How ceramides orchestrate cardiometabolic health—an ode to physically active living

Review Article

Author(s):
Carrard, Justin; Gallart-Ayala, Hector; Weber, Nadia; Colledge, Flora; Streese, Lukas; Hanssen, Henner; Schmied, Christian; Ivanisevic, Julijana; Schmidt-Trucksäss, Arno

Review

How Ceramides Orchestrate Cardiometabolic Health—An Ode to Physically Active Living

Justin Carrard [1,*], Hector Gallart-Ayala [2], Nadia Weber [3], Flora Colledge [4], Lukas Streese [1], Henner Hanssen [1], Christian Schmied [5], Julijana Ivanisevic [2,†] and Arno Schmidt-Trucksäss [1,†]

1   Division of Sports and Exercise Medicine, Department of Sport, Exercise and Health, University of Basel, Birsstrasse 320B, 4052 Basel, Switzerland; lukas.streese@unibas.ch (L.S.); henner.hanssen@unibas.ch (H.H.); arno.schmidt-trucksaess@unibas.ch (A.S.-T.)
2   Metabolomics Platform, Faculty of Biology and Medicine, University of Lausanne, Quartier UNIL-CHUV, Rue du Bugnon 19, 1005 Lausanne, Switzerland; hector.gallartayala@unil.ch (H.G.-A.); julijana.ivanisevic@unil.ch (J.I.)
3   Medical School, Department of Health Sciences and Technology, Swiss Federal Institute of Technology, Universitätstrasse 2, 8092 Zurich, Switzerland; naweber@student.ethz.ch
4   Division of Sports Science, Department of Sport, Exercise and Health, University of Basel, Birsstrasse 320B, 4052 Basel, Switzerland; flora.colledge@unibas.ch
5   Sports Cardiology Section, Department of Cardiology, University Heart Center Zurich, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland; christian.schmied@usz.ch
*   Correspondence: justin.carrard@unibas.ch
†   Last authors, equally contributed.

Abstract: Cardiometabolic diseases (CMD) represent a growing socioeconomic burden and concern for healthcare systems worldwide. Improving patients' metabolic phenotyping in clinical practice will enable clinicians to better tailor prevention and treatment strategy to individual needs. Recently, elevated levels of specific lipid species, known as ceramides, were shown to predict cardiometabolic outcomes beyond traditional biomarkers such as cholesterol. Preliminary data showed that physical activity, a potent, low-cost, and patient-empowering means to reduce CMD-related burden, influences ceramide levels. While a single bout of physical exercise increases circulating and muscular ceramide levels, regular exercise reduces ceramide content. Additionally, several ceramide species have been reported to be negatively associated with cardiorespiratory fitness, which is a potent health marker reflecting training level. Thus, regular exercise could optimize cardiometabolic health, partly by reversing altered ceramide profiles. This short review provides an overview of ceramide metabolism and its role in cardiometabolic health and diseases, before presenting the effects of exercise on ceramides in humans.

Keywords: ceramides; sphingolipids; cardiometabolic health; cardiovascular health; metabolism; insulin; physical activity; exercise; cardiorespiratory fitness

1. Introduction

According to the Global Burden of Diseases, Injuries and Risk Factor Study, the rate of mortality from noncommunicable diseases (NCD) has increased from 1990 to 2019, to account for 74% of total mortality [1,2]. Strikingly, about one-half of NCD-related deaths are still attributable to cardiometabolic diseases (CMD), encompassing cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) [3]. Whereas age-standardized death rates for CMD have decreased since 1990 in high-income countries, absolute number of CMD-related deaths are still increasing in these regions, mainly due to population aging [4]. Simultaneously, about two thirds of all CMD-related deaths are now occurring in low- and middle-income countries [5]. To combat the growing burden of CMD worldwide, it is urgent to improve patients' stratification with respect

to cardiometabolic health decline and CMD onset, which will enable the development of more precise and personalized prevention and treatment strategies [5].

Blood lipid analysis has been part of cardiometabolic risk stratification strategy since the middle of the last century [6]. Despite technological advances in mass-spectrometry and bioinformatics, clinical lipid analysis is still mostly restricted to total triglycerides, cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) [7,8]. Although these biomarkers provide an acceptable risk assessment, growing evidence shows that lower-abundant specific sphingolipid species, known as ceramides, predict cardiometabolic outcomes more precisely in patients with and without coronary artery disease [9–12]. As ceramides could eventually replace cholesterol in clinical practice [13], it is important to determine whether and how physical activity, as a powerful prevention and treatment strategy, can influence ceramide levels.

Regular physical activity is, indeed, essential to maintain general health [14–17], prevent and treat CVD [18–21], insulin resistance and T2DM [22–24] as well as NAFLD [25]. Adding to its appeal, physical activity is a simple, low-cost and patient-empowering means to reduce CMD-related burden in low-income, middle-income, and high-income countries [26]. Exercise, as a subset of physical activity that is structured in order to improve or maintain physical fitness [27], not only mitigates traditional risk factors for CVD but also directly improves cardiometabolic health. For instance, exercise optimizes vascular endothelial function, stimulates secretion of cardioprotective myokines, but also acts through other mechanisms not fully elucidated yet, which might include changes in ceramide metabolism [19]. This short review provides an overview on ceramide metabolism and its role in human cardiometabolic health and diseases before presenting the effects of physical exercise on ceramides.

2. Ceramide Metabolism

Ceramides are synthesized through three major metabolic pathways: the de novo pathway that mainly results from the combination of serine and palmitoyl-CoA in the endoplasmic reticulum, the salvage pathway, which consists of the breakdown of sphingomyelins or glycosphingolipids in lysosomes, and the sphingomyelinase pathway, where sphingomyelins are converted into ceramides in cell membrane (Figure 1) [28,29]. Due to the specific subcellular localization of the enzymes controlling ceramides metabolism, the latter is highly compartmentalized [29].

In the Golgi apparatus, ceramides are transformed into various complex sphingolipids such as ceramide-1-phosphate, sphingomyelin or glycosphingolipids [28,29] Ceramides can also be acylated to form acylceramides, which accumulate in intracellular lipid droplets [29,30]. Finally, the only 'exit' from ceramide metabolism results in the production of fatty aldehydes and ethanolamine phosphate by the action of a sphingosine-1-phosphate (S1P) lyase on sphingoid base phosphates [29].

