Circulation

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Ceramides and Atherosclerotic Cardiovascular Disease: A Current Perspective

Qingzhang Zhu, PhD; Philipp E. Scherer, PhD

Cardiovascular disease has been a leading cause of mortality worldwide since the 1950s, currently with an estimated 17.9 million deaths annually. Early assessment of atherosclerotic cardiovascular disease (ASCVD) risk is critical for preventive interventions. Advances in plasma lipidomics have highlighted circulating ceramides as risk predictors for ASCVD. Ceramides, a class of sphingolipids, play a role beyond mere structural components of biomembranes; they are bioactive molecules involved in diverse cellular processes, including apoptosis, mitochondrial damage, inflammation, and insulin resistance (Figure).

Ceramides contain a sphingosine backbone and a fatty acid moiety linked by an amide bond. Understanding how ceramide homeostasis is regulated at the cellular level and systemically is necessary for appreciating the role of ceramides in the pathophysiology of ASCVD and for developing specific therapeutic interventions.[1] At the cellular level, 3 aspects are critical concerning the specific ceramide effects: the species, the location, and the signaling potential. Ceramide species are determined by 6 ceramide synthases (CerS1–6), exhibiting cell-specific expression patterns and incorporating distinct fatty acyl-CoAs. Despite some promiscuity in ceramide synthase specificity, the enzymatic reactions are largely substrate driven. These pathways originate in distinct subcellular compartments, resulting in high local ceramide concentrations due to the biophysical properties of both acceptors and donors of hydrogen bonds. In the myocardium, ceramide overload compromises mitochondrial function and triggers inflammation and apoptosis, leading to heart failure. It is interesting that ceramides can be generated in mitochondria through specific ceramide synthases in an "organelle autonomous" manner. Moreover, ceramides initiate distinct molecular signaling events and play distinct roles, depending on their species and the context. Therefore, examining imbalances in local ceramide levels and their association with cellular dysfunction (ie, mitochondrial function) and intercellular communication (eg, between vascular smooth muscle cells, endothelial cells, and macrophages) is critical.

CERAMIDE-BASED RISK SCORES

Ceramide scores such as Coronary Event Risk Test (CERT) 1 and CERT2 (scale 0–12 in 4 risk categories) have been developed. Distinct from classic risk factors, CERT1 relies on 4 plasma ceramide measures and their ratios, whereas CERT2 incorporates additional phosphatidylcholines (Figure). These models have been validated in several large-scale studies for primary and secondary ASCVD risk stratification. For instance, the FINRISK study monitors risk factors in non-communicable diseases in the Finnish population every 5 years since 1972. In the 2002 FINRISK study involving 7324 primary prevention individuals, both CERT2 and CERT1 were strongly linked to ASCVD incidence (hazard ratio, 1.49 versus 1.35) and cardiovascular death (hazard ratio,1.72 versus 1.52), resulting in a clinical net reclassification improvement of 7.5% compared with cholesterol-based biomarkers such as low-density lipoprotein cholesterol (hazard ratio, 1.13 and 1.30 for ASCVD incidence and cardiovascular death, respectively).[2] A recent meta-analysis of ≈27 000 patients

Key Words: cardiovascular diseases ■ ceramides ■ risk factors

Correspondence to: Philipp E. Scherer, PhD, University of Texas Southwestern Medical Center, Touchstone Diabetes Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8549. Email philipp.scherer@utsouthwestern.edu

For Sources of Funding and Disclosures, see page 1626.

