ceramides in insulin resistance. *Front Endocrinol*. 2019;10(577).
16. Schmitz-Peiffer C. Targeting ceramide synthesis to reverse insulin resistance. *Diabetes*. 2010;59(10):2351-2353.
17. Andrieu-Abadie N, Gouazé V, Salvayre R, Levade T. Ceramide in apoptosis signaling: relationship with oxidative stress. *Free Radic Biol Med*. 2001;31(6):717-728.
18. Bismuth J, Lin P, Yao Q, Chen C. Ceramide: a common pathway for atherosclerosis? *Atherosclerosis*. 2008;196(2):497-504.
19. de Wit NM, den Hoedt S, Martinez-Martinez P, Rozemuller AJ, Mulder MT, de Vries HE. Astrocytic ceramide as possible indicator of neuroinflammation. *J Neuroinflammation*. 2019;16(1):48.
20. Schneider PB, Kennedy EP. Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease. *J Lipid Res*. 1967;8(3):202-209.
21. Rao BG, Spence MW. Sphingomyelinase activity at pH 7.4 in human brain and a comparison to activity at pH 5.0. *J Lipid Res*. 1976;17(5):506-515.
22. Clarke CJ, Hannun YA. Neutral sphingomyelinases and nSMase2: bridging the gaps. *Biochim Biophys Acta*. 2006;1758(12):1893-1901.
23. Shamseddine AA, Airola MV, Hannun YA. Roles and regulation of neutral sphingomyelinase-2 in cellular and pathological processes. *Adv Biol Regul*. 2015;57:24-41.
24. Clarke CJ, Snook CF, Tani M, Matmati N, Marchesini N, Hannun YA. The extended family of neutral sphingomyelinases. *Biochemistry*. 2006;45(38):11247-11256.
25. Karakashian AA, Giltiay NV, Smith GM, Nikolova-Karakashian MN. Expression of neutral sphingomyelinase-2 (NSMase-2) in primary rat hepatocytes modulates IL-beta-induced JNK activation. *FASEB J*. 2004;18(9):968-970.
26. Airola MV, Shanbhogue P, Shamseddine AA, et al. Structure of human nSMase2 reveals an interdomain allosteric activation mechanism for ceramide generation. *Proc Natl Acad Sci U S A*. 2017;114(28):E5549-E5558.
27. Clarke CJ, Cloessner EA, Roddy PL, Hannun YA. Neutral sphingomyelinase 2 (nSMase2) is the primary neutral sphingomyelinase isoform activated by tumour necrosis factor-alpha in MCF-7 cells. *Biochem J*. 2011;435(2):381-390.
28. Kim MY, Linardic C, Obeid L, Hannun Y. Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. *J Biol Chem*. 1991;266(1):484-489.
29. Stoffel W, Jenke B, Block B, Zumbansen M, Koebke J. Neutral sphingomyelinase 2 (smpd3) in the control of postnatal growth and development. *Proc Natl Acad Sci U S A*. 2005;102(12):4554-4559.
30. Khavandgar Z, Poirier C, Clarke CJ, et al. A cell-autonomous requirement for neutral sphingomyelinase 2 in bone mineralization. *J Cell Biol*. 2011;194(2):277-289.
31. Strum JC, Ghosh S, Bell RM. Lipid second messengers. A role in cell growth regulation and cell cycle progression. *Adv Exp Med Biol*. 1997;407:421-431.
32. Devillard R, Galvani S, Thiers JC, et al. Stress-induced sphingolipid signaling: role of type-2 neutral sphingomyelinase in murine cell apoptosis and proliferation. *PLoS One*. 2010;5(3):e9826.
33. Trayssac M, Hannun YA, Obeid LM. Role of sphingolipids in senescence: implication in aging and age-related diseases. *J Clin Invest*. 2018;128(7):2702-2712.
34. Jarvis WD, Kolesnick RN, Fornari FA, Traylor RS, Gewirtz DA, Grant S. Induction of apoptotic DNA damage and cell death by activation of the sphingomyelin pathway. *Proc Natl Acad Sci U S a*. 1994;91(1):73-77.
35. Gills JJ, Zhang C, Abu-Asab MS, et al. Ceramide mediates nanovesicle shedding and cell death in response to phosphatidylinositol ether lipid analogs and perifosine. *Cell Death Dis*. 2012;3:e340.
36. Back MJ, Ha HC, Fu Z, et al. Activation of neutral sphingomyelinase 2 by starvation induces cell-protective autophagy via an increase in Golgi-localized ceramide. *Cell Death Dis*. 2018;9(6):670.
37. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002;2(8):569-579.
38. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. *J Biol Chem*. 2013;288(15):10849-10859.
39. Yang Z, Costanzo M, Golde DW, Kolesnick RN. Tumor necrosis factor activation of the sphingomyelin pathway signals nuclear factor kappa B translocation in intact HL-60 cells. *J Biol Chem*. 1993;268(27):20520-20523.
40. Szabo G, Csak T. Inflammasomes in liver diseases. *J Hepatol*. 2012;57(3):642-654.
41. Dobierzewska A, Shi L, Karakashian AA, Nikolova-Karakashian MN. Interleukin 1beta regulation of FoxO1 protein content and localization: evidence for a novel ceramide-dependent mechanism. *J Biol Chem*. 2012;287(53):44749-44760.