Less frequently, substrates other than serine and palmitoyl-CoA, such as alanine or glycine plus stearate or myristate, can also enter the de novo pathway [29]. This leads to the formation of structurally different sphingoid bases, namely deoxysphingoid and 1-deoxymethyl sphingoid bases [29]. Distinct isoforms of ceramide synthases also add variations in the acyl groups of sphingoid bases, which further increases diversity and complexity of ceramides structures [29]. Additionally, hydroxylases and desaturases can modify acyl groups; thus, ceramides should be regarded as a family of related but structurally distinct lipid species [29]. These structural variations are at the origin of the distinct biological functions attributed to the different ceramide species [29].
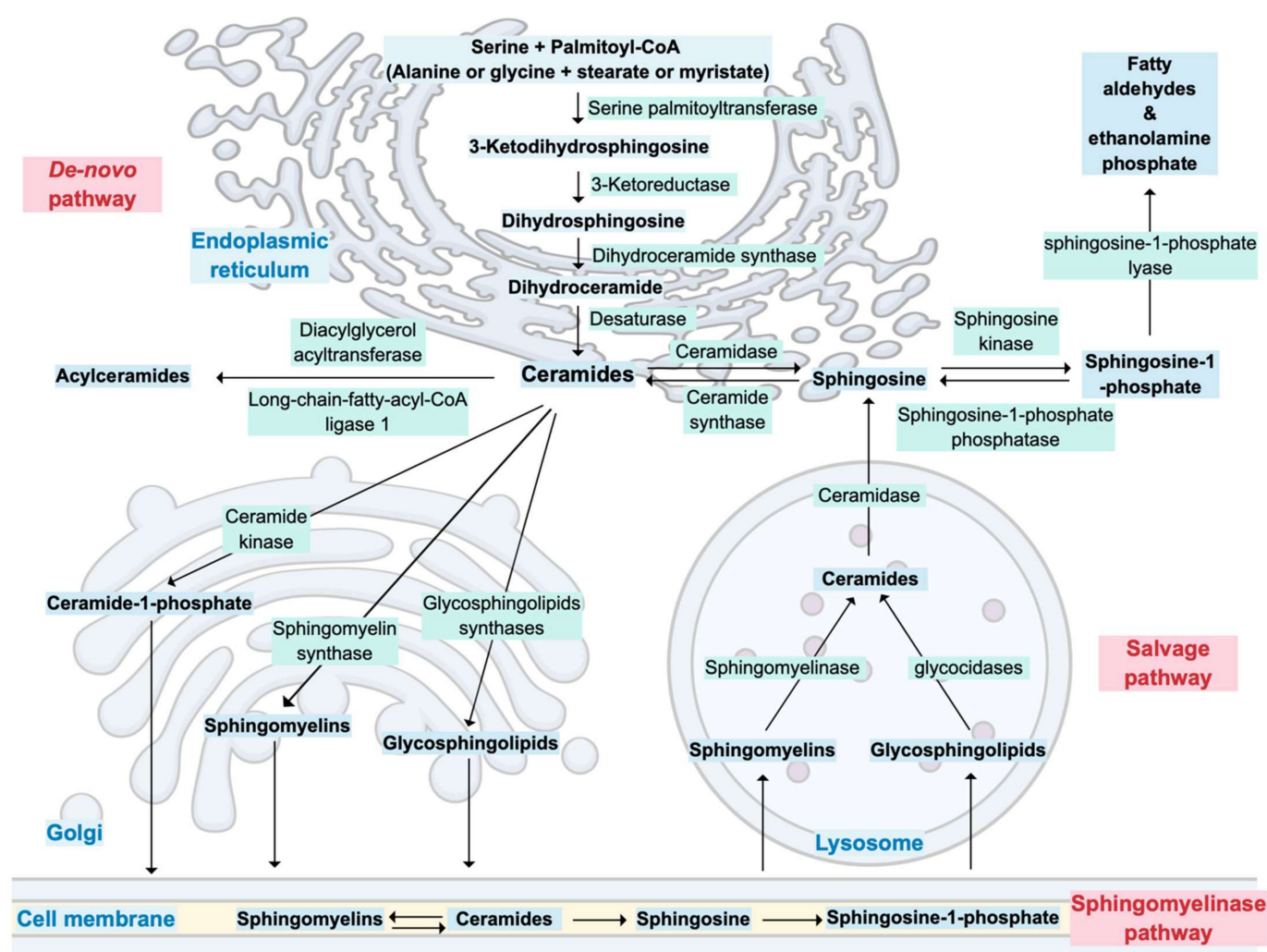
Figure 1. Overview of ceramide metabolism and its cellular compartmentalization.

3. Ceramides in Cardiometabolic Health and Diseases

Ceramides and their derivatives are involved in many, if not all, essential cellular processes such as cell growth, cell adhesion and migration, senescence, apoptosis, inflammation, immune responses, and angiogenesis [28,29]. They are also part of cell membrane, where they influence vesicular transport, membrane receptors, and exosome secretion [29]. Thus, ceramides are implicated in both intracellular and cell-to-cell signaling [31]. Ceramides synthesis and accumulation are influenced by multiple factors, which include excessive supply of substrates, systemic inflammation, oxidative stress and the microbiome [31]. In obesity, levels of Tumor Necrosis Factor-Alpha (TNF-α) are elevated, and Toll-like receptors (TLRs) are stimulated by excess of fatty acids [32]. Both TNF-α and TLRs can stimulate ceramide synthesis enzymes [32]. Ceramides will then activate inflammasomes (specifically the NLRP3 inflammasome in adipocytes), which lead to an increase in pro-inflammatory cytokine secretion [32]. This inflammatory response can be modulated by the adipokine adiponectin through activation of the ceramidase activity of the adiponectin receptors [33]. Therefore, the many roles played by ceramides in cardiometabolic health and diseases are associated with this connection between excessive supply of lipids and inflammation [32].

Changes in ceramide levels have been observed in diverse pathological processes such as NAFLD [34,35], obesity [36,37], insulin resistance [38,39], vascular inflammation, and atherosclerosis (Figure 2) [40–42]. Patients suffering from NAFLD display elevated levels of circulating and liver ceramides [34]. Hepatocellular ceramides stimulate fatty acid

uptake, by increasing the translocation of fatty acid transporters to the plasma membrane, and facilitate their transformation into intracellular triglycerides [34]. Ceramides also decrease glucose uptake, by inhibiting Akt/protein kinase B (PKB) activity, which inhibits translocation of glucose transporters (GLUT4) to the cell membrane and reduce hepatic gluconeogenesis [34,39,43]. This enables hepatocytes to favor fatty acids over glucose as a preferred energy source and leads to NAFLD [34].
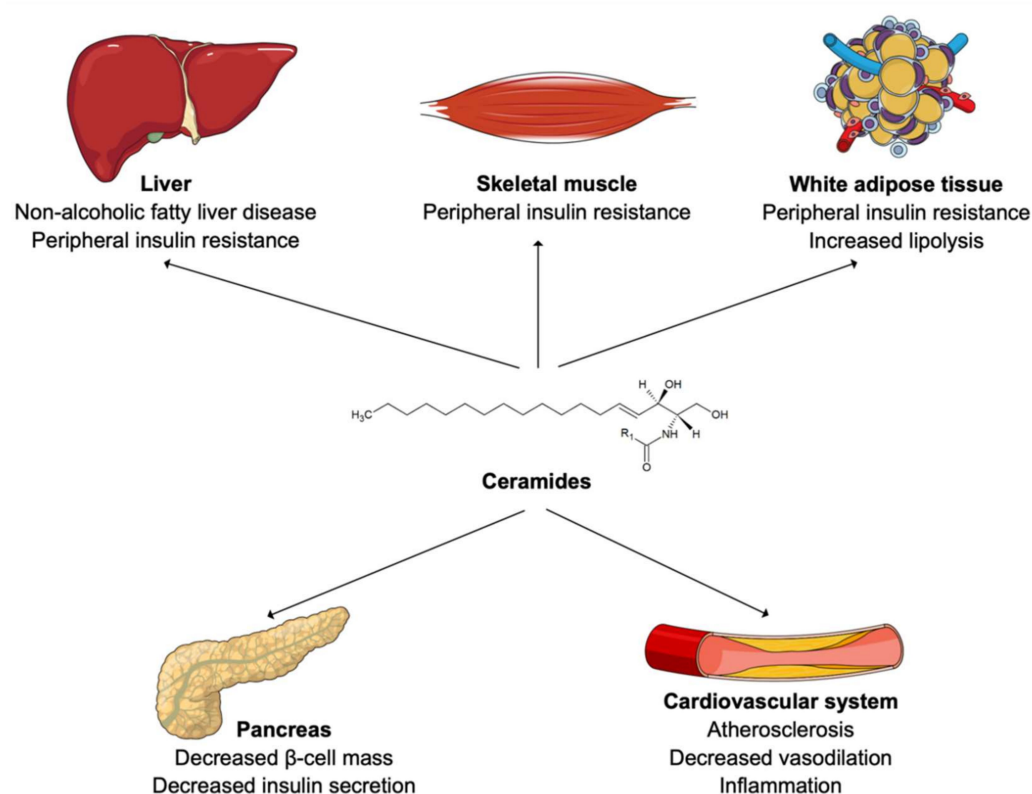

Figure 2. Ceramides in human cardiometabolic diseases.

Elevated circulating ceramide levels have been reported in obese patients with T2DM [44,45]. Furthermore, LDL were shown to be enriched in ceramides in lean patients with T2DM compared to healthy individuals [46]. In fact, an accumulation of ceramides in muscle cells and adipocytes also inhibits Akt/PKB, reduces GLUT4 translocation to the membrane, and ultimately decreases glucose uptake [43,47,48]. In adipocytes, ceramides also slow down fatty acid oxidation by inhibiting specific hormone-sensitive lipases [49]. This results in peripheral insulin resistance [42]. In addition, ceramide accumulation is believed to induce β-cell dysfunction and reduce insulin secretion [50,51]. Looking at species level, long-chain ceramides, such as Cer (18:1;2/16:0) or Cer (18:1;2/18:0), have been shown to correlate the most with insulin resistance and NAFLD [52,53]. Treatment with the glucagon-like peptide-1 receptor agonist liraglutide was recently shown to reduce ceramide levels in patient with T2DM [54]. The authors of this study then hypothesized that the cardiovascular benefit of this treatment observed within patients with T2DM might be partially due to this reduction in ceramide content [54].