Circulation is available at www.ahajournals.org/journal/circ

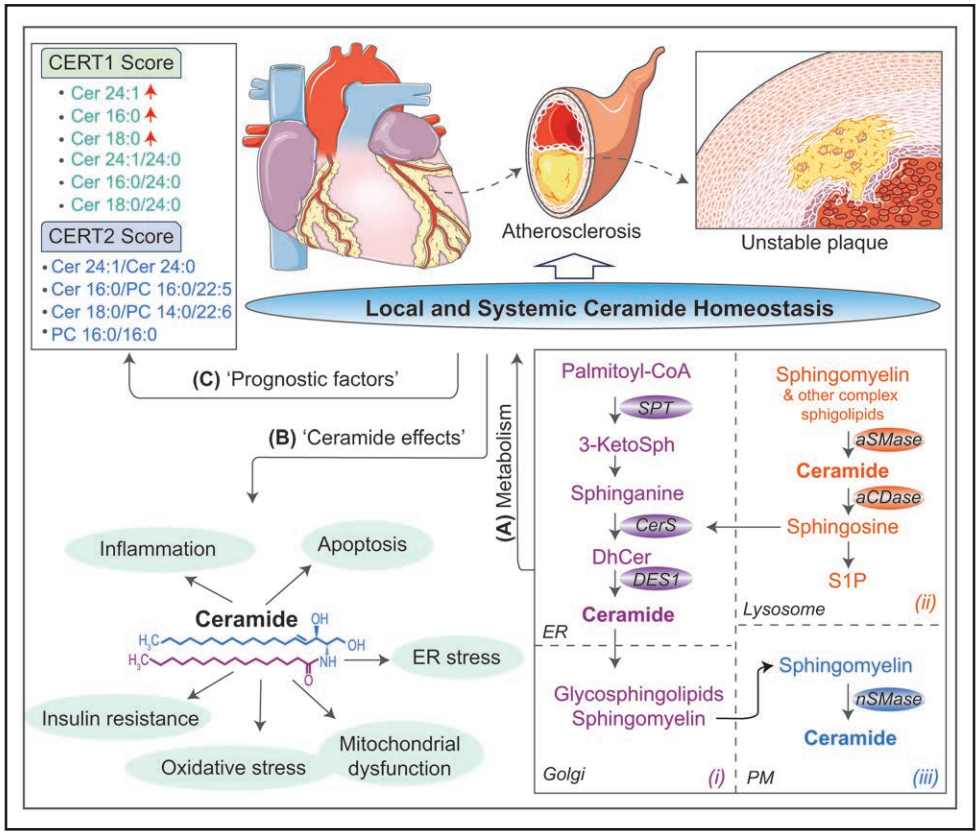

Figure. Ceramide homeostasis in atherosclerosis.
Ceramide (Cer) metabolism (**A**). Ceramide generation is initiated in separate cellular compartments from 3 distinct pathways: (**i**) de novo biosynthesis, which occurs in endoplasmic reticulum (ER), that can further form sphingomyelin and other complex glycosphingolipids in the Golgi apparatus; (**ii**) the salvage pathway, which recycles the sphingosine backbone by degrading the complex sphingolipids in the lysosome; and (**iii**) sphingomyelin hydrolysis, occurring mainly in the plasma membrane (PM). Key rate-limiting enzymes in the metabolic pathways that may serve as potential therapeutic targets are indicated. Ceramide effects (**B**). In cells, ceramide accumulation induces multiple negative effects depending on the species, location, and signaling capacity of the molecules. Ceramides as prognostic factors (**C**). Disruption of ceramide metabolism alters their cellular levels locally and in circulation. Several circulating ceramide species, including Cer 24:1, Cer 16:0, and Cer 18:0, are increased in cardiovascular disease. These circulating ceramide species, along with their ratios normalized to Cer 24:0 (as reflected in the Coronary Event Risk Test [CERT] 1 score) and levels of critical phosphatidylcholines (as captured by the CERT2 score), serve as powerful predictors for atherosclerotic cardiovascular disease risk. Ceramides contain 1 sphingosine (blue) and 1 fatty acid residue (purple) with variable lengths and degrees of saturation. 3-KetoSph, 3-ketosphinganine; aCDase indicates acid ceramidase; aSMase, acid sphingomyelinase; CerS, ceramide synthase; DES1, dihydroceramide desaturase 1; DhCer, dihydroceramide; nSMase, neutral sphingomyelinase; PC, phosphatidylcholine; S1P, sphingosine-1-phosphate; and SPT, serine palmitoyl transferase. Portions of this figure were created with pictures from Servier Medical Art (https://smart.servier.com) using a Creative Commons Attribution 3.0 unported license.

with established coronary artery disease reaffirmed the prognostic value of CERT2.[3] In this study, a significantly higher risk of major adverse atherosclerotic cardiovascular outcomes (defined as myocardial infarction, stroke, or cardiovascular death) was projected when CERT2 score was >3. It is important to note that ceramide scores offer a complementary tool in residual ASCVD risk prediction. Integrating CERT2 with other risk predictors or risk assessment models such as high-sensitivity troponin assays further improves the prognostic potential.[2,3]

Although the associations between plasma ceramides and ASCVD are strong, a direct causal relationship remains to be established. Understanding the process of ceramide release into the circulation and the regulation of local ceramide levels under metabolic stress is essential. As highly hydrophobic lipids, ceramide secretion from cells requires protein carriers. Conventionally, the liver has been considered a major source of plasma ceramides because they are transported by very-low-density lipoprotein and low-density lipoprotein particles. However, recent studies reveal small extracellular vesicles (secreted lipid-bound small particles), particularly from adipose tissue, as an important source of circulating ceramides.[4] Ceramide abundance in small extracellular vesicles varies across different fat depots, and small extracellular vesicle–based ceramides, derived particularly from thoracic fat, influence the vascular redox state and correlate with a worse outcome in obese patients with atherosclerosis. Furthermore, the release rate of small extracellular vesicles depends on the functional

integrity of adipocyte mitochondria. This aspect is particularly relevant in the context of local paracrine crosstalk between the heart and epicardial and pericardial adipose tissue.