42. Henry B, Moller C, Dimanche-Boitrel MT, Gulbins E, Becker KA. Targeting the ceramide system in cancer. *Cancer Lett*. 2013;332(2):286-294.
43. Park B, Lee YM, Kim JS, et al. Neutral sphingomyelinase 2 modulates cytotoxic effects of protopanaxadiol on different human cancer cells. *BMC Complement Altern Med*. 2013;13:194.
44. Farkas E, Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol*. 2001;64(6):575-611.
45. Saxena S, Caroni P. Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. *Neuron*. 2011;71(1):35-48.
46. Poirier C, Berdyshev EV, Dimitropoulou C, Bogatcheva NV, Biddinger PW, Verin AD. Neutral sphingomyelinase 2 deficiency is associated with lung anomalies similar to emphysema. *Mamm Genome*. 2012;23(11–12):758-763.
47. Cogolludo A, Moreno L, Frazziano G, et al. Activation of neutral sphingomyelinase is involved in acute hypoxic pulmonary vasoconstriction. *Cardiovasc Res*. 2009;82(2):296-302.
48. Smith AR, Visioli F, Frei B, Hagen TM. Age-related changes in endothelial nitric oxide synthase phosphorylation and nitric oxide dependent vasodilation: evidence for a novel mechanism involving sphingomyelinase and ceramide-activated phosphatase 2A. *Aging Cell*. 2006;5(5):391-400.
49. Mogami K, Kishi H, Kobayashi S. Sphingomyelinase causes endothelium-dependent vasorelaxation through endothelial nitric oxide production without cytosolic Ca(2+) elevation. *FEBS Lett*. 2005;579(2):393-397.
50. Ohanian J, Forman SP, Katzenberg G, Ohanian V. Endothelin-1 stimulates small artery VCAM-1 expression through p38MAPK-dependent neutral sphingomyelinase. *J Vasc Res*. 2012;49(4):353-362.

51. Altura BM, Shah NC, Shah GJ, et al. Magnesium deficiency upregulates sphingomyelinases in cardiovascular tissues and cells: cross-talk among proto-oncogenes, Mg(2+), NF-kappaB and ceramide and their potential relationships to resistant hypertension, atherogenesis and cardiac failure. *Int J Clin Exp Med*. 2013;6(10):861-879.
52. Altura BM, Shah NC, Shah GJ, et al. Short-term Mg deficiency upregulates protein kinase C isoforms in cardiovascular tissues and cells; relation to NF-kB, cytokines, ceramide salvage sphingolipid pathway and PKC-zeta: hypothesis and review. *Int J Clin Exp Med*. 2014;7(1):1-21.
53. Shah NC, Shah GJ, Li Z, Jiang XC, Altura BT, Altura BM. Short-term magnesium deficiency downregulates telomerase, upregulates neutral sphingomyelinase and induces oxidative DNA damage in cardiovascular tissues: relevance to atherogenesis, cardiovascular diseases and aging. *Int J Clin Exp Med*. 2014;7(3):497-514.
54. Hernandez OM, Discher DJ, Bishopric NH, Webster KA. Rapid activation of neutral sphingomyelinase by hypoxia-reoxygenation of cardiac myocytes. *Circ Res*. 2000;86(2):198-204.
55. Baranowski M, Blachnio-Zabielska A, Hirnle T, et al. Myocardium of type 2 diabetic and obese patients is characterized by alterations in sphingolipid metabolic enzymes but not by accumulation of ceramide. *J Lipid Res*. 2010;51(1):74-80.
56. Thudichum JLW. *A Treatise on the Chemical Constitution of Brain*. Vol. 22. London: Bailliere, Tindall, and Cox; 1884:146.
57. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol*. 2008;9(2):139-150.
58. Hannun YA, Luberto C. Lipid metabolism: ceramide transfer protein adds a new dimension. *Curr Biol*. 2004;14(4):R163-R165.
59. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol*. 2010;688:1-23.
60. Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. *Nat Rev Mol Cell Biol*. 2018;19(3):175-191.
61. Menaldino DS, Bushnev A, Sun A, et al. Sphingoid bases and de novo ceramide synthesis: enzymes involved, pharmacology and mechanisms of action. *Pharmacol Res*. 2003;47(5):373-381.
62. Kolter T, Sandhoff K. Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu Rev Cell Dev Biol*. 2005;21:81-103.
63. Snook CF, Jones JA, Hannun YA. Sphingolipid-binding proteins. *Biochim Biophys Acta*. 2006;1761(8):927-946.
64. Zeidan YH, Hannun YA. Translational aspects of sphingolipid metabolism. *Trends Mol Med*. 2007;13(8):327-336.
65. Aburasayn H, Al Batran R, Ussher JR. Targeting ceramide metabolism in obesity. *Am J Physiol Endocrinol Metab*. 2016;311(2):E423-E435.
66. Garcia-Gonzalez V, Diaz-Villanueva JF, Galindo-Hernandez O, Martinez-Navarro I, Hurtado-Ureta G, Perez-Arias AA. Ceramide metabolism balance, a multifaceted factor in critical steps of breast cancer development. *Int J Mol Sci*. 2018;19(9):2527.
