Cardiovascular Implications of Sphingomyelin Presence in Biological Membranes

Petros Kikas, George Chalikias, and Dimitrios Tziakas

Democritus University of Thrace, Alexandroupolis, Greece

Abstract

Sphingomyelin (SM) is a type of sphingolipid found within plasma, cellular membranes and plasma lipoproteins. Here we highlight the basic biochemical features of SMs and their role in biological membranes. We further discuss evidence of the association between SM and cardiovascular diseases such as atherosclerosis, valvular disease, heart failure and diabetes mellitus.

Keywords

Sphingomyelin, sphingolipids, cardiovascular implications

Disclosure: The authors have no conflicts of interest to declare.
Received: 07 Sept 2017 **Accepted:** 07 March 2018 **Citation:** *European Cardiology Review* 2018;**13**(1):42–5. DOI: https://doi.org/10.15420/ecr.2017:20:3
Correspondence: Professor Dimitrios Tziakas, Cardiology Department, University Hospital of Alexandroupolis, Dragana, Alexandroupolis 68100, Greece.
E: dtziakas@med.duth.gr

Sphingolipids are one of the major categories of lipids and beyond their role as structural membrane components they have important functions as signalling molecules in a wide array of biological processes. They are composed of two key lipid building blocks – long-chain bases (usually sphingosine or 1,3-dihydroxy-2-amino-4-octadecene) and fatty acids – and use a glycerol-based backbone to which acyl chains are attached.[1,2]

Sphingomyelins (SMs) are among the most common sphingolipids in many mammalian cells and tissues, especially in the membranous myelin sheath that surrounds nerve cell axons.[3] SMs have significant structural and functional roles in the cell such as creating unique lateral structures (lipid rafts and ordered domains) in membranes, binding to and functional regulation of membrane-spanning proteins and involvement in cell signalling events.[3] They play an important role in the regulation of plasma membrane and cell cholesterol homeostasis.[3]

SM was first isolated from brain tissue by German chemist Johann LW Thudicum in the 1880s.[4] In 1927 the structure of SM was first reported by Pick and Bielschowsky; SM is an N-acyl-sphingosine-1-phosphorylcholine, consisting of a phosphocholine head group, a sphingosine and a fatty acid.[5] The most common long-chain base in SM is 1,3-dihydroxy-2-amino-4-octadecene (sphingosine or d18:1). The most common N-linked acyl chain of SMs in mammalian peripheral cells is palmitic acid (16:0), whereas stearic acid (18:0) is more common in neural tissue SMs. Long-chain fatty acids (e.g. 24:0 and 24:1) are also common constituents of the SMs found in most tissues.[3]

SMs are synthesised in the endoplasmic reticulum, where they can be found in low levels, and in the trans Golgi.[6] The first step in SM synthesis is the condensation of L-serine and palmitoyl-coenzyme A. This reaction is catalysed by serine palmitoyltransferase and yields 3-keto-dihydrosphingosine, which is reduced to dihydrosphingosine. Dihydrosphingosine undergoes N-acylation followed by desaturation to generate ceramide, which has a central role in sphingolipid metabolism.[3,7] These reactions occur on the cytosolic surface of the endoplasmic reticulum. Subsequently, ceramide is delivered to the Golgi apparatus where it is converted to SM or glucosylceramide. In most mammalian cell types the majority of ceramide is converted to SM in the lumen of the trans Golgi by an SM synthase (SMS) enzyme named SMS1, which catalyses the transfer of phosphocholine from phosphatidylcholine to ceramide, yielding a diacylglycerol side product.[3,7] A second enzyme, SMS2, resides at the plasma membrane and may convert locally produced ceramide to SM.[3,7] An alternative pathway of SM synthesis has been postulated in which ceramide is first converted to ethanolamine phosphorylceramide via transfer of the head group from phosphatidylethanolamine.[7] Ethanolamine phosphorylceramide is then converted to SM by stepwise methylation. Although this pathway has been demonstrated in isolated membrane fractions from rat brain and liver, its precise contribution to the *de novo* synthesis of SM remains to be established.[7]

