Research Article

Plasma Ceramides and Risk of Impaired Lower-Extremity Function in Older Adults: A Nested Case–Control Study

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Abstract

Background: Higher levels of ceramides have been linked to several chronic diseases; also there is emerging cross-sectional evidence that ceramides are associated with lower physical functioning. This research assessed for the first time the prospective relationship between ceramide species and impaired lower-extremity function (ILEF) in older adults.

Methods: Case–control study with 43 cases of ILEF and 86 age- and sex-matched controls, which was nested in the Seniors-ENRICA cohort of community-dwelling older adults. Incident ILEF from 2015 to 2017 was ascertained with the Short Physical Performance Battery. In 2015, 27 ceramide species were measured in plasma by liquid chromatography-tandem mass spectrometry. Conditional logistic regression models were used to assess the longitudinal relationship between ceramides concentration and incidence of ILEF.

Results: After adjusting for education level, body mass index, alcohol and total energy intake, physical activity, and presence of chronic conditions, some ceramide species were related to 2-year incidence of ILEF. Specifically, the odds ratios of ILEF per 1-SD increase in ceramide concentration were: 1.66 [95% CI = (1.03, 2.68)] for ceramide C14:0, 1.61 (1.00, 2.59) for ceramide C16:0, and 1.64 (1.03, 2.60) for ceramide C16:1 (n-7). In the case of ceramides C16:0 and C16:1 (n-7), a stronger relationship was found in those with a higher body mass index; systolic blood pressure could also mediate the relationship between ceramide C16:1 (n-7) and ILEF (*p* for interaction = .03).

Conclusions: Higher plasma levels of ceramides C14:0, C16:0, and C16:1 (n-7) are associated with higher risk of ILEF, and might serve as risk markers for functional decline in older adults.

Keywords: Aging; Case–control design; Ceramides; Impaired lower-extremity function; Physical function

Ceramides have recently been proposed as promising biomarkers of functional decline (1). Ceramides are the central molecular species of the sphingolipid pathway and have a potential influence on age-related cellular processes as inflammation (2) and apoptosis (3), as a key regulatory mechanism that commits cells to life or death. In previous studies, higher levels of ceramide lipids have been associated with the development and progression of multiple age-related conditions, such as type 2 diabetes (4), cardiovascular risk (5), and low cardiorespiratory fitness (6). Higher levels of ceramides have been also related to types of cancer as chordoma (7), breast cancer (8), and prostate cancer (9).

Ceramides have been found to induce atrophy, proteolysis, and necrosis in cellular muscle models (10,11). Higher plasma levels of ceramides have also been associated with the development of insulin resistance (12), one of the pathways to muscle deterioration. In a cross-sectional study, ceramide C16:0 was associated with slower gait speed, decreased cadence, and increased double support time (13). In the same line, whether levels of ceramides might be early biomarkers of impaired physical function and subsequent disability is unknown. The aim of the present study was to assess comprehensively the potential role of several ceramide species on the risk of impaired lower-extremity function (ILEF). For this purpose, we

used a case-control design nested in a cohort of community-dwelling older adults; this design made an efficient use of the data by limiting the amount of metabolomic measurements needed and, at the same time, benefited from the longitudinal cohort study by selecting only incident cases of physical function impairment.

Method

Study Design

A nested case-control study was designed using data from the Spanish Seniors-ENRICA (Study on Nutrition and Cardiovascular Risk) cohort (14). This cohort comprised noninstitutionalized individuals aged ≥ 60 years at the time of baseline data collection, in 2008–2010. The Clinical Research Ethics Committee of “La Paz” University Hospital in Madrid approved the study protocol (registration number: 2144). For the purposes of the present study, a subset of participants gave their written consent for extraction of blood and use of plasma samples in 2015 (this assessment has been considered as baseline in the present article), and their lifestyles, morbidity, and physical function were also assessed. Blood was collected by trained nurses at the participants’ home, under standardized conditions including 12-hour fasting. After 2 years, in 2017, a new phase of data collection was carried out in order to assess the change in physical function of the participants included in the study.

Before obtaining our sample of 129 participants, a total of 1138 participants in the Seniors-ENRICA cohort were interviewed by phone in 2015. Among them, 965 provided their consent for physical exams at home (with blood samples). Then, these participants were followed until 2017. After excluding those who died or those who were lost to follow-up, we had 519 participants available in 2017. Prevalent cases were also excluded and 43 participants were finally identified as new cases of physical impairment. We randomly selected a 1:2 design to choose the 86 controls, paired by age group (<65 vs ≥ 65 years in the first wave of ENRICA conducted in 2008–2010) and sex.