From a cardiovascular perspective, ceramides located at the surface of LDL drive their transcytosis through the endothelium and uptake into macrophages, which results in foam cell formation, vascular inflammation, and atherosclerosis [40–42]. Thoracic adipose tissue of obese individuals secretes a particular ceramide species, named Cer (18:1;2/16:0), which acts on endothelial cells to reduce vasodilatation, induce inflammation, oxidative stress, and finally increase risk of cardiovascular death [55]. Again, circulating long-chain ceramides, such as Cer (18:1;2/16:0), Cer (18:1;2/18:0) and Cer (18:1;2/24:1), seem to be the species

most frequently associated with cardiovascular morbidity and mortality [10,31,56]. Statin therapy showed conflicting results on ceramide levels. A recent study reported no reduction in absolute ceramide content within LDL particles, or even an increase of its relative concentration as other lipid species composing LDL, such as lysophospholipids, were reduced [57]. The authors of this study hypothesized that ceramides might be responsible for residual cardiovascular risk on statin therapy [57]. Conversely, other studies found a decrease in circulating ceramide levels on simvastatin or rosuvastatin therapy [58,59] as well as on proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [60]. Ezetimibe therapy did not lead to a reduction in circulating ceramides [59]. Thus, larger population trials are necessary to unravel effects of lipid-lowering drugs on ceramide levels [61].

4. Ceramide Measurement in Clinical Research and Practice

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the technique of choice for ceramide measurement as it offers a high sensitivity to detect low abundant species and a large breadth of coverage with respect to chemical diversity [62,63]. Currently, hurdles remain in the translation of lipidomic output, obtained by LC-MS/MS, to clinical practice [64]. First, harmonization of data quality assessment is necessary to allow for measurements comparison between laboratories using different technologies and methodologies [64]. Several ring trials are currently ongoing to optimize and standardize this analytical aspect [64]. The next step forward is the establishment of biological reference intervals for the species, which are amenable to be used in clinical practice. Establishing these reference values through large-scale population studies, while taking variables such as age, sex, physical activity, diet, medication, and circadian rhythm into consideration, is required to distinguish physiological from pathological variations [64,65].

Nevertheless, plasma ceramides are already measured in daily practice in some clinical facilities, such as at the Mayo Clinic (Rochester, Minnesota, USA) [66,67]. Concretely, the Coronary Event Risk Test 1 (CERT1) is calculated based on concentrations of Cer (18:1;2/16:0), Cer (18:1;2/18:0), Cer (18:1/24:1) and on the ratios Cer (18:1;2/16:0) to Cer (18:1;2/24:0), Cer (18:1;2/18:0) to Cer (18:1;2/24:0) and Cer (18:1;2/24:1) to Cer (18:1;2/24:0) [9–12,56,68–73]. One or two points are attributed for each result above the median or the third quartile, respectively [66]. This results in a 12-point scale categorizing the risk of myocardial infarction, acute coronary syndromes, and mortality within 1 to 5 years [66]. This score was improved by adding some specific phosphatidylcholine (PC) species to it, resulting in the Coronary Event Risk Test 2 (CERT2) [12]. This score consists of the ratios Cer (18:1;2/24:1) to Cer (18:1;2/24:0), Cer (18:1;2/16:0) to PC (16:0/22:5), Cer (18:1;2/16:0) to PC (14:0/22:6) and the concentration of PC 16:0/16:0 [12]. CERT2 was shown to efficiently predict residual cardiovascular events in patients with coronary artery disease (CAD) [12], to identify high-risk patients among patients with stable CAD [74] and to predict the risk of cardiovascular death in patients with acute coronary syndrome, independently of established risk factors [75]. Both CERT1 and CERT2 were shown to be superior to the Systematic COronary Risk Evaluation (SCORE) with regard to prediction of cardiovascular events, cardiovascular mortality, and overall mortality in patients with CVD, with CERT2 displaying better results than CERT1 [76]. Predictive performance of CERT2 was further improved once combined with SCORE [76]. As SCORE was recently updated to SCORE2, it might be worthful to repeat these comparisons and likely to integrate ceramide and phosphatidylcholine items in the next version of SCORE [77].

Overall, circulating ceramides can predict cardiovascular outcomes beyond traditional cardiovascular risk factors in healthy individuals and patients with CAD (Figure 3) [9,10,12,78–81]. On a mechanistic level, however, ceramide metabolism, along with its role in cardiometabolic health, is not as deeply understood as that of cholesterol [13]. Therefore, further mechanistic, epidemiological, and interventional studies are required to advance this field of research [13]. Conversely to CVD, no ceramides-based risk assessment tool is available for insulin resistance, type 2 diabetes and NAFLD yet, although circulating ceramides have been associated with these pathologies in clinical cohort studies [34,44,82,83]. Finally,

ceramide metabolism might represent a future therapeutic target, as exemplified by studies in rodents demonstrating that inhibition of ceramide synthesis reduces incidence of hypertension, type 2 diabetes mellitus, NAFLD, atherosclerosis and heart failure [49,61].
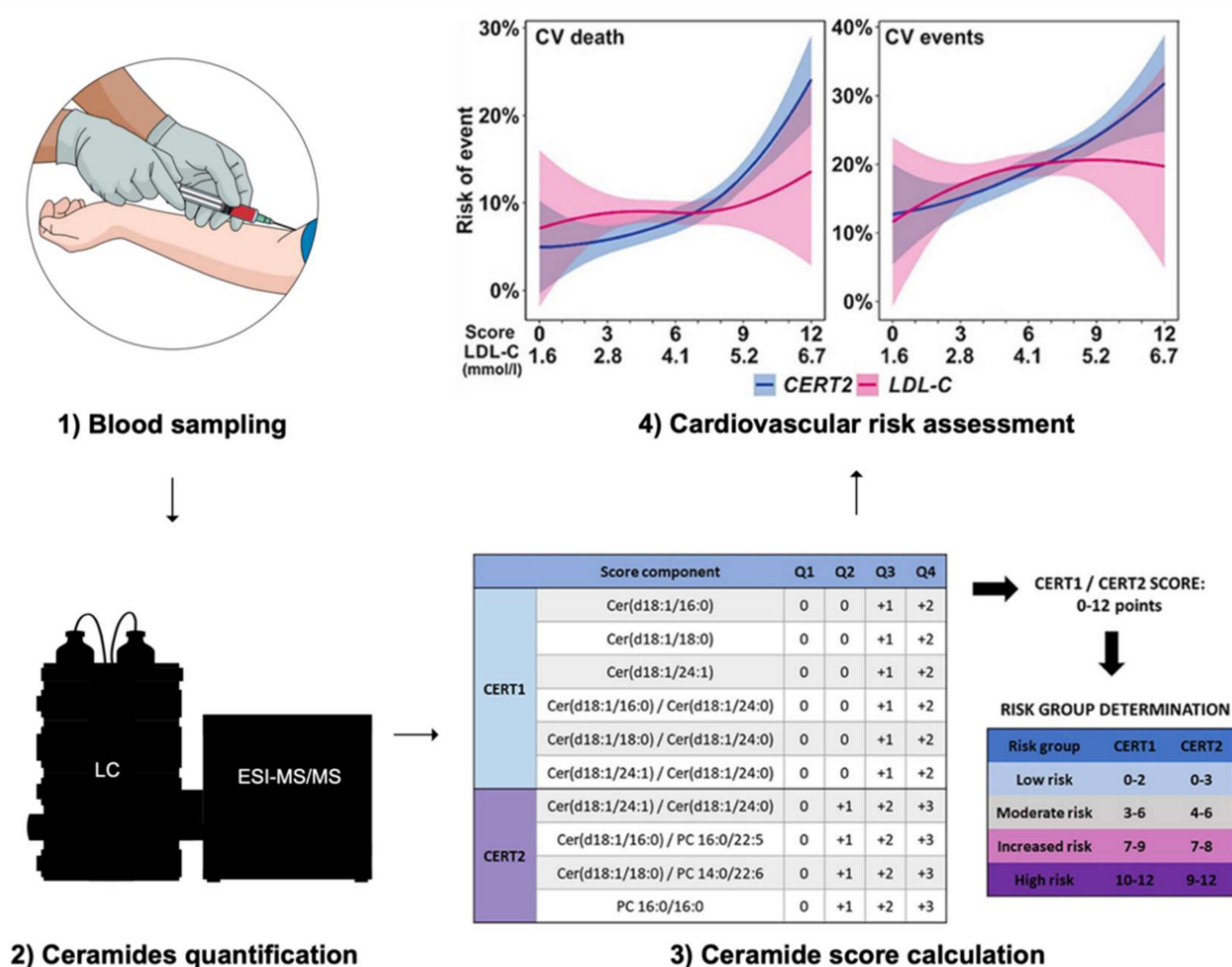