Since major SMS activity is found in the luminal trans Golgi and the plasma membranes, it is not surprising that SM is enriched in membranes derived from trans Golgi and in the plasma membrane (including the endosomal recycling compartment). The asymmetric location of SMS activity (luminal trans Golgi and outer leaflet of plasma membranes) may explain why SMs are enriched at the plasma membrane with a greater concentration on the outer than the inner leaflet.[3,6,8] In humans, SMs comprise nearly 85 % of all sphingolipids and 10–20 mol % of the total plasma membrane lipids. SMs comprise 4–18 % of all sarcolemma membrane lipids with 93 % present in the outer leaflet.[8]

The Role of Sphingomyelin in Biological Membranes

The presence of SM in biological membranes is associated with various critical functions. SMs accumulate in the exoplasmic leaflet of the

plasma membrane where their high packing density and affinity for sterols help create a rigid barrier to the extracellular environment.[7] Under this notion, the membranous myelin sheath that surrounds nerve cell axons is particularly rich in SMs, which may suggest a role as an insulator of nerve fibres.[9,10]

SM presence in the plasma membrane directly affects cholesterol homeostasis.[11,12] The addition of SM to cells has been shown to increase cholesterol biosynthesis and affect LDL binding to cell surface receptors and subsequent internalisation.[13] Together they form SM/sterol-rich domains that often are more ordered than the surrounding phase in biological membranes.[14] The growing body of evidence regarding their favourable interaction with sterols indicates that SM may be a key a regulator of cholesterol distribution within cellular membranes and cholesterol homeostasis in cells.[14] In addition, excess levels of SM in the red blood cell membrane (i.e. abetalipoproteinemia) cause increased lipid accumulation in the outer leaflet of the red blood cell plasma membrane, which results in abnormally shaped red cells called acanthocytes.[15]

The SM pool in the plasma membrane acts as a reservoir of lipid signalling molecules, the liberation of which is catalysed by acidic or neutral SMSs in response to a variety of biological stimuli. SM metabolites such as ceramide,[16] sphingosine[17,18] and sphingosine 1-phosphate[17,18] are emerging as critical regulators of cell proliferation, differentiation and apoptosis.[7] The ability of SM to function as a precursor of signalling molecules in a wide array of biological processes is extensively reviewed elsewhere.[19]

As SMs have a strong, inherent capacity to form microdomains, their production in the trans Golgi may affect the lateral organisation of other membrane molecules and are thus associated with the formation of lipid rafts, caveoles and other micro-membrane structures involved in various cellular processes (including endocytosis, intracellular trafficking, and signal transduction by membrane receptors, cell–cell communication and host–pathogen interactions).[7,10,20-23]

SM synthesis in the trans Golgi may create a local pool of diacylglycerol, which leads to protein kinase D recruitment and the formation of secretory vesicles.[7,10,24]

The proportion of SM within a membrane as well as SM structural configuration contribute to membrane thickness and recruitment of specific integral proteins, thereby modulating membrane structural integrity and function.[25,10] Furthermore, published evidence suggests that SM interacts with these integral membrane proteins, modulating their functional role.[10]

Interdigitation occurs when a long acyl chain in SM located in one leaflet penetrates through the bilayer into the opposite leaflet. Such interdigitation has been shown to affect the diffusion rates in opposing glycerophospholipid leaflets, suggesting interleaflet coupling with possible biological significance.[26,10]

Cardiovascular Implications Associated with Sphingomyelin

Atherosclerosis/Thrombosis

A study of human carotid plaques showed that levels of several sphingolipids (including SM) were increased in plaques associated with symptoms and correlated with inflammatory cytokines.[27] All sphingolipids were correlated with histological markers of plaque instability and also induced an inflammatory response in human coronary artery smooth muscle cells. SM and ceramide were also found to be positively correlated with lipid plaque burden.[27] A study of hypercholesterolaemic rabbits found that SMs were relatively abundant within atherosclerotic lesions compared with other lipids.[28] Furthermore, strikingly elevated intra-platelet levels of triglycerides and SM were found in patients with ST-elevation MI in comparison with matched controls.[29]