67. Weiss B, Stoffel W. Human and murine serine-palmitoyl-CoA transferase—cloning, expression and characterization of the key enzyme in sphingolipid synthesis. *Eur J Biochem*. 1997;249(1):239-247.
68. Hannun YA, Obeid LM. Ceramide: an intracellular signal for apoptosis. *Trends Biochem Sci*. 1995;20(2):73-77.
69. Kitatani K, Idkowiak-Baldys J, Hannun YA. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal*. 2008;20(6):1010-1018.
70. Goni FM, Alonso A. Sphingomyelinases: enzymology and membrane activity. *FEBS Lett*. 2002;531(1):38-46.
71. Marchesini N, Hannun YA. Acid and neutral sphingomyelinases: roles and mechanisms of regulation. *Biochem Cell Biol*. 2004;82(1):27-44.
72. Henry B, Ziobro R, Becker KA, Kolesnick R, Gulbins E. Acid sphingomyelinase. *Handb Exp Pharmacol*. 2013;215:77-88.
73. Barnholz Y, Roitman A, Gatt S. Enzymatic hydrolysis of sphingolipids. II. Hydrolysis of sphingomyelin by an enzyme from rat brain. *J Biol Chem*. 1966;241(16):3731-3737.
74. Tabas I. Secretory sphingomyelinase. *Chem Phys Lipids*. 1999;102(1–2):123-130.
75. Schissel SL, Keesler GA, Schuchman EH, Williams KJ, Tabas I. The cellular trafficking and zinc dependence of secretory and lysosomal sphingomyelinase, two products of the acid sphingomyelinase gene. *J Biol Chem*. 1998;273(29):18250-18259.
76. Schissel SL, Jiang X, Tweedie-Hardman J, et al. Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH. Implications for atherosclerotic lesion development. *J Biol Chem*. 1998;273(5):2738-2746.
77. Krut O, Wiegmann K, Kashkar H, Yazdanpanah B, Kronke M. Novel tumor necrosis factor-responsive mammalian neutral sphingomyelinase-3 is a C-tail-anchored protein. *J Biol Chem*. 2006;281(19):13784-13793.
78. Tani M, Ito M, Igarashi Y. Ceramide/sphingosine/sphingosine 1-phosphate metabolism on the cell surface and in the extracellular space. *Cell Signal*. 2007;19(2):229-237.
79. Andrieu-Abadie N, Levade T. Sphingomyelin hydrolysis during apoptosis. *Biochim Biophys Acta*. 2002;1585(2–3):126-134.
80. Czarny M, Schnitzer JE. Neutral sphingomyelinase inhibitor scyphostatin prevents and ceramide mimics mechanotransduction in vascular endothelium. *Am J Physiol Heart Circ Physiol*. 2004;287(3):H1344-H1352.
81. Auge N, Andrieu N, Negre-Salvayre A, Thiers JC, Levade T, Salvayre R. The sphingomyelin-ceramide signaling pathway is involved in oxidized low density lipoprotein-induced cell proliferation. *J Biol Chem*. 1996;271(32):19251-19255.
82. Chatterjee S. Neutral sphingomyelinase: past, present and future. *Chem Phys Lipids*. 1999;102(1–2):79-96.
83. Ghosh N, Sabbadini R, Chatterjee S. Identification, partial purification, and localization of a neutral sphingomyelinase in rabbit skeletal muscle: neutral sphingomyelinase in skeletal muscle. *Mol Cell Biochem*. 1998;189(1–2):161-168.
84. Okazaki T, Bielawska A, Domae N, Bell RM, Hannun YA. Characteristics and partial purification of a novel cytosolic, magnesium-independent, neutral sphingomyelinase activated in the early signal transduction of 1 alpha,25-dihydroxyvitamin D3-induced HL-60 cell differentiation. *J Biol Chem*. 1994;269(6):4070-4077.
85. Nilsson A, Duan RD. Alkaline sphingomyelinases and ceramidases of the gastrointestinal tract. *Chem Phys Lipids*. 1999;102(1–2):97-105.
86. Cheng Y, Nilsson A, Tomquist E, Duan RD. Purification, characterization, and expression of rat intestinal alkaline sphingomyelinase. *J Lipid Res*. 2002;43(2):316-324.
87. Duan RD. Alkaline sphingomyelinase: an old enzyme with novel implications. *Biochim Biophys Acta*. 2006;1761(3):281-291.
88. Liu B, Hassler DF, Smith GK, Weaver K, Hannun YA. Purification and characterization of a membrane bound neutral pH optimum magnesium-dependent and phosphatidylserine-stimulated sphingomyelinase from rat brain. *J Biol Chem*. 1998;273(51):34472-34479.
89. Bernardo K, Krut O, Wiegmann K, et al. Purification and characterization of a magnesium-dependent neutral sphingomyelinase from bovine brain. *J Biol Chem*. 2000;275(11):7641-7647.
90. Tomiuk S, Zumbansen M, Stoffel W. Characterization and subcellular localization of murine and human magnesium-dependent neutral sphingomyelinase. *J Biol Chem*. 2000;275(8):5710-5717.
91. Hofmann K, Tomiuk S, Wolff G, Stoffel W. Cloning and characterization of the mammalian brain-specific, Mg2+-dependent neutral sphingomyelinase. *Proc Natl Acad Sci U S A*. 2000;97(11):5895-5900.