Figure 3. Cardiovascular risk assessment using ceramides-based scores. Abbreviations: LC = liquid chromatography, ESI-MS/MS = electrospray ionization tandem-mass spectrometry, CV = cardiovascular, LDL-C = low-density lipoprotein cholesterol, CERT1 = Ceramide Test Score 1, CERT2 = Ceramide Test Score 2. The figures illustrating the stages 3 and 4 are reproduced, without modification, under the terms of the Creative Commons Attribution License from Hilvo et al. [84].

5. Exercise-A Modulator of Ceramide Levels

Physical activity and exercise represent cost-effective means to prevent and treat NCD [15,26,85–87]. While physical activity notoriously mitigates traditional cardiometabolic risk factors, mechanisms through which exercise directly improves cardiometabolic health remain poorly understood [19]. Preliminary data suggests that exercise could influence ceramide metabolism and thereby optimize human health.

Circulating ceramide levels were increased following a single session of moderate-intensity continuous training (MICT) in endurance athletes, sedentary obese individuals and patients with T2DM [88]. Likewise, muscle ceramide levels increased after three hours of cycling [89]. Conversely, a several-week period of MICT lowered circulating ceramide levels in patients suffering from obesity or T2DM [90,91]. Regular MICT or high-intensity interval training (HIIT) also lead to a reduction in muscle ceramides [91–93].

Cardiorespiratory fitness (CRF), defined as the peak oxygen uptake (VO$_2$peak), is inversely associated with incidence of cancer, cardiometabolic diseases and all-cause mortality [94–99]. CRF has been shown to be a better predictor of morbidity and mor-

tality than physical activity itself [100–102]. Thus, the American Heart Association even recommends assessing CRF as a vital sign in clinical practice [97]. Strikingly, several ceramide species were reported to be negatively associated with CRF: Cer (18:0;2/22:1) [103], Cer (18:0;2/24:1) [103], Cer (18:1;2/14:0) [103], Cer (18:1;2/16:0) [103–105], Cer (18:1;2/18:0) [105,106], Cer (18:1;2/20:0) [105,106], Cer (18:1;2/22:1) [103] and Cer (18:1;2/24:1) [105]. The three cardiometabolically deleterious ceramide species clinically used in the ceramide-phospholipid score (Cer (18:1;2/16:0), Cer (18:1;2/18:0) and Cer (18:1;2/24:1)) were notably reported to be negatively associated with CRF [12,79]. Finally, some glycosphingolipids have also been negatively associated with CRF, such as HexCer (18:1;2/16:0) [103,107], HexCer (18:1;2/18:0) [103,105], LacCer (18:1;2/18:1) [103] and LacCer (18:1;2/22:0) [103].

To summarize, acute bouts of physical activity tend to elevate both circulating and muscle ceramide levels, while regular exercise leads to a reduction of ceramides in the circulation and myocytes (Figure 4). It has been hypothesized that the increase in ceramide could inhibit insulin action, promote fatty acid oxidation and thereby favor lipids as an energy source during exercise [28]. The effect of regular exercise on ceramides is also supported by the reported negative associations between ceramides and CRF. Therefore, it can be hypothesized that regular exercise, leading to an improvement of CRF, optimizes cardiometabolic health, partly by reversing altered ceramide profiles.
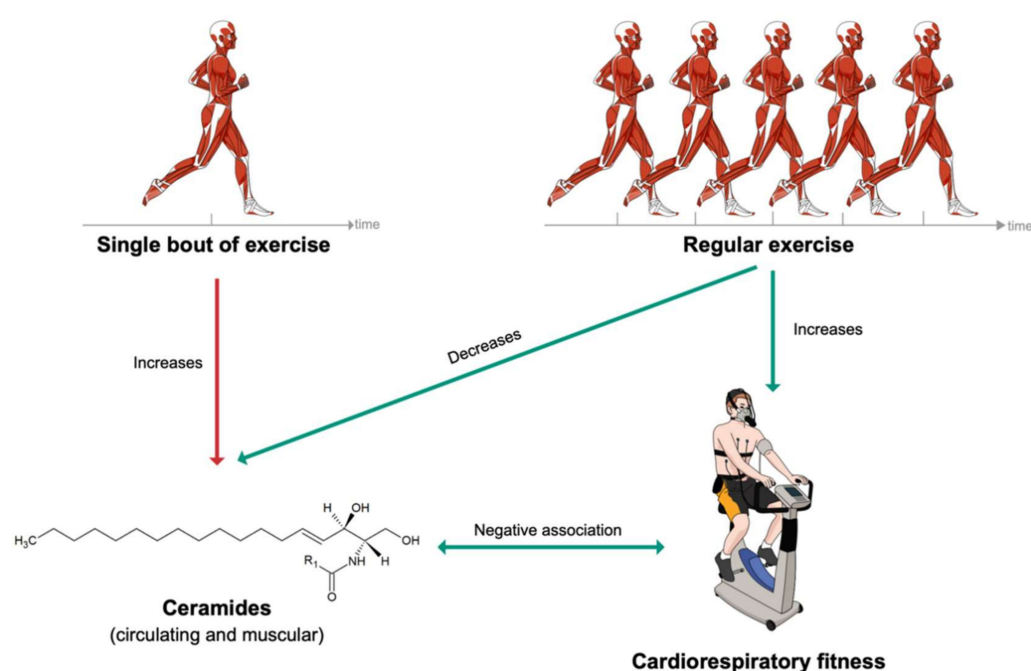

Figure 4. Short- and long-term effects of exercise on ceramide levels.

6. Conclusions

Upgrading patients' metabolic stratification in clinical practice has the potential to improve personalization of CMD prevention and treatment. Specific ceramide species were shown to predict cardiometabolic outcomes beyond classical biomarkers such as cholesterol. While an acute bout of physical exercise tends to increase circulating and muscular ceramide levels, regular exercise leads to a reduction of ceramide levels. This is also reflected by the fact that CRF, a powerful marker reflecting cardiometabolic health and training status, has been negatively associated with several ceramide species. Preventive and therapeutic effectiveness of a well-conducted exercise intervention to mitigate ceramides profile remains to be investigated.

Author Contributions: Conceptualization, J.C. and N.W.; resources, A.S.-T.; writing—original draft preparation, J.C. and N.W.; writing—review and editing, J.C., H.G.-A., N.W., L.S., H.H., F.C., C.S.

References

1. Lozano, R.; Naghavi, M.; Foreman, K.; Lim, S.; Shibuya, K.; Aboyans, V.; Abraham, J.; Adair, T.; Aggarwal, R.; Ahn, S.Y.; et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. *Lancet* **2012**, *380*, 2095–2128. [CrossRef]

2. Murray, C.J.B.R.; Foreman, K.J.; GBD 2019 DALYs and HALE Collaborator. Global, regional, and national disability-adjusted life years (dalys) for diseases and injuries and healthy life expectancy (hale), 1990 to 2019: Quantifying the epidemiological transition. In *GBD Compare Data Visualization*; Institute for Health Metrics and Evaluation, University of Washington: Seattle, WA, USA, 2019.