Several studies have assessed the association between circulating plasma SM levels and incidence of cardiovascular disease, with conflicting results. Fernandez et al. showed that SM (38:2) levels was the only lipid species of its class to be associated with increased risk of future cardiovascular disease.[30] In another study, patients with stable angina, unstable angina or acute MI showed higher plasma SM and ceramide levels as well as higher SMS activity compared with healthy individuals.[31] However, in the population-based Multi-Ethnic Study of Atherosclerosis, a high plasma SM level was not associated with an increased risk of incident coronary heart disease in adults free of clinical cardiovascular disease at baseline.[32]

Recently, erythrocytes have received considerable attention as significant players in accelerating plaque progression, with observations of erythrocytes being 'driven' to the plaque lipid core via intraplaque haemorrhages.[33] Assessing the sphingolipid content of erythrocyte membranes, Zhang et al. demonstrated that SM levels were elevated in patients with an acute coronary syndrome compared with patients with stable angina.[34] However, no differences were found between patients with stable coronary artery disease and healthy individuals regarding SM content in erythrocyte membranes. In the same study, a significant correlation between plasma SM levels, erythrocyte membrane SM content and lipoprotein alpha(a) levels were shown. These observations suggest a link between SM content of erythrocyte membrane and clinical instability in coronary artery disease, which may reflect another potential source of atheromatous plaque instability via intraplaque haemorrhages. Therefore, the SM content of erythrocyte membranes could serve as a marker for coronary artery disease activity independent of other risk factors and clinical features.[34]

Diabetes

As part of the Finnish Diabetic Nephropathy Study, 326 patients with type 1 diabetes were studied for presence of diabetic renal disease.[35] Serum levels of SM and large HDL particles were the strongest predictors after adjustment of established kidney injury biomarkers. In addition, SM levels assessed in serum was correlated significantly with 24 hour albumin excretion rate. In contrast, another study demonstrated that compared with healthy controls, patients with impaired fasting glucose levels or type 2 diabetes had significantly reduced serum concentrations of glycerophospholipids and SMs, even after adjusting for age, gender and BMI or even when separately analysed for those not receiving lipid-modifying medications.[36] The authors suggested that the decrease in the SM pool in diabetes could potentially contribute to increased oxidative stress and reduced insulin secretion, resulting in hyperglycaemia secondary to an inability to compensate for reduced insulin sensitivity.[36]

Lipoproteins

SM is the most abundant sphingolipid in lipoproteins. Very low density liopoprotein/LDL and HDL comprise approximately 63–75 % and

25–35 % of SMs, respectively.[37] SM is slightly more abundant (13 %) in large light HDL2 particles than in small dense HDL3 particles per millilitre of plasma.[38] The decrease in SM content in HDL particles associated with smaller and more dense HDL molecules was shown to correlate with the atheroprotective functionality of HDL, such as cholesterol efflux capacity, antioxidative activity toward LDL oxidation, antithrombotic activity in human platelets, anti-inflammatory activity and anti-apoptotic activity.[38] In another study, anti-apoptotic and anti-oxidative activities of small dense HDL particles were associated with depletion in SM within the same molecules.[39] This association between structural reformation of HDL molecules and their anti-atherogenic properties (mainly their capacity for cholesterol efflux) was explained by the negative impact that SM had on molecular surface fluidity and lecithin:cholesterol acyltransferase activity.

LDL present in atherosclerotic plaques has higher SM levels than plasma LDL; this is mainly due to *de novo* synthesis in the aorta.[37,40] Furthermore, early studies have shown that advanced aortic wall atherosclerosis is characterised by increased SM proportion.[41] Schissel et al. have shown that in rabbit aorta and human atherosclerotic lesions, arterial-wall SMS activity hydrolyses the SM of retained LDL leading to LDL aggregation, an important event regarding the initiation and progression of atherosclerosis.[37,42]