92. Aubin I, Adams CP, Opsahl S, et al. A deletion in the gene encoding sphingomyelin phosphodiesterase 3 (Smpd3) results in osteogenesis and dentinogenesis imperfecta in the mouse. *Nat Genet*. 2005;37(8):803-805.
93. Marchesini N, Osta W, Bielawski J, Luberto C, Obeid LM, Hannun YA. Role for mammalian neutral sphingomyelinase 2 in confluence-induced growth arrest of MCF7 cells. *J Biol Chem*. 2004;279(24):25101-25111.
94. Tani M, Hannun YA. Analysis of membrane topology of neutral sphingomyelinase 2. *FEBS Lett*. 2007;581(7):1323-1328.
95. Ito H, Tanaka K, Hagiwara K, et al. Transcriptional regulation of neutral sphingomyelinase 2 in all-trans retinoic acid-treated human breast cancer cell line, MCF-7. *J Biochem*. 2012;151(6):599-610.
96. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes*. 2006;55(9):2579-2587.
97. Blachnio-Zabielska AU, Koutsari C, Tchkonia T, Jensen MD. Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance. *Obesity (Silver Spring)*. 2012;20(12):2341-2347.
98. Levy M, Castillo SS, Goldkorn T. nSMase2 activation and trafficking are modulated by oxidative stress to induce apoptosis. *Biochem Biophys Res Commun*. 2006;344(3):900-905.
99. Clarke CJ, Truong TG, Hannun YA. Role for neutral sphingomyelinase-2 in tumor necrosis factor alpha-stimulated expression of vascular cell adhesion molecule-1 (VCAM) and intercellular adhesion molecule-1 (ICAM) in lung epithelial cells: p38 MAPK is an upstream regulator of nSMase2. *J Biol Chem*. 2007;282(2):1384-1396.
100. Clarke CJ, Guthrie JM, Hannun YA. Regulation of neutral sphingomyelinase-2 (nSMase2) by tumor necrosis factor-alpha involves protein kinase C-delta in lung epithelial cells. *Mol Pharmacol*. 2008;74(4):1022-1032.
101. Horres CR, Hannun YA. The roles of neutral sphingomyelinases in neurological pathologies. *Neurochem Res*. 2012;37(6):1137-1149.
102. Rutkute K, Asmis RH, Nikolova-Karakashian MN. Regulation of neutral sphingomyelinase-2 by GSH: a new insight to the role of oxidative stress in aging-associated inflammation. *J Lipid Res*. 2007;48(11):2443-2452.
103. Luberto C, Hassler DF, Signorelli P, et al. Inhibition of tumor necrosis factor-induced cell death in MCF7 by a novel inhibitor of neutral sphingomyelinase. *J Biol Chem*. 2002;277(43):41128-41139.
104. de Palma C, Meacci E, Perrotta C, Bruni P, Clementi E. Endothelial nitric oxide synthase activation by tumor necrosis factor alpha through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 phosphate receptors: a novel pathway relevant to the pathophysiology of endothelium. *Arterioscler Thromb Vasc Biol*. 2006;26(1):99-105.
105. Zeng C, Lee JT, Chen H, Chen S, Hsu CY, Xu J. Amyloid-beta peptide enhances tumor necrosis factor-alpha-induced iNOS through neutral sphingomyelinase/ceramide pathway in oligodendrocytes. *J Neurochem*. 2005;94(3):703-712.
106. Marchesini N, Luberto C, Hannun YA. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. *J Biol Chem*. 2003;278(16):13775-13783.
107. Ito H, Murakami M, Furuhata A, et al. Transcriptional regulation of neutral sphingomyelinase 2 gene expression of a human breast cancer cell line, MCF-7, induced by the anti-cancer drug, daunorubicin. *Biochim Biophys Acta*. 2009;1789(11–12):681-690.
108. Dressler KA, Mathias S, Kolesnick RN. Tumor necrosis factor-alpha activates the sphingomyelin signal transduction pathway in a cell-free system. *Science*. 1992;255(5052):1715-1718.
109. Wiegmann K, Schutze S, Machleidt T, Witte D, Kronke M. Functional dichotomy of neutral and acidic sphingomyelinases in tumor necrosis factor signaling. *Cell*. 1994;78(6):1005-1015.
110. Ishii T, Warabi E. Mechanism of rapid nuclear factor-E2-related factor 2 (Nrf2) activation via membrane-associated estrogen receptors: roles of NADPH oxidase 1, neutral sphingomyelinase 2 and epidermal growth factor receptor (EGFR). *Antioxidants (Basel)*. 2019;8(3):69.
111. Grimm MO, Grimm HS, Patzold AJ, et al. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol*. 2005;7(11):1118-1123.
112. Filosto S, Ashfaq M, Chung S, Fry W, Goldkorn T. Neutral sphingomyelinase 2 activity and protein stability are modulated by phosphorylation of five conserved serines. *J Biol Chem*. 2012;287(1):514-522.
113. Filosto S, Fry W, Knowlton AA, Goldkorn T. Neutral sphingomyelinase 2 (nSMase2) is a phosphoprotein regulated by calcineurin (PP2B). *J Biol Chem*. 2010;285(14):10213-10222.
114. Liu B, Andrieu-Abadie N, Levade T, Zhang P, Obeid LM, Hannun YA. Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death. *J Biol Chem*. 1998;273(18):11313-11320.