3. Hunter, D.J.; Reddy, K.S. Noncommunicable diseases. *N. Engl. J. Med.* **2013**, *369*, 1336–1343. [CrossRef]

4. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: A systematic analysis for the global burden of disease study 2013. *Lancet* **2015**, *385*, 117–171. [CrossRef]

5. Benziger, C.P.; Roth, G.A.; Moran, A.E. The global burden of disease study and the preventable burden of ncd. *Glob. Heart* **2016**, *11*, 393–397. [CrossRef]

6. Morrison, L.M.; Hall, L.; Chaney, A.L. Cholesterol metabolism and its relationship to atherosclerosis, coronary artery disease, and arteriosclerosis. *Am. J. Med.* **1948**, *4*, 616. [CrossRef]

7. Kuijpers, P. History in medicine: The story of cholesterol, lipids and cardiology. *J. Cradiol. Pract.* **2021**, *19*, 1–5.

8. Wang, M.; Wang, C.; Han, R.H.; Han, X. Novel advances in shotgun lipidomics for biology and medicine. *Prog. Lipid Res.* **2016**, *61*, 83–108. [CrossRef] [PubMed]

9. Laaksonen, R.; Ekroos, K.; Sysi-Aho, M.; Hilvo, M.; Vihervaara, T.; Kauhanen, D.; Suoniemi, M.; Hurme, R.; Marz, W.; Scharnagl, H.; 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*, 1967–1976. [CrossRef]

10. Havulinna, A.S.; Sysi-Aho, M.; Hilvo, M.; Kauhanen, D.; Hurme, R.; Ekroos, K.; Salomaa, V.; Laaksonen, R. Circulating ceramides predict cardiovascular outcomes in the population-based finrisk 2002 cohort. *Arterioscler. Thromb. Vasc. Biol.* **2016**, *36*, 2424–2430. [CrossRef]

11. Meeusen, J.W.; Donato, L.J.; Bryant, S.C.; Baudhuin, L.M.; Berger, P.B.; Jaffe, A.S. Plasma ceramides. *Arterioscler. Thromb. Vasc. Biol.* **2018**, *38*, 1933–1939. [CrossRef]

12. Hilvo, M.; Meikle, P.J.; Pedersen, E.R.; Tell, G.S.; Dhar, I.; Brenner, H.; Schöttker, B.; Lääperi, M.; Kauhanen, D.; Koistinen, K.M.; et al. Development and validation of a ceramide- and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. *Eur. Heart J.* **2019**, *41*, 371–380. [CrossRef] [PubMed]

13. Summers, S.A. Could ceramides become the new cholesterol? *Cell Metab.* **2018**, *27*, 276–280. [CrossRef]

14. Bull, F.C.; Al-Ansari, S.S.; Biddle, S.; Borodulin, K.; Buman, M.P.; Cardon, G.; Carty, C.; Chaput, J.P.; Chastin, S.; Chou, R.; et al. World health organization 2020 guidelines on physical activity and sedentary behaviour. *Br. J. Sports Med.* **2020**, *54*, 1451–1462. [CrossRef] [PubMed]

15. Khan, K.M.; Thompson, A.M.; Blair, S.N.; Sallis, J.F.; Powell, K.E.; Bull, F.C.; Bauman, A.E. Sport and exercise as contributors to the health of nations. *Lancet* **2012**, *380*, 59–64. [CrossRef]

16. Kohl, H.W., III; Craig, C.L.; Lambert, E.V.; Inoue, S.; Alkandari, J.R.; Leetongin, G.; Kahlmeier, S. The pandemic of physical inactivity: Global action for public health. *Lancet* **2012**, *380*, 294–305. [CrossRef]

17. Lee, I.M.; Shiroma, E.J.; Lobelo, F.; Puska, P.; Blair, S.N.; Katzmarzyk, P.T. Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. *Lancet* **2012**, *380*, 219–229. [CrossRef]

18. Pelliccia, A.; Sharma, S.; Gati, S.; Bäck, M.; Börjesson, M.; Caselli, S.; Collet, J.P.; Corrado, D.; Drezner, J.A.; Halle, M.; et al. 2020 esc guidelines on sports cardiology and exercise in patients with cardiovascular disease. *Eur. Heart J.* **2021**, *42*, 17–96. [CrossRef]

19. Fiuza-Luces, C.; Santos-Lozano, A.; Joyner, M.; Carrera-Bastos, P.; Picazo, O.; Zugaza, J.L.; Izquierdo, M.; Ruilope, L.M.; Lucia, A. Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. *Nat. Rev. Cardiol.* **2018**, *15*, 731–743. [CrossRef]

20. Lavie, C.J.; Ozemek, C.; Carbone, S.; Katzmarzyk, P.T.; Blair, S.N. Sedentary behavior, exercise, and cardiovascular health. *Circ. Res.* **2019**, *124*, 799–815. [CrossRef]

21. Pedersen, B.K.; Saltin, B. Exercise as medicine—Evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand. J. Med. Sci. Sports* **2015**, *25*, 1–72. [CrossRef] [PubMed]

22. Cosentino, F.; Grant, P.J.; Aboyans, V.; Bailey, C.J.; Ceriello, A.; Delgado, V.; Federici, M.; Filippatos, G.; Grobbee, D.E.; Hansen, T.B.; et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD). *Eur. Heart J.* **2020**, *41*, 255–323. [CrossRef]

23. Magkos, F.; Hjorth, M.F.; Astrup, A. Diet and exercise in the prevention and treatment of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **2020**, *16*, 545–555. [CrossRef]

24. Slentz, C.A.; Bateman, L.A.; Willis, L.H.; Granville, E.O.; Piner, L.W.; Samsa, G.P.; Setji, T.L.; Muehlbauer, M.J.; Huffman, K.M.; Bales, C.W.; et al. Effects of exercise training alone vs a combined exercise and nutritional lifestyle intervention on glucose homeostasis in prediabetic individuals: A randomised controlled trial. *Diabetologia* **2016**, *59*, 2088–2098. [CrossRef]

25. Farzanegi, P.; Dana, A.; Ebrahimpoor, Z.; Asadi, M.; Azarbayjani, M.A. Mechanisms of beneficial effects of exercise training on non-alcoholic fatty liver disease (nafld): Roles of oxidative stress and inflammation. *Eur. J. Sport Sci.* **2019**, *19*, 994–1003. [CrossRef]

26. Lear, S.A.; Hu, W.; Rangarajan, S.; Gasevic, D.; Leong, D.; Iqbal, R.; Casanova, A.; Swaminathan, S.; Anjana, R.M.; Kumar, R.; et al. The effect of physical activity on mortality and cardiovascular disease in 130,000 people from 17 high-income, middle-income, and low-income countries: The pure study. *Lancet* **2017**, *390*, 2643–2654. [CrossRef]

27. Caspersen, C.J.; Powell, K.E.; Christenson, G.M. Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. *Public Health Rep.* **1985**, *100*, 126–131. [PubMed]

28. Tan-Chen, S.; Guitton, J.; Bourron, O.; Le Stunff, H.; Hajduch, E. Sphingolipid metabolism and signaling in skeletal muscle: From physiology to physiopathology. *Front. Endocrinol.* **2020**, *11*, 491. [CrossRef]

29. Hannun, Y.A.; Obeid, L.M. Sphingolipids and their metabolism in physiology and disease. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 175–191. [CrossRef] [PubMed]

30. Senkal, C.E.; Salama, M.F.; Snider, A.J.; Allopenna, J.J.; Rana, N.A.; Koller, A.; Hannun, Y.A.; Obeid, L.M. Ceramide is metabolized to acylceramide and stored in lipid droplets. *Cell Metab.* **2017**, *25*, 686–697. [CrossRef] [PubMed]

31. Green, C.D.; Maceyka, M.; Cowart, L.A.; Spiegel, S. Sphingolipids in metabolic disease: The good, the bad, and the unknown. *Cell Metab.* **2021**, *33*, 1293–1306. [CrossRef]

32. Chaurasia, B.; Talbot, C.L.; Summers, S.A. Adipocyte ceramides—The nexus of inflammation and metabolic disease. *Front. Immunol.* **2020**, *11*, 2282. [CrossRef] [PubMed]

33. Field, B.C.; Gordillo, R.; Scherer, P.E. The role of ceramides in diabetes and cardiovascular disease regulation of ceramides by adipokines. *Front. Endocrinol.* **2020**, *11*, 763. [CrossRef]