Valvular Disease

Recently, the presence of intra-leaflet haemorrhages and the associated increase in intra-leaflet lipid accumulation has been recognised as a novel mechanism for valve tissue degeneration. In a study by Lehti et al., higher SM:phosphatidylcholine ratio as well as higher proportions of lysophosphatidylcholine and unesterified cholesterol were found in lipid particles isolated from patients with stenotic aortic valves compared with non-stenotic counterparts.[43] Under the same notion, using a metabolomics approach in aortic tissue, Doppler et al. showed that levels of SM were significantly increased in patients with bicuspid aortic valve disease undergoing ascending thoracic aorta surgery and with tricuspid aortic valve associated aortic dissection compared with controls.[44]

Niemann–Pick disease is a rare lysosomal storage disease caused by deficient activity of acid SMS and the accumulation of SM within cells; these abnormalities occur especially in the monocyte–macrophage system. In a prospective, cross-sectional survey study, McGovern et al. demonstrated that most patients with Niemann–Pick disease had low HDL cholesterol, high total cholesterol, high triglyceride and high LDL levels compared with control subjects.[45] Two-dimensional echocardiograms performed during the study, showed abnormalities in the majority of patients with Niemann–Pick disease, most commonly mitral valve regurgitation. Furthermore, several published cases of patients with Niemman–Pick disease report severe mitral regurgitation as a clinical manifestation of the disease, thus suggesting an association between progressive accumulation of SM in lysosomes and degeneration of cardiac valves.[46]

Cardiomyopathies

Ceramides are produced by either *de novo* synthesis or hydrolysis of SM catalysed by acid and/or neutral SMS.[47] Accumulation of cardiac ceramides in the post-ischaemic heart is mediated by acid SMS and not by *de novo* sphingolipid synthesis.[48] Therefore, the presence of ceramides in the post-ischaemic myocardium is most likely due to SM degradation by SMS, thus suggesting a detrimental role of SM in a wide variety of cardiomyopathies, especially in ischaemic cardiomyopathy. However, a recent study in mice showed that inhibition of acid SMS does not result in improved heart function or survival after an induced MI despite reducing ischaemia-induced ceramide accumulation.[49] In a rodent model of lipotoxic cardiomyopathy, mice exhibited increased lipid uptake and oxidation, ceramide accumulation and a dilated cardiomyopathy, with decreased functional cardiomyocyte shortening.[49] In contrast, we must emphasise that within this cardiolipotoxic model, ceramides were *de novo* synthesised within the myocardium and that the mechanism of this cardiac dysfunction was not clear.

As part of the Alberta Heart Failure Etiology and Analysis Research Team (HEART) project, ambulatory patients with clinical diagnosis of heart failure (HF; with preserved or reduced ejection fraction) and age-matched non-HF controls were selected for metabolomic analysis.[50] Compared with non-HF control subjects, patients with HF with preserved ejection fraction had lower levels of phosphatidylcholines and SMs. Furthermore, in a recent study enrolling patients with either ischaemic or non-ischaemic cardiomyopathy, healthy controls and patients with pulmonary diseases, three specific metabolomic features belonging to the lipid classes of SMs, triglycerides and phosphatidylcholines together with N-terminal pro-B-type natriuretic peptide (NT-proBNP) distinguished patients with HF from healthy controls.[51] In addition, the diagnostic accuracy of this combination was significantly superior compared with the diagnostic accuracy of NT-proBNP alone.[51]

Conclusion

Dysregulation of sphingolipid synthesis and transport is associated with cardiovascular disease, diabetes and other metabolic disorders. However, further studies are needed to identify the molecular and pathophysiological pathways by which certain sphingolipids species are associated with different pathologies. ■