115. Airola MV, Hannun YA. Sphingolipid metabolism and neutral sphingomyelinases. *Handb Exp Pharmacol*. 2013;215:57-76.
116. Shanbhogue P, Hoffmann RM, Airola MV, et al. The juxtamembrane linker in neutral sphingomyelinase-2 functions as an intramolecular allosteric switch that activates the enzyme. *J Biol Chem*. 2019;294(18):7488-7502.
117. Ago H, Oda M, Takahashi M, et al. Structural basis of the sphingomyelin phosphodiesterase activity in neutral sphingomyelinase from Bacillus cereus. *J Biol Chem*. 2006;281(23):16157-16167.
118. Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *J Lipid Res*. 2009;50 Suppl(Suppl):S91-S96.
119. Pavoine C, Pecker F. Sphingomyelinases: their regulation and roles in cardiovascular pathophysiology. *Cardiovasc Res*. 2009;82(2):175-183.
120. Wu H, Ballantyne CM. Metabolic inflammation and insulin resistance in obesity. *Circ Res*. 2020;126(11):1549-1564.
121. Li Y, Talbot CL, Chaurasia B. Ceramides in adipose tissue. *Front Endocr (Lausanne)*. 2020;11:407.
122. Nikolova-Karakashian M, Karakashian A, Rutkute K. Role of neutral sphingomyelinases in aging and inflammation. *Subcell Biochem*. 2008;49:469-486.
123. Ghosh N, Patel N, Jiang K, et al. Ceramide-activated protein phosphatase involvement in insulin resistance via Akt, serine/arginine-rich protein 40, and ribonucleic acid splicing in L6 skeletal muscle cells. *Endocrinology*. 2007;148(3):1359-1366.
124. Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol*. 2003;23(21):7794-7808.
125. Chavez JA, Knotts TA, Wang LP, et al. A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J Biol Chem*. 2003;278(12):10297-10303.
126. Resjo S, Goransson O, Harndahl L, Zolnierowicz S, Manganiello V, Degerman E. Protein phosphatase 2A is the main phosphatase involved in the regulation of protein kinase B in rat adipocytes. *Cell Signal*. 2002;14(3):231-238.
127. Turpin SM, Nicholls HT, Willmes DM, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab*. 2014;20(4):678-686.
128. Konstantynowicz-Nowicka K, Harasim E, Baranowski M, Chabowski A. New evidence for the role of ceramide in the development of hepatic insulin resistance. *PLoS One*. 2015;10(1):e0116858.

129. Fernandez-Veledo S, Hernandez R, Teruel T, Mas JA, Ros M, Lorenzo M. Ceramide mediates TNF-alpha-induced insulin resistance on GLUT4 gene expression in brown adipocytes. *Arch Physiol Biochem*. 2006;112(1):13-22.
130. Hajduch E, Balendran A, Batty IH, et al. Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia*. 2001;44(2):173-183.
131. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science*. 2004;306 (5695):457-461.
132. Verma MK, Yateesh AN, Neelima K, et al. Inhibition of neutral sphingomyelinases in skeletal muscle attenuates fatty-acid induced defects in metabolism and stress. *Springerplus*. 2014;3:255.
133. Al-Rashed F, Ahmad Z, Thomas R, et al. Neutral sphingomyelinase 2 regulates inflammatory responses in monocytes/macrophages induced by TNF-α. *Sci Rep*. 2020;10(1):16802.
134. Holland WL, Brozinick JT, Wang LP, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab*. 2007;5(3):167-179.
135. Miyazaki Y, Matsuda M, DeFronzo RA. Dose-response effect of pioglitazone on insulin sensitivity and insulin secretion in type 2 diabetes. *Diabetes Care*. 2002;25(3):517-523.
136. Sakamoto J, Kimura H, Moriyama S, et al. Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. *Biochem Biophys Res Commun*. 2000;278(3):704-711.
137. Smith U. Pioglitazone: mechanism of action. *Int J Clin Pract Suppl*. 2001;121:13-18.
138. Murase K, Odaka H, Suzuki M, Tayuki N, Ikeda H. Pioglitazone time-dependently reduces tumour necrosis factor-alpha level in muscle and improves metabolic abnormalities in Wistar fatty rats. *Diabetologia*. 1998;41(3):257-264.
139. Chaurasia B, Tippetts TS, Mayoral Monibas R, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science*. 2019;365(6451):386-392.
140. Apostolopoulou M, Gordillo R, Gancheva S, et al. Role of ceramide-to-dihydroceramide ratios for insulin resistance and non-alcoholic fatty liver disease in humans. *BMJ Open Diabetes Res Care*. 2020;8 (2):e001860.
141. Apostolopoulou M, Gordillo R, Koliaki C, et al. Specific hepatic sphingolipids relate to insulin resistance, oxidative stress, and inflammation in nonalcoholic steatohepatitis. *Diabetes Care*. 2018; 41(6):1235-1243.
142. Kolak M, Westerbacka J, Velagapudi VR, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes*. 2007;56(8): 1960-1968.
143. Xia JY, Holland WL, Kusminski CM, et al. Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab*. 2015;22(2):266-278.
144. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest*. 2006;116(7):1802-1812.