Study Variables

Impaired lower-extremity function

Impaired lower-extremity function was identified with the Short Physical Performance Battery test (15). This instrument comprises 3 different components: gait speed, standing balance, and ability to rise from a chair. Gait speed was calculated over a distance of 2.44 m, while the ability to rise from a chair was assessed by the time required to stand up and sit down from a chair 5 consecutive times without helping with the arms. In the standing balance test, participants attempted to maintain 3 hierarchical tandem positions: side-by-side, semi-tandem, and full-tandem. Each component was scored between 0 and 4, with higher scores reflecting a better lower-extremity performance. A global score was then obtained, after adding up the scores in each component, ranging between 0 and 12, with higher scores indicating better lower-extremity performance. Although the habitual cutoff to define impaired function in the clinical setting is 9 (16), we used a cutoff point of 6 to identify incident ILEF, in order to improve the specificity of the test, since the study population comprised fairly healthy community-dwelling older people (17).

Ceramide species

Blood samples were collected in EDTA (ethylenediamine tetraacetic acid) tubes and were centrifuged (3000 rpm during 10 minutes)

and sent to “La Paz” University Hospital on a cool pack for aliquoting in the following 24 hours from collection. The plasma samples were stored at -70°C at the Universidad Autónoma de Madrid Biobank until their processing, at the Metabolomics Core, Biomedical Research Core Facilities of Michigan University. A total of 27 ceramide species were measured by liquid chromatography-tandem mass spectrometry. The process was as follows: lipids were extracted from 50 μL of plasma samples following Bligh and Dyer’s method of solvent partition with some modifications (18). The volumes of the samples were increased to 600 μL with water followed by addition of 2.25 mL of a mixture of chloroform-methanol (1:2) containing 0.01% of butylated hydroxytoluene and 10 μL of 4 mM heptadecanoic (C17:0) acid as an internal standard. The mixtures were homogenized on vortex mixture. The homogenized samples were further treated with 0.75 mL each of chloroform and NaCl (0.9%) solution followed by mixing and centrifugation at ~ 3000 rpm for 5–6 minutes on a table top centrifuge. The upper aqueous layers were removed and the lower organic (chloroform) layer containing the lipids was separated out and saved at -20°C .

The fatty acid portions of the lipids were converted into their methyl esters via transesterification with BF_3 -methanol as described by Morrison and Smith (19), with slight modification. The solvents of the lipid extracts were removed under nitrogen. To the dry residues, 2 mL of Boron trifluoride-methanol (BF_3 -methanol, 14% solution from Sigma) were added; the mixtures were incubated at 60°C for 2 to 2.5 hours. The fatty acid methyl esters were extracted in hexane by adding 2 mL of hexane and 1 mL of water, mixing and centrifuging. The crude fatty acid methyl esters were purified on a thin-layer chromatographic plate (20 $\times$ 20 cm, silica gel 60, Merck, Germany) by spotting them as a band. The plate was developed with a solvent mixture of hexane-diethyl ether-acetic acid (80-20-1.5, v/v). Methyl ester bands were identified by comparing the retention flow of the authentic standard and the contents from the thin-layer chromatographic powders were extracted with chloroform. The solvents were removed under nitrogen, methyl esters were re-dissolved in a small volume of hexane (100–200 μL depending upon the starting material), and the fatty acid compositions of the lipids were analyzed by gas chromatography. Gas chromatography was performed with an Agilent system, model 6890N equipped with flame ionization detector, an auto sampler and ChemStation software for data analysis. The gas chromatography column used was Agilent HP 88, 30 m, 0.25 mm ID, and 0.20 μm film thickness. Hydrogen was used as a carrier gas, while nitrogen was employed as a make-up gas. Analyses were carried out with a temperature program of 125°C to 220°C . One microliters of sample was injected via the auto sampler. A calibration curve was prepared using known amounts of C17:0 (internal standard) and other authentic methyl esters. A mixture of authentic methyl esters was also run side by side with the unknown samples to identify the components in the unknown by comparing their retention times. The fatty acids were quantified with respect to the amounts of C17:0 internal standard added and the calibration curve prepared. The coefficient of variation for gas chromatography analyses was found to range between 2.3% and 3.7% across ceramide species.