1. Breslow DK, Weissman JS. Membranes in balance: mechanisms of sphingolipid homeostasis. *Mol Cell* 2010;**40**:267–79. https://doi.org/10.1016/j.molcel.2010.10.005; PMID: 20965421.
2. Barenholz Y, Thompson TE. Sphingomyelins in bilayers and biological membranes. *Biochim Biophys Acta* 1980;**604**:129–58. https://doi.org/10.1016/0005-2736(80)90572-6; PMID: 7000188.
3. Slotte JP. Molecular properties of various structurally defined sphingomyelins -- correlation of structure with function. *Prog Lipid Res* 2013;**52**:206–19. https://doi.org/10.1016/j.plipres.2012.12.001; PMID: 23295259.
4. Thudicum JL. A treatise on the chemical constitution of brain. London: Bailliere, Tindall and Cox, 1884.
5. Pick L, Bielschowsky M. Verhandlungen ärztlicher gesellschaften. *Klin Wochenschr* 1927;**6**:1631–7 [in German]. https://doi.org/10.1007/BF01467780.
6. Testi R. Sphingomyelin breakdown and cell fate. *Trends Biochem Sci* 1996;**21**:468–71. https://doi.org/10.1016/S0968-0004(96)10056-6; PMID: 9009829.
7. Tafesse FG, Ternes P, Holthuis JC. The multigenic sphingomyelin synthase family. *J Biol Chem* 2006;**281**:29,421–5. https://doi.org/10.1074/jbc.R600021200; PMID: 16905542.
8. D'Avanzo N. Lipid regulation of sodium channels. *Curr Top in Membr* 2016;**78**:353–407. https://doi.org/10.1016/bs.ctm.2016.04.003 PMID: 27586290.
9. Stoffel W, Bosi A. Myelin glycolipids and their functions. *Curr Opin. Neurobiol* 1997;**7**:654–61. https://doi.org/10.1016/S0959-4388(97)80085-2; PMID: 9384539.
10. Voet DJ, Voet JG, Pratt CW. Lipids, Bilayers and Membranes. In: *Principles of Biochemistry*. 3rd ed. New York: Wiley, 2008.
11. Patton S. Correlative relationship of cholesterol and sphingomyelin in cell membranes. *J Theor Biol* 1970;**29**:489–91. https://doi.org/10.1016/0022-5193(70)90111-6; PMID: 5492999.
12. Slotte JP. Biological functions of sphingomyelins. *Prog Lipid Res* 2013;**52**:424–37. https://doi.org/10.1016/j.plipres.2013.05.001; PMID: 23684760.
13. Gatt S, Bierman EL. Sphingomyelin suppresses the binding and utilization of low density lipoproteins by skin fibroblasts. *J Biol Chem* 1980;**255**:3371–6. PMID: 7364747.
14. Slotte JP. Molecular properties of various structurally defined sphingomyelins – correlation of structure with function. *Prog Lipid Res* 2013;**52**:206–19. https://doi.org/10.1016/j.plipres.2012.12.001; PMID: 23295259.
15. Welty FK. Hypobetalipoproteinemia and abetalipoproteinemia. *Curr Opin Lipidol* 2014;**25**:161–8. https://doi.org/10.1097/MOL.0000000000000072; PMID: 24751931.
16. Deng X, Yin X, Allan R, et al. Ceramide biogenesis is required for radiation-induced apoptosis in the germ line of C. elegans. *Science* 2008;**322**:110–5. https://doi.org/10.1126/science.1158111; PMID: 18832646.
17. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat Rev Mol Cell Biol* 200;**4**:397–407. https://doi.org/10.1038/nrm1103; PMID: 12728273.
18. Fyrst H, Saba JD. An update on sphingosine-1-phosphate and other sphingolipid mediators. *Nat Chem Biol* 2010;**6**:489–97. https://doi.org/10.1038/nchembio.392; PMID: 20559316.
19. Kolesnick RN. Sphingomyelin and derivatives as