145. Prentki M, Peyot ML, Masiello P, Madiraju SRM. Nutrient-induced metabolic stress, adaptation, detoxification, and toxicity in the pancreatic β-cell. *Diabetes*. 2020;69(3):279-290.
146. Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. *J Biol Chem*. 1998;273(49):32487-32490.
147. Boslem E, Meikle PJ, Biden TJ. Roles of ceramide and sphingolipids in pancreatic β-cell function and dysfunction. *Islets*. 2012;4(3): 177-187.
148. Lei X, Bone RN, Ali T, et al. Evidence of contribution of iPLA2beta-mediated events during islet beta-cell apoptosis due to proinflammatory cytokines suggests a role for iPLA2beta in T1D development. *Endocrinology*. 2014;155(9):3352-3364.
149. Ying L, Tippetts TS, Chaurasia B. Ceramide dependent lipotoxicity in metabolic diseases. *Nutr Heal Ag*. 2019;5(1):1-12.
150. Véret J, Bellini L, Giussani P, Ng C, Magnan C, Stunff HL. Roles of sphingolipid metabolism in pancreatic β cell dysfunction induced by lipotoxicity. *J Clin Med*. 2014;3(2):646-662.
151. Chipuk JE, McStay GP, Bharti A, et al. Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell*. 2012;148(5):988-1000.
152. Janikiewicz J, Hanzelka K, Kozinski K, Kolczynska K, Dobrzyn A. Islet β-cell failure in type 2 diabetes—within the network of toxic lipids. *Biochem Biophys Res Commun*. 2015;460(3):491-496.
153. Lupi R, Dotta F, Marselli L, et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes*. 2002;51(5):1437-1442.
154. Yano M, Watanabe K, Yamamoto T, et al. Mitochondrial dysfunction and increased reactive oxygen species impair insulin secretion in sphingomyelin synthase 1-null mice. *J Biol Chem*. 2011;286(5): 3992-4002.
155. Tong X, Chaudhry Z, Lee CC, et al. Cigarette smoke exposure impairs beta-cell function through activation of oxidative stress and ceramide accumulation. *Mol Metab*. 2020;37:100975.
156. Kovilakath A, Cowart LA. Sphingolipid mediators of myocardial pathology. *J Lipi Atheroscle*. 2020;9(1):23-49.
157. Canals D, Perry DM, Jenkins RW, Hannun YA. Drug targeting of sphingolipid metabolism: sphingomyelinases and ceramidases. *Br J Pharmacol*. 2011;163(4):694-712.
158. Deng P, Hoffman JB, Petriello MC, et al. Dietary inulin decreases circulating ceramides by suppressing neutral sphingomyelinase expression and activity in mice. *J Lipid Res*. 2020;61(1):45-53.
159. Simon J, Ouro A, Ala-Ibanibo L, Presa N, Delgado TC, Martinez-Chantar ML. Sphingolipids in non-alcoholic fatty liver disease and hepatocellular carcinoma: ceramide turnover. *Int J Mol Sci*. 2019;21 (1):40.
160. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism*. 2016;65 (8):1038-1048.
161. Masarone M, Rosato V, Dallio M, et al. Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. *Oxid Med Cell Longev*. 2018;2018:9547613.
162. Lebeaupin C, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J Hepatol*. 2018;69(4): 927-947.
163. Wandrer F, Liebig S, Marhenke S, et al. TNF-receptor-1 inhibition reduces liver steatosis, hepatocellular injury and fibrosis in NAFLD mice. *Cell Death Dis*. 2020;11(3):212.
164. Niederreiter L, Tilg H. Cytokines and fatty liver diseases. *Liver Research*. 2018;2(1):14-20.
165. Mendez-Sanchez N, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP, Barranco-Fragoso B, Cordova-Gallardo J. New aspects of lipotoxicity in nonalcoholic steatohepatitis. *Int J Mol Sci*. 2018;19(7): 2034.
166. Di Sessa A, Cirillo G, Guarino S, Marzuillo P, Miraglia Del Giudice E. Pediatric non-alcoholic fatty liver disease: current perspectives on diagnosis and management. *Pediatric Health Med Ther*. 2019;10: 89-97.
167. Mauer AS, Hirsova P, Maiers JL, Shah VH, Malhi H. Inhibition of sphingosine 1-phosphate signaling ameliorates murine nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol*. 2017;312(3): G300-G313.

168. Raichur S, Wang ST, Chan PW, et al. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab*. 2014;20(4):687-695.
169. Lee AY, Lee JW, Kim JE, et al. Dihydroceramide is a key metabolite that regulates autophagy and promotes fibrosis in hepatic steatosis model. *Biochem Biophys Res Commun*. 2017;494(3–4):460-469.
170. Kasumov T, Li L, Li M, et al. Ceramide as a mediator of non-alcoholic Fatty liver disease and associated atherosclerosis. *PLoS One*. 2015;10(5):e0126910.
171. Chaurasia B, Kaddai VA, Lancaster GI, et al. Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism. *Cell Metab*. 2016;24(6):820-834.
172. Sanyal AJ, Pacana T. A lipidomic readout of disease progression in a diet-induced mouse model of nonalcoholic fatty liver disease. *Trans Am Clin Climatol Assoc*. 2015;126:271-288.