TARGETING CERAMIDE PATHWAYS IN ASCVD

Although still in early stages of development, strategies aimed at lowering ceramide levels, by either inhibiting biogenesis or enhancing degradation, offer valuable therapeutic opportunities. Adiponectin, a hormone originating from adipose tissue, has broad benefits, including insulin-sensitizing, anti-inflammatory, and antifibrotic effects.[1] It also displays cardioprotective roles by increasing the intrinsic ceramidase activity of its receptors AdipoR1 and AdipoR2, thereby reducing cellular ceramide stress. Therapeutically, overexpressing acid ceramidase in the heart by synthetic modified mRNA reduces cardiac ceramide species, alleviating inflammation and apoptosis; it also improves heart function and survival rate in a mouse model of myocardial infarction.[5] Moreover, targeting other key enzymes in the ceramide pathway, including serine palmitoyltransferase, ceramide synthases, dihydroceramide desaturase 1, and sphingomyelinase, is a promising therapeutic avenue according to preclinical models.[1] Although no clinical trials specifically targeting the sphingolipid pathway in ASCVD are underway, the ongoing SphingoFIT trial (Reducing Circulating Sphingolipid Levels to Optimise Cardiometabolic Health; ClinicalTrials.gov NCT06024291) aims to enhance cardiometabolic health by reducing circulating sphingolipid levels. It is interesting that antidiabetic sodium/glucose cotransporter 2 inhibitors and low-density lipoprotein cholesterol–lowering drugs such as statins and proprotein convertase subtilisin/kexin type 9 inhibitors also reduce ceramide levels in the heart or in circulation while concurrently reducing cardiovascular events.[1] These effects may be due to improvements in ceramide-mediated insulin resistance and dyslipidemia.

In summary, with ceramide scores showing exciting prognostic value, further research is needed to elucidate the mechanisms by which local and circulating ceramides contribute to the pathogenesis of atherosclerosis and whether strategies to improve the ceramide profile can directly benefit ASCVD. Ceramide lowering may provide additional benefits when combined with current interventions. Given the heterogeneity of the ceramide species and their complex biogenesis, interventions must be carefully analyzed. However, given the clinical correlations and promising preclinical data, ceramide-lowering approaches hold significant potential for the future of ASCVD interventions.

ARTICLE INFORMATION

Affiliation

Touchstone Diabetes Center, University of Texas Southwestern Medical Center, Dallas.

Sources of Funding

The authors are supported by US National Institutes of Health grants RC2-DK118620, R01-DK55758, R01-DK099110, R01-DK127274, P01-AG051459, and R01-DK131537 to Dr Scherer and American Heart Association Career Development Award 855170 to Dr Zhu.

Disclosures

None.

REFERENCES

1. Choi RH, Tatum SM, Symons JD, Summers SA, Holland WL. Ceramides and other sphingolipids as drivers of cardiovascular disease. *Nat Rev Cardiol*. 2021;18:701–711. doi: 10.1038/s41569-021-00536-1
2. Hilvo M, Jylhä A, Lääperi M, Jousilahti P, Laaksonen R. Absolute and relative risk prediction in cardiovascular primary prevention with a modified SCORE chart incorporating ceramide-phospholipid risk score and diabetes mellitus. *Eur Heart J Open*. 2021;13:oeab010. doi: 10.1093/ehjopen/oeab010
3. Papazoglou AS, Stalikas N, Moysidis DV, Otountzidis N, Kartas A, Karagiannidis E, Giannakoulas G, Sianos G. CERT2 ceramide- and phospholipid-based risk score and major adverse cardiovascular events: a systematic review and meta-analysis. *J Clin Lipidol*. 2022;16:272–276. doi: 10.1016/j.jacl.2022.02.001
4. Akawi N, Checa A, Antonopoulos AS, Akoumianakis I, Daskalaki E, Kotanidis CP, Kondo H, Lee K, Yesilyurt D, Badi I, et al. Fat-secreted ceramides regulate vascular redox state and influence outcomes in patients with cardiovascular disease. *J Am Coll Cardiol*. 2021;77:2494–2513. doi: 10.1016/j.jacc.2021.03.314
5. Hadas Y, Vincek AS, Youssef E, Żak MM, Chepurko E, Sultana N, Sharkar MTK, Guo N, Komargodski R, Kurian AA, et al. Altering sphingolipid metabolism attenuates cell death and inflammatory response after myocardial infarction. *Circulation*. 2020;141:916–930. doi: 10.1161/CIRCULATIONAHA.119.041882