cellular signals. *Prog Lipid Res* 1991;**30**:1–38. https://doi.org/10.1016/0163-7827(91)90005-P; PMID: 1771169.
20. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science* 2010;**327**:46–50. https://doi.org/10.1126/science.1174621; PMID: 20044567.
21. Lippincott-Schwartz J, Phair RD. Lipids and cholesterol as regulators of traffic in the endomembrane system. *Annu Rev Biophys* 2010;**39**:559–78. https://doi.org/10.1146/annurev.biophys.093008.131357; PMID: 20192772.
22. Lopez PH, Schnaar RL. Gangliosides in cell recognition and membrane protein regulation. *Curr Opin Struct Biol* 2009;**19**:549–57. https://doi.org/10.1016/j.sbi.2009.06.001; PMID: 19608407.
23. Tsai B, Gilbert JM, Stehle T, et al. Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J* 2003;**22**:4346–55. https://doi.org/10.1093/emboj/cdg439; PMID: 12941687.
24. Baron CL, Malhotra V. Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane. *Science* 2002;**295**:325–8. https://doi.org/10.1126/science.1066759; PMID: 11729268.
25. Holthuis JC, Pomorski T, Raggers RJ, et al. The organizing potential of sphingolipids in intracellular membrane transport. *Physiol Rev* 2001;**81**:1689–723. https://doi.org/10.1152/physrev.2001.81.4.1689; PMID: 11581500.
26. Jaikishan S, Slotte JP. Effect of hydrophobic mismatch and interdigitation on sterol / sphingomyelin interaction in ternary bilayer membranes. *Biochim Biophys Acta* 2011;**1808**:1940–5. https://doi.org/10.1016/j.bbamem.2011.04.004; PMID: 21515240.
27. Edsfeldt A, Dunér P, Ståhlman M, et al. Sphingolipids contribute to human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol* 2016;**36**:1132–40. https://doi.org/10.1161/ATVBAHA.116.305675; PMID: 27055903.
28. Bojic LA, McLaren DG, Shah V, et al. Lipidome of atherosclerotic plaques from hypercholesterolemic rabbits. *Int J Mol Sci* 2014;**15**:23283–93. https://doi.org/10.3390/ijms151223283; PMID: 25517033.
29. Chatterjee M, Rath D, Schlotterbeck J, et al. Regulation of oxidized platelet lipidome: implications for coronary artery disease. *Eur Heart J* 2017;**38**:1993–2005. https://doi.org/10.1093/eurheartj/ehx146; PMID: 28431006.
30. Fernandez C, Sandin M, Sampaio JL, et al. Plasma lipid composition and risk of developing cardiovascular disease. *PLoS One* 2013;**8**:e71846. https://doi.org/10.1371/journal.pone.0071846; PMID: 23967253.
31. Pan W, Yu J, Shi R, et al. Elevation of ceramide and activation of secretory acid sphingomyelinase in patients with acute coronary syndromes. *Coron Artery Dis* 2014;**25**:230–5. https://doi.org/10.1097/MCA.0000000000000079; PMID: 24589572.
32. Yeboah J, McNamara C, Jiang XC, et al. Association of plasma sphingomyelin levels and incident coronary heart disease events in an adult population: Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010;**30**:628–33. https://doi.org/10.1161/ATVBAHA.109.199281; PMID: 20032291.
33. Michel JB, Martin-Ventura JL, Nicoletti A, Ho-Tin-Noé B. Pathology of human plaque vulnerability: mechanisms and consequences of intraplaque haemorrhages. *Atherosclerosis* 2014;**234**:311–9. https://doi.org/10.1016/j.atherosclerosis.2014.03.020; PMID: 24726899.
34. Zhang J, Tu K, Xu Y, et al. Sphingomyelin in erythrocyte membranes increases the total cholesterol content of erythrocyte membranes in patients with acute coronary syndrome. *Coron Artery Dis* 2013;**24**:361–7. https://doi.org/10.1097/MCA.0b013e328362228f; PMID: 23652364.
35. Makinen VP, Tynkkynen T, Soininen P, et al. Sphingomyelin is associated with kidney disease in type 1 diabetes (The FinnDiane Study). *Metabolomics* 2012;**8**:369–75. https://doi.org/10.1007/s11306-011-0343-y; PMID: 22661917.