173. Sajan MP, Ivey RA, Lee MC, Farese RV. Hepatic insulin resistance in ob/ob mice involves increases in ceramide, aPKC activity, and selective impairment of Akt-dependent FoxO1 phosphorylation. *J Lipid Res*. 2015;56(1):70-80.
174. Ito M, Suzuki J, Tsujioka S, et al. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res*. 2007;37(1):50-57.
175. Jiang M, Li C, Liu Q, Wang A, Lei M. Inhibiting ceramide synthesis attenuates hepatic steatosis and fibrosis in rats with non-alcoholic fatty liver disease. *Front Endocrinol*. 2019;10(665).
176. Kurek K, Piotrowska D, Wiesiołek P, et al. Inhibition of ceramide de novo synthesis reduces liver lipid accumulation in rats with non-alcoholic fatty liver disease. *Liver Intern: Official J Intern Asso Stud Liv*. 2013;34.
177. Taltavull N, Ras R, Marine S, et al. Protective effects of fish oil on pre-diabetes: a lipidomic analysis of liver ceramides in rats. *Food Funct*. 2016;7(9):3981-3988.
178. Lieber CS, Leo MA, Mak KM, et al. Model of nonalcoholic steatohepatitis. *Am J Clin Nutr*. 2004;79(3):502-509.
179. Weyler J, Verrijken A, Hornemann T, et al. Association of 1-deoxy-sphingolipids with steatosis but not steatohepatitis nor fibrosis in non-alcoholic fatty liver disease. *Acta Diabetol*. 2021;58(3):319–327.
180. Zitomer NC, Mitchell T, Voss KA, et al. Ceramide synthase inhibition by fumonisin B1 causes accumulation of 1-deoxysphinganine: a novel category of bioactive 1-deoxysphingoid bases and 1-deoxydihydroceramides biosynthesized by mammalian cell lines and animals. *J Biol Chem*. 2009;284(8):4786-4795.
181. Penno A, Reilly MM, Houlden H, et al. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. *J Biol Chem*. 2010;285(15):11178-11187.
182. Alecu I, Othman A, Penno A, et al. Cytotoxic 1-deoxysphingolipids are metabolized by a cytochrome P450-dependent pathway. *J Lipid Res*. 2017;58(1):60-71.
183. Bertea M, Rutti MF, Othman A, et al. Deoxysphingoid bases as plasma markers in diabetes mellitus. *Lipids Health Dis*. 2010;9:84.
184. Othman A, Saely CH, Muendlein A, et al. Plasma 1-deoxysphingolipids are predictive biomarkers for type 2 diabetes mellitus. *BMJ Open Diabetes Res Care*. 2015;3(1):e000073.
185. Gorden DL, Myers DS, Ivanova PT, et al. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res*. 2015;56(3):722-736.
186. Dohrn MF, Othman A, Hirshman SK, et al. Elevation of plasma 1-deoxy-sphingolipids in type 2 diabetes mellitus: a susceptibility to neuropathy? *Eur J Neurol*. 2015;22(5):806-814, e855.
187. Insausti-Urkia N, Solsona-Vilarrasa E, Garcia-Ruiz C, Fernandez-Checa JC. Sphingomyelinases and liver diseases. *Biomolecules*. 2020;10(11):1497.
188. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(5):G1281-G1287.
189. Chiappini F, Barrier A, Saffroy R, et al. Exploration of global gene expression in human liver steatosis by high-density oligonucleotide microarray. *Lab Invest*. 2006;86(2):154-165.
190. Deevska G, Sunkara M, Karakashian C, Peppers B, Morris AJ, Nikolova-Karakashian MN. Effect of procysteine on aging-associated changes in hepatic GSH and SMase: evidence for transcriptional regulation of smpd3. *J Lipid Res*. 2014;55(10):2041-2052.
191. Griffin JH, Fernandez JA, Deguchi H. Plasma lipoproteins, hemostasis and thrombosis. *Thromb Haemost*. 2001;86(1):386-394.
192. Oörni K, Posio P, Ala-Korpela M, Jauhiainen M, Kovanen PT. Sphingomyelinase induces aggregation and fusion of small very low-density lipoprotein and intermediate-density lipoprotein particles and increases their retention to human arterial proteoglycans. *Arterioscler Thromb Vasc Biol*. 2005;25(8):1678-1683.
193. Kinnunen PK, Holopainen JM. Sphingomyelinase activity of LDL: a link between atherosclerosis, ceramide, and apoptosis? *Trends Cardiovasc Med*. 2002;12(1):37-42.
194. Chatterjee SB, Dey S, Shi WY, Thomas K, Hutchins GM. Accumulation of glycosphingolipids in human atherosclerotic plaque and unaffected aorta tissues. *Glycobiology*. 1997;7(1):57-65.
195. Levade T, Auge N, Veldman RJ, Cuvillier O, Negre-Salvayre A, Salvayre R. Sphingolipid mediators in cardiovascular cell biology and pathology. *Circ Res*. 2001;89(11):957-968.
196. Martin SF, Williams N, Chatterjee S. Lactosylceramide is required in apoptosis induced by N-Smase. *Glycoconj J*. 2006;23(3):147-157.