34. Poss, A.M.; Summers, S.A. Too much of a good thing? An evolutionary theory to explain the role of ceramides in nafld. *Front. Endocrinol.* **2020**, *11*, 505. [CrossRef]

35. Yazıcı, D.; Sezer, H. Insulin resistance, obesity and lipotoxicity. *Adv. Exp. Med. Biol.* **2017**, *960*, 277–304. [CrossRef]

36. Heras, V.; Castellano, J.M.; Fernandois, D.; Velasco, I.; Rodríguez-Vazquez, E.; Roa, J.; Vazquez, M.J.; Ruiz-Pino, F.; Rubio, M.; Pineda, R.; et al. Central ceramide signaling mediates obesity-induced precocious puberty. *Cell Metab.* **2020**, *32*, 951–966.e958. [CrossRef]

37. Aburasayn, H.; Al Batran, R.; Ussher, J.R. Targeting ceramide metabolism in obesity. *Am. J. Physiol. Endocrinol. Metab.* **2016**, *311*, E423–E435. [CrossRef] [PubMed]

38. Amati, F.; Dubé, J.J.; Alvarez-Carnero, E.; Edreira, M.M.; Chomentowski, P.; Coen, P.M.; Switzer, G.E.; Bickel, P.E.; Stefanovic-Racic, M.; Toledo, F.G.S.; et al. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: Another paradox in endurance-trained athletes? *Diabetes* **2011**, *60*, 2588–2597. [CrossRef]

39. Holland, W.L.; Brozinick, J.T.; Wang, L.-P.; Hawkins, E.D.; Sargent, K.M.; Liu, Y.; Narra, K.; Hoehn, K.L.; Knotts, T.A.; Siesky, A.; et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* **2007**, *5*, 167–179. [CrossRef]

40. Li, W.; Yang, X.; Xing, S.; Bian, F.; Yao, W.; Bai, X.; Zheng, T.; Wu, G.; Jin, S. Endogenous ceramide contributes to the transcytosis of oxldl across endothelial cells and promotes its subendothelial retention in vascular wall. *Oxid. Med. Cell Longev.* **2014**, *2014*, 823071. [CrossRef]

41. Zhang, X.; Zhang, Y.; Wang, P.; Zhang, S.-Y.; Dong, Y.; Zeng, G.; Yan, Y.; Sun, L.; Wu, Q.; Liu, H.; et al. Adipocyte hypoxia-inducible factor 2α suppresses atherosclerosis by promoting adipose ceramide catabolism. *Cell Metab.* **2019**, *30*, 937–951.e5. [CrossRef] [PubMed]

42. Chaurasia, B.; Summers, S.A. Ceramides—Lipotoxic inducers of metabolic disorders. *Trends Endocrinol. Metab.* **2015**, *26*, 538–550. [CrossRef]

43. Chavez, J.A.; Knotts, T.A.; Wang, L.-P.; Li, G.; Dobrowsky, R.T.; Florant, G.L.; Summers, S.A. A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J. Biol. Chem.* **2003**, *278*, 10297–10303. [CrossRef]

44. Lemaitre, R.N.; Yu, C.; Hoofnagle, A.; Hari, N.; Jensen, P.N.; Fretts, A.M.; Umans, J.G.; Howard, B.V.; Sitlani, C.M.; Siscovick, D.S.; et al. Circulating sphingolipids, insulin, homa-ir, and homa-b: The strong heart family study. *Diabetes* **2018**, *67*, 1663–1672. [CrossRef]

45. Haus, J.M.; Kashyap, S.R.; Kasumov, T.; Zhang, R.; Kelly, K.R.; Defronzo, R.A.; Kirwan, J.P. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* **2009**, *58*, 337–343. [CrossRef]

46. Boon, J.; Hoy, A.J.; Stark, R.; Brown, R.D.; Meex, R.C.; Henstridge, D.C.; Schenk, S.; Meikle, P.J.; Horowitz, J.F.; Kingwell, B.A.; et al. Ceramides contained in ldl are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes* **2013**, *62*, 401–410. [CrossRef] [PubMed]

47. Zierath, J.R. The path to insulin resistance: Paved with ceramides? *Cell Metab.* **2007**, *5*, 161–163. [CrossRef] [PubMed]

48. Reidy, P.T.; Mahmassani, Z.S.; McKenzie, A.I.; Petrocelli, J.J.; Summers, S.A.; Drummond, M.J. Influence of exercise training on skeletal muscle insulin resistance in aging: Spotlight on muscle ceramides. *Int. J. Mol. Sci.* **2020**, *21*, 1514. [CrossRef] [PubMed]

49. Chaurasia, B.; Tippetts, T.S.; Monibas, R.M.; Liu, J.; Li, Y.; Wang, L.; Wilkerson, J.L.; Sweeney, C.R.; Pereira, R.F.; Sumida, D.H.; et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science* **2019**, *365*, 386–392. [CrossRef]

50. Kowluru, A.; Kowluru, R.A. Racking up ceramide-induced islet β-cell dysfunction. *Biochem. Pharmacol.* **2018**, *154*, 161–169. [CrossRef]

51. Lang, F.; Ullrich, S.; Gulbins, E. Ceramide formation as a target in beta-cell survival and function. *Expert Opin. Ther. Targets* **2011**, *15*, 1061–1071. [CrossRef]

52. Turpin, S.M.; Nicholls, H.T.; Willmes, D.M.; Mourier, A.; Brodesser, S.; Wunderlich, C.M.; Mauer, J.; Xu, E.; Hammerschmidt, P.; Brönneke, H.S.; et al. Obesity-induced cers6-dependent c16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* **2014**, *20*, 678–686. [CrossRef] [PubMed]

53. Bergman, B.C.; Brozinick, J.T.; Strauss, A.; Bacon, S.; Kerege, A.; Bui, H.H.; Sanders, P.; Siddall, P.; Wei, T.; Thomas, M.K.; et al. Muscle sphingolipids during rest and exercise: A c18:0 signature for insulin resistance in humans. *Diabetologia* **2016**, *59*, 785–798. [CrossRef]

54. Zobel, E.H.; Wretlind, A.; Ripa, R.S.; Rotbain Curovic, V.; von Scholten, B.J.; Suvitaival, T.; Hansen, T.W.; Kjær, A.; Legido-Quigley, C.; Rossing, P. Ceramides and phospholipids are downregulated with liraglutide treatment: Results from the liraflame randomized controlled trial. *BMJ Open Diabetes Res. Care* **2021**, *9*, e002395. [CrossRef] [PubMed]

55. Akawi, N.; Checa, A.; Antonopoulos, A.S.; Akoumianakis, I.; Daskalaki, E.; Kotanidis, C.P.; 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. [CrossRef]

56. Wang, D.D.; Toledo, E.; Hruby, A.; Rosner, B.A.; Willett, W.C.; Sun, Q.; Razquin, C.; Zheng, Y.; Ruiz-Canela, M.; Guasch-Ferré, M.; et al. Plasma ceramides, mediterranean diet, and incident cardiovascular disease in the predimed trial (prevención con dieta mediterránea). *Circulation* **2017**, *135*, 2028–2040. [CrossRef]

57. Chapman, M.J.; Orsoni, A.; Tan, R.; Mellett, N.A.; Nguyen, A.; Robillard, P.; Giral, P.; Thérond, P.; Meikle, P.J. Ldl subclass lipidomics in atherogenic dyslipidemia: Effect of statin therapy on bioactive lipids and dense ldl[s]. *J. Lipid Res.* **2020**, *61*, 911–932. [CrossRef]

58. Ng, T.W.K.; Ooi, E.M.M.; Watts, G.F.; Chan, D.C.; Weir, J.M.; Meikle, P.J.; Barrett, P.H.R. Dose-dependent effects of rosuvastatin on the plasma sphingolipidome and phospholipidome in the metabolic syndrome. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E2335–E2340. [CrossRef]

59. Tarasov, K.; Ekroos, K.; Suoniemi, M.; Kauhanen, D.; Sylvänne, T.; Hurme, R.; Gouni-Berthold, I.; Berthold, H.K.; Kleber, M.E.; Laaksonen, R.; et al. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and pcsk9 deficiency. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E45–E52. [CrossRef] [PubMed]