36. Xu F, Tavintharan S, Sum CF, et al. Metabolic signature shift in type 2 diabetes mellitus revealed by mass spectrometry-based metabolomics. *J Clin Endocrinol Metab* 2013;**98**:E1060–5. https://doi.org/10.1210/jc.2012-4132; PMID: 23633210.
37. Iqbal J, Walsh MT, Hammad SM, et al. sphingolipids and lipoproteins in health and metabolic disorders. *Trends Endocrinol Metab* 2017;**28**:506–18. https://doi.org/10.1016/j.tem.2017.03.005; PMID: 28462811.
38. Camont L, Lhomme M, Rached F, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. *Arterioscler Thromb Vasc Biol* 2013;**33**:2715–23. https://doi.org/10.1161/ATVBAHA.113.301468; PMID: 24092747.
39. Kontush A, Therond P, Zerrad A, et al. Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. *Arterioscler Thromb Vasc Biol* 2007;**27**:1843–9. https://doi.org/10.1161/ATVBAHA.107.145672; PMID: 17569880.
40. Guyton JR, Klemp KF. Development of the lipid-rich core in human atherosclerosis *Arterioscler Thromb Vasc Biol* 1996;**16**:4–11. https://doi.org/10.1161/01.ATV.16.1.4; PMID: 8548424.
41. Bottcher CJ, Vangent CM. Changes in the composition of phospholipids and of phospholipid fatty acids associated with atherosclerosis in the human aortic wall. *J Atheroscler Res* 1961;**1**:36–46. https://doi.org/10.1016/S0368-1319(61)80052-5.
42. Schissel SL, Tweedie-Hardman J, Rapp JH, et al. Rabbit aorta and human atherosclerotic lesions hydrolyze the sphingomyelin of retained low-density lipoprotein. Proposed role for arterial-wall sphingomyelinase in subendothelial retention and aggregation of atherogenic lipoproteins. *J Clin Invest* 1996;**98**:1455–64. https://doi.org/10.1172/JCI118934; PMID: 8823312.
43. Lehti S, Käkelä R, Hörkkö S, et al. Modified lipoprotein-derived lipid particles accumulate in human stenotic aortic valves. *PLoS One* 2013;**8**:e65810. https://doi.org/10.1371/journal.pone.0065810; PMID: 23762432.
44. Doppler C, Arnhard K, Dumfarth J, et al. Metabolomic profiling of ascending thoracic aortic aneurysms and dissections - Implications for pathophysiology and biomarker discovery. *PLoS One* 2017;**12**:e0176727. https://doi.org/10.1371/journal.pone.0176727; PMID: 28467501.
45. McGovern MM, Wasserstein MP, Giugliani R, et al. A prospective, cross-sectional survey study of the natural history of Niemann-Pick disease type B. *Pediatrics* 2008;**122**:e341–9. https://doi.org/10.1542/peds.2007-3016; PMID: 18625664.
46. Fotoulaki M, Schuchman EH, Simonaro CM, et al. Acid sphingomyelinase-deficient Niemann-Pick disease: novel findings in a Greek child. *J Inherit Metab Dis* 2007;**30**:986. https://doi.org/10.1007/s10545-007-0557-3; PMID: 17876723.
47. Nilsson A, Duan RD. Absorption and lipoprotein transport of sphingomyelin. *J Lipid Res* 2006;**47**:154–71. https://doi.org/10.1194/jlr.M500357-JLR200; PMID: 16251722.
48. Klevstig M, Ståhlman M, Lundqvist A, et al. Targeting acid sphingomyelinase reduces cardiac ceramide accumulation in the post-ischemic heart. *J Mol Cell Cardiol* 2016;**93**:69–72. https://doi.org/10.1016/j.yjmcc.2016.02.019; PMID: 26930027.
49. Park TS, Hu Y, Noh HL, et al. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res* 2008;**49**:2101–12. https://doi.org/10.1194/jlr.M800147-JLR200; PMID: 18515784.
50. Zordoky BN, Sung MM, Ezekowitz J, et al. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PLoS One* 2015;**10**:e0124844. https://doi.org/10.1371/journal.pone.0124844; PMID: 26010610.
51. Mueller-Hennessen M, Düngen HD, Lutz M, et al. A novel lipid biomarker panel for the detection of heart failure with reduced ejection fraction. *Clin Chem* 2017;**63**:267–77. https://doi.org/10.1373/clinchem.2016.257279; PMID: 28062623.