197. Mu H, Wang X, Wang H, Lin P, Yao Q, Chen C. Lactosylceramide promotes cell migration and proliferation through activation of ERK1/2 in human aortic smooth muscle cells. *Am J Physiol Heart Circ Physiol*. 2009;297(1):H400-H408.
198. Yang G, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol Endocrinol Metab*. 2009;297(1):E211-E224.
199. Lallemand T, Rouahi M, Swiader A, et al. nSMase2 (type 2-neutral sphingomyelinase) deficiency or inhibition by GW4869 reduces inflammation and atherosclerosis in apoe(−/−) mice. *Arterioscler Thromb Vasc Biol*. 2018;38(7):1479-1492.
200. Park TS, Hu Y, Noh HL, et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res*. 2008;49(10):2101-2112.
201. Laaksonen R, Ekroos K, Sysi-Aho M, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur Heart J*. 2016;37(25):1967-1976.
202. de Carvalho LP, Tan SH, Ow GS, et al. Plasma ceramides as prognostic biomarkers and their arterial and myocardial tissue correlates in acute myocardial infarction. *JACC Basic Transl Sci*. 2018;3(2):163-175.
203. Dotson PP II, Karakashian Alexander A, Nikolova-Karakashian MN. Neutral sphingomyelinase-2 is a redox sensitive enzyme: role of catalytic cysteine residues in regulation of enzymatic activity through changes in oligomeric state. *Biochem J*. 2015;465(3):371-382.
204. Tellier E, Negre-Salvayre A, Bocquet B, et al. Role for furin in tumor necrosis factor alpha-induced activation of the matrix metalloproteinase/sphingolipid mitogenic pathway. *Mol Cell Biol*. 2007;27(8):2997-3007.
205. Philipp S, Puchert M, Adam-Klages S, et al. The polycomb group protein EED couples TNF receptor 1 to neutral sphingomyelinase. *Proc Natl Acad Sci U S A*. 2010;107(3):1112-1117.
206. Adamy C, Mulder P, Khouzami L, et al. Neutral sphingomyelinase inhibition participates to the benefits of N-acetylcysteine treatment in post-myocardial infarction failing heart rats. *J Mol Cell Cardiol*. 2007;43(3):344-353.

207. Smith AR, Visioli F, Frei B, Hagen TM. Lipoic acid significantly restores, in rats, the age-related decline in vasomotion. *Br J Pharmacol*. 2008;153(8):1615-1622.
208. Anderson HC. Vesicles associated with calcification in the matrix of epiphyseal cartilage. *J Cell Biol*. 1969;41(1):59-72.
209. Egea-Jimenez AL, Zimmermann P. Lipids in exosome biology. *Handb Exp Pharmacol*. 2020;259:309-336.
210. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neurooncol*. 2013;113(1):1-11.
211. Engin A. Dark-side of exosomes. *Adv Exp Med Biol*. 2021;1275:101-131.
212. Trajkovic K, Hsu C, Chiantia S, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*. 2008;319(5867):1244-1247.
213. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol*. 2014;29:116-125.
214. Llorente A, Skotland T, Sylvänne T, et al. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim Biophys Acta*. 2013;1831(7):1302-1309.
215. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem*. 2010;285(23):17442-17452.
216. Singh R, Pochampally R, Watabe K, Lu Z, Mo YY. Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. *Mol Cancer*. 2014;13:256.
217. Sackmann V, Sinha MS, Sackmann C, et al. Inhibition of nSMase2 reduces the transfer of oligomeric α-synuclein irrespective of hypoxia. *Fronti Molecu Neurosc*. 2019;12:200.
218. Bilousova T, Simmons BJ, Knapp RR, et al. Dual neutral sphingomyelinase-2/acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. *ACS Chem Biol*. 2020;15(6):1671-1684.
219. Guo BB, Bellingham SA, Hill AF. The neutral sphingomyelinase pathway regulates packaging of the prion protein into exosomes. *J Biol Chem*. 2015;290(6):3455-3467.
220. Liu Y, Wang Y, Wang C, et al. Maternal obesity increases the risk of fetal cardiac dysfunction via visceral adipose tissue derived exosomes. *Placenta*. 2021;105:85-93.
221. Gu H, Yang K, Shen Z, et al. ER stress-induced adipocytes secrete-aldo-keto reductase 1B7-containing exosomes that cause non-alcoholic steatohepatitis in mice. *Free Radic Biol Med*. 2021;163:220-233.
222. Kumar A, Sundaram K, Mu J, et al. High-fat diet-induced upregulation of exosomal phosphatidylcholine contributes to insulin resistance. *Nat Commun*. 2021;12(1):213.
223. Li J, Zhang Y, Ye Y, et al. Pancreatic β cells control glucose homeostasis via the secretion of exosomal miR-29 family. *J Extracel Ves*. 2021;10(3):e12055.
224. Burillo J, Fernández-Rhodes M, Piquero M, et al. Human amylin aggregates release within exosomes as a protective mechanism in pancreatic β cells: Pancreatic β-hippocampal cell communication. *Biochimica et Biophysica Acta Mole Cell Res*. 1868;2021(5):118971.
225. Ying W, Gao H, Dos Reis FCG, et al. MiR-690, an exosomal-derived miRNA from M2-polarized macrophages, improves insulin sensitivity in obese mice. *Cell Metab*. 2021. https://doi.org/10.1016/j.cmet.2020.12.019

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