60. Ye, Q.; Svatikova, A.; Meeusen, J.W.; Kludtke, E.L.; Kopecky, S.L. Effect of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma ceramide levels. *Am. J. Cardiol.* **2020**, *128*, 163–167. [CrossRef]

61. Choi, R.H.; Tatum, S.M.; Symons, J.D.; Summers, S.A.; Holland, W.L. Ceramides and other sphingolipids as drivers of cardiovascular disease. *Nat. Rev. Cardiol.* **2021**, *18*, 701–711. [CrossRef]

62. Burla, B.; Muralidharan, S.; Wenk, M.R.; Torta, F. Sphingolipid analysis in clinical research. In *Clinical Metabolomics: Methods and Protocols*; Giera, M., Ed.; Springer: New York, NY, USA, 2018; pp. 135–162. [CrossRef]

63. Wang, J.-R.; Zhang, H.; Yau, L.F.; Mi, J.-N.; Lee, S.; Lee, K.C.; Hu, P.; Liu, L.; Jiang, Z.-H. Improved sphingolipidomic approach based on ultra-high performance liquid chromatography and multiple mass spectrometries with application to cellular neurotoxicity. *Anal. Chem.* **2014**, *86*, 5688–5696. [CrossRef]

64. Burla, B.; Arita, M.; Arita, M.; Bendt, A.K.; Cazenave-Gassiot, A.; Dennis, E.A.; Ekroos, K.; Han, X.; Ikeda, K.; Liebisch, G.; et al. Ms-based lipidomics of human blood plasma: A community-initiated position paper to develop accepted guidelines1. *J. Lipid Res.* **2018**, *59*, 2001–2017. [CrossRef]

65. Carrard, J.; Gallart-Ayala, H.; Infanger, D.; Teav, T.; Wagner, J.; Knaier, R.; Colledge, F.; Streese, L.; Königstein, K.; Hinrichs, T.; et al. Metabolic view on human healthspan: A lipidome-wide association study. *Metabolites* **2021**, *11*, 287. [CrossRef] [PubMed]

66. Ceramides: A Class of Lipids with Links to Heart Disease. Available online: https://www.mayoclinic.org/medical-professionals/cardiovascular-diseases/news/ceramides-a-class-of-lipids-with-links-to-heart-disease/mac-20429577 (accessed on 1 September 2021).

67. Mi-Heart Ceramides. Be in the Know. *Now.* Available online: https://news.mayocliniclabs.com/ceramides-miheart/#form-row-right (accessed on 1 September 2021).

68. Peterson, L.R.; Xanthakis, V.; Duncan, M.S.; Gross, S.; Friedrich, N.; Völzke, H.; Felix, S.B.; Jiang, H.; Sidhu, R.; Nauck, M.; et al. Ceramide remodeling and risk of cardiovascular events and mortality. *J. Am. Heart Assoc.* **2018**, *7*, e007931. [CrossRef]

69. Alshehry, Z.H.; Mundra, P.A.; Barlow, C.K.; Mellett, N.A.; Wong, G.; McConville, M.J.; Simes, J.; Tonkin, A.M.; Sullivan, D.R.; Barnes, E.H.; et al. Plasma lipidomic profiles improve on traditional risk factors for the prediction of cardiovascular events in type 2 diabetes mellitus. *Circulation* **2016**, *134*, 1637–1650. [CrossRef] [PubMed]

70. Anroedh, S.; Hilvo, M.; Akkerhuis, K.M.; Kauhanen, D.; Koistinen, K.; Oemrawsingh, R.; Serruys, P.; van Geuns, R.J.; Boersma, E.; Laaksonen, R.; et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J. Lipid Res.* **2018**, *59*, 1729–1737. [CrossRef]

71. Lemaitre, R.N.; Jensen, P.N.; Hoofnagle, A.; McKnight, B.; Fretts, A.M.; King, I.B.; Siscovick, D.S.; Psaty, B.M.; Heckbert, S.R.; Mozaffarian, D.; et al. Plasma ceramides and sphingomyelins in relation to heart failure risk. *Circ. Heart Fail.* **2019**, *12*, e005708. [CrossRef]

72. Peterson, L.R.; Jiang, X.; Chen, L.; Goldberg, A.C.; Farmer, M.S.; Ory, D.S.; Schaffer, J.E. Alterations in plasma triglycerides and ceramides: Links with cardiac function in humans with type 2 diabetes. *J. Lipid Res.* **2020**, *61*, 1065–1074. [CrossRef] [PubMed]

73. Nwabuo, C.C.; Duncan, M.; Xanthakis, V.; Peterson, L.R.; Mitchell, G.F.; McManus, D.; Cheng, S.; Vasan, R.S. Association of circulating ceramides with cardiac structure and function in the community: The framingham heart study. *J. Am. Heart Assoc.* **2019**, *8*, e013050. [CrossRef]

74. Hilvo, M.; Lääperi, M.; Jylhä, A.; Kleber, M.E.; Hurme, R.; Scharnagl, H.; März, W.; Sinisalo, J.; Laaksonen, R. Prior myocardial infarction, coronary artery disease extent, diabetes mellitus, and cert2 score for risk stratification in stable coronary artery disease. *Eur. J. Prev. Cardiol.* **2021**, zwab122. [CrossRef]

75. Gencer, B.; Morrow, D.A.; Braunwald, E.; Goodrich, E.L.; Hilvo, M.; Kauhanen, D.; Sabatine, M.S.; Laaksonen, R.; O'Donoghue, M.L. Plasma ceramide and phospholipid-based risk score and the risk of cardiovascular death in patients after acute coronary syndrome. *Eur J. Prev. Cardiol.* **2020**, zwaa143. [CrossRef] [PubMed]

76. Leiherer, A.; Mündlein, A.; Laaksonen, R.; Lääperi, M.; Jylhä, A.; Fraunberger, P.; Drexel, H. Comparison of recent ceramide-based coronary risk prediction scores in cardiovascular disease patients. *Eur. J. Prev. Cardiol.* **2021**, zwab112. [CrossRef] [PubMed]

77. SCORE2 Working Group; ESC Cardiovascular Risk Collaboration. Score2 risk prediction algorithms: New models to estimate 10-year risk of cardiovascular disease in Europe. *Eur. Heart J.* **2021**, *42*, 2439–2454. [CrossRef] [PubMed]

78. Cao, R.; Fang, Z.; Li, S.; Xu, M.; Zhang, J.; Han, D.; Hu, W.; Yan, L.; Wang, Y.; Fan, L.; et al. Circulating ceramide: A new cardiometabolic biomarker in patients with comorbid acute coronary syndrome and type 2 diabetes mellitus. *Front. Physiol.* **2020**, *11*, 1104. [CrossRef] [PubMed]

79. Hilvo, M.; Wallentin, L.; Lakic, T.G.; Held, C.; Kauhanen, D.; Jylhä, A.; Lindbäck, J.; Siegbahn, A.; Granger, C.B.; Koenig, W.; et al. Prediction of residual risk by ceramide-phospholipid score in patients with stable coronary heart disease on optimal medical therapy. *J. Am. Heart Assoc.* **2020**, *9*, e015258. [CrossRef]

80. Mantovani, A.; Dugo, C. Ceramides and risk of major adverse cardiovascular events: A meta-analysis of longitudinal studies. *J. Clin. Lipidol.* **2020**, *14*, 176–185. [CrossRef]

81. Poss, A.M.; Holland, W.L.; Summers, S.A. Risky lipids: Refining the ceramide score that measures cardiovascular health. *Eur. Heart J.* **2019**, *41*, 381–382. [CrossRef]

82. Thorens, B.; Rodriguez, A.; Cruciani-Guglielmacci, C.; Wigger, L.; Ibberson, M.; Magnan, C. Use of preclinical models to identify markers of type 2 diabetes susceptibility and novel regulators of insulin secretion—A step towards precision medicine. *Mol. Metab.* **2019**, *27*, S147–S154. [CrossRef] [PubMed]

83. Wigger, L.; Cruciani-Guglielmacci, C.; Nicolas, A.; Denom, J.; Fernandez, N.; Fumeron, F.; Marques-Vidal, P.; Ktorza, A.; Kramer, W.; Schulte, A.; et al. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep.* **2017**, *18*, 2269–2279. [CrossRef]

84. Hilvo, M.; Vasile, V.C.; Donato, L.J.; Hurme, R.; Laaksonen, R. Ceramides and ceramide scores: Clinical applications for cardiometabolic risk stratification. *Front. Endocrinol.* **2020**, *11*, 628. [CrossRef]

85. Ding, D.; Lawson, K.D.; Kolbe-Alexander, T.L.; Finkelstein, E.A.; Katzmarzyk, P.T.; Van Mechelen, W.; Pratt, M. Lancet Physical Activity Series 2 Executive Committee. The economic burden of physical inactivity: A global analysis of major non-communicable diseases. *Lancet* **2016**, *388*, 1311–1324. [CrossRef]

86. Merkur, S.; Sassi, F.; McDaid, D. *Promoting Health, Preventing Disease: Is there an Economic Case?* WHO: Copenhagen, Denmark, 2013; p. 72.

87. Gojanovic, B. Physical activity is an opportunity for the health of nations: What should we do next? *Praxis* **2018**, *107*, 943–949. [CrossRef] [PubMed]

88. Bergman, B.C.; Brozinick, J.T.; Strauss, A.; Bacon, S.; Kerege, A.; Bui, H.H.; Sanders, P.; Siddall, P.; Kuo, M.S.; Perreault, L. Serum sphingolipids: Relationships to insulin sensitivity and changes with exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* **2015**, *309*, E398–E408. [CrossRef] [PubMed]

89. Helge, J.W.; Dobrzyn, A.; Saltin, B.; Gorski, J. Exercise and training effects on ceramide metabolism in human skeletal muscle. *Exp. Physiol.* **2004**, *89*, 119–127. [CrossRef]

90. Kasumov, T.; Solomon, T.P.J.; Hwang, C.; Huang, H.; Haus, J.M.; Zhang, R.; Kirwan, J.P. Improved insulin sensitivity after exercise training is linked to reduced plasma c14:0 ceramide in obesity and type 2 diabetes. *Obesity* **2015**, *23*, 1414–1421. [CrossRef] [PubMed]

91. Shepherd, S.O.; Cocks, M.; Meikle, P.J.; Mellett, N.A.; Ranasinghe, A.M.; Barker, T.A.; Wagenmakers, A.J.M.; Shaw, C.S. Lipid droplet remodelling and reduced muscle ceramides following sprint interval and moderate-intensity continuous exercise training in obese males. *Int. J. Obes.* **2017**, *41*, 1745–1754. [CrossRef]

92. Bruce, C.R.; Thrush, A.B.; Mertz, V.A.; Bezaire, V.; Chabowski, A.; Heigenhauser, G.J.; Dyck, D.J. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *291*, E99–E107. [CrossRef]

93. Dubé, J.J.; Amati, F.; Stefanovic-Racic, M.; Toledo, F.G.; Sauers, S.E.; Goodpaster, B.H. Exercise-induced alterations in intramyocellular lipids and insulin resistance: The athlete's paradox revisited. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *294*, E882–E888. [CrossRef]

94. Robsahm, T.E.; Falk, R.S.; Heir, T.; Sandvik, L.; Vos, L.; Erikssen, J.E.; Tretli, S. Measured cardiorespiratory fitness and self-reported physical activity: Associations with cancer risk and death in a long-term prospective cohort study. *Cancer Med.* **2016**, *5*, 2136–2144. [CrossRef]

95. Gander, J.C.; Sui, X.; Hébert, J.R.; Hazlett, L.J.; Cai, B.; Lavie, C.J.; Blair, S.N. Association of cardiorespiratory fitness with coronary heart disease in asymptomatic men. *Mayo Clin. Proc.* **2015**, *90*, 1372–1379. [CrossRef]

96. Farrell, S.W.; Finley, C.E.; Radford, N.B.; Haskell, W.L. Cardiorespiratory fitness, body mass index, and heart failure mortality in men. *Circ. Heart Fail.* **2013**, *6*, 898–905. [CrossRef]

97. Ross, R.; Blair, S.N.; Arena, R.; Church, T.S.; Despres, J.P.; Franklin, B.A.; Haskell, W.L.; Kaminsky, L.A.; Levine, B.D.; Lavie, C.J.; et al. Importance of assessing cardiorespiratory fitness in clinical practice: A case for fitness as a clinical vital sign: A scientific statement from the american heart association. *Circulation* **2016**, *134*, e653–e699. [CrossRef] [PubMed]

98. Juraschek, S.P.; Blaha, M.J.; Whelton, S.P.; Blumenthal, R.; Jones, S.R.; Keteyian, S.J.; Schairer, J.; Brawner, C.A.; Al-Mallah, M.H. Physical fitness and hypertension in a population at risk for cardiovascular disease: The henry ford exercise testing (fit) project. *J. Am. Heart Assoc.* **2014**, *3*, e001268. [CrossRef]

99. Rizzo, N.S.; Ruiz, J.R.; Hurtig-Wennlöf, A.; Ortega, F.B.; Sjöström, M. Relationship of physical activity, fitness, and fatness with clustered metabolic risk in children and adolescents: The european youth heart study. *J. Pediatr.* **2007**, *150*, 388–394. [CrossRef] [PubMed]

100. Myers, J.; Kaykha, A.; George, S.; Abella, J.; Zaheer, N.; Lear, S.; Yamazaki, T.; Froelicher, V. Fitness versus physical activity patterns in predicting mortality in men. *Am. J. Med.* **2004**, *117*, 912–918. [CrossRef]

101. Lee, D.-C.; Sui, X.; Ortega, F.B.; Kim, Y.-S.; Church, T.S.; Winett, R.A.; Ekelund, U.; Katzmarzyk, P.T.; Blair, S.N. Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. *Br. J. Sports Med.* **2011**, *45*, 504–510. [CrossRef] [PubMed]

102. Williams, P.T. Physical fitness and activity as separate heart disease risk factors: A meta-analysis. *Med. Sci. Sports Exerc.* **2001**, *33*, 754–761. [CrossRef]

103. Contrepois, K.; Wu, S.; Moneghetti, K.J.; Hornburg, D.; Ahadi, S.; Tsai, M.S.; Metwally, A.A.; Wei, E.; Lee-McMullen, B.; Quijada, J.V.; et al. Molecular choreography of acute exercise. *Cell* **2020**, *181*, 1112.e1116–1130.e1116. [CrossRef]

104. Nayor, M.; Shah, R.V.; Miller, P.E.; Blodgett, J.B.; Tanguay, M.; Pico, A.R.; Murthy, V.L.; Malhotra, R.; Houstis, N.E.; Deik, A.; et al. Metabolic architecture of acute exercise response in middle-aged adults in the community. *Circulation* **2020**, *142*, 1905–1924. [CrossRef]

105. Saleem, M.; Herrmann, N.; Dinoff, A.; Marzolini, S.; Mielke, M.M.; Andreazza, A.; Oh, P.I.; Vattem Venkata, S.L.; Haughey, N.J.; Lanctôt, K.L. Association between sphingolipids and cardiopulmonary fitness in coronary artery disease patients undertaking cardiac rehabilitation. *J. Gerontol. A Biol. Sci. Med. Sci.* **2018**, *75*, 671–679. [CrossRef]

106. Fabbri, E.; Yang, A.; Simonsick, E.M.; Chia, C.W.; Zoli, M.; Haughey, N.J.; Mielke, M.M.; Ferrucci, L.; Coen, P.M. Circulating ceramides are inversely associated with cardiorespiratory fitness in participants aged 54-96 years from the baltimore longitudinal study of aging. *Aging Cell* **2016**, *15*, 825–831. [CrossRef] [PubMed]

107. Contaifer, D.; Buckley, L.F.; Wohlford, G.; Kumar, N.G.; Morriss, J.M.; Ranasinghe, A.D.; Carbone, S.; Canada, J.M.; Trankle, C.; Abbate, A.; et al. Metabolic modulation predicts heart failure tests performance. *PLoS ONE* **2019**, *14*, e218153. [CrossRef] [PubMed]