The metabolites assessed were ceramides C14:0, C14:1 (n-5), C15:0, C16:0, C16:1 (n-7), C18:0, C18:1 (n-7), C18:1 (n-9), C18:2 (n-6), C18:3 (n-3), C18:3 (n-6), C19:0, C20:0, C20:1, C20:2, C20:3 (n-6), C20:4 (n-6), C20:5 (n-3), C21:0, C22:0, C22:1, C22:2 (n-6), C22:4 (n-6), C22:5 (n-3), C22:6 (n-3), C24:0, and C24:1.

Covariates

Participants reported their highest educational level attained. Weight and height were measured in standardized conditions, using electronic scales (model Seca 841, precision to 0.1 kg), and portable extendable stadiometers (model Ka We 44 444Seca). The body mass index (BMI) was calculated as weight (kg) divided by squared height (m). Total energy intake (kcal/day) and alcohol consumption (g/day) were assessed with a validated computerized diet history (20). Physical activity was ascertained with a validated questionnaire (21) and expressed in metabolic equivalents of task (METs) given in hours/week. Participants also reported the following physician-diagnosed diseases: diabetes, cardiovascular disease (heart failure, heart attack, atrial fibrillation, and stroke), or musculoskeletal disease (arthritis, osteoarthritis, and hip fracture). Fasting blood glucose was measured by the glucose oxidase method (22,23), and blood pressure was measured under standardized conditions with validated automatic sphygmomanometers (24).

Statistical Analyses

One plasma sample was spoiled during lab procedures, so the statistical analyses were conducted with 128 participants (43 incident ILEF cases and 85 controls). Baseline characteristics in both cases and control group were described and compared. Geometric means on the raw values of ceramides measured and 95% confidence intervals (CIs) for these means were provided for both groups. The rank-based inverse normal transformation (25) was applied to the raw values of ceramides in order to mitigate potential deviations from the normal distribution. Then unpaired *t* tests for statistical comparisons between the transformed ceramide values were employed.

Conditional logistic regression models were used to assess the association between each ceramide and the incidence of ILEF after the 2 years of follow-up, using age group and sex as matching variables. Odds ratios (ORs) and 95% CIs per 1-SD increase in ceramides were provided for each model. To assess model fit, we used the Somers' *D*, which estimates the rank correlation of the predicted probabilities (according to the model) and the incidence of ILEF (26). Crude (Model 1) and adjusted (Model 2 or fully adjusted model) models were built to assess the association between each ceramide and ILEF. The fully adjusted models included the following potential confounders: educational level (primary education or less, secondary school, university), BMI (<25, 25–30, ≥30 kg/m²), alcohol and total energy intake (tertiles in both cases), physical activity (tertiles), and presence of chronic conditions, including diabetes, cardiovascular disease, and musculoskeletal disease (yes/no). As an additional analysis to confirm trends, a backward stepwise selection was conducted over the covariates included in the fully adjusted model, removing covariates if the significance level was higher than 0.20 (Model 3).

A similar analysis was conducted over several ratios of ceramides that have been previously assessed in the literature and could have a potential relationship with other health outcomes, such as cardiovascular disease (5,27). These ratios of ceramides were: C16:0/C24:0, C18:0/C24:0, C22:0/C24:0, and C24:1/C24:0.

As a sensitivity analysis conducted over ceramides which were found to be significantly related to ILEF, an interaction term for sex and the corresponding ceramide was included in the fully adjusted model, in order to assess potential different relationships between those ceramide species and ILEF. Regarding age, it was added as a covariate to the fully adjusted model in order to adjust for potential differences between cases and control group when dealing with age as a continuous variable.

Finally, stratified analyses were done by BMI, energy intake, physical activity, glucose level, and systolic blood pressure (below vs above the median value in each case) to assess differential relationships between ceramide species and incidence of ILEF, based on those which previously had shown a significant association with ILEF. The Wald test for the interaction term was used to examine whether the results varied across strata in each variable. Statistical analyses were performed with Stata (version 15.1; Stata Corp., College Station, TX).

Results

Of the 128 participants, 57.8% were women and the mean age of the overall sample was 72.4 years (*SD* = 4.8). Primary education was the highest educational level for 59 participants (46.1% of the sample), while 35 participants (27.3%) completed secondary education. The remaining 34 ones (26.6%) reached university studies. Significant differences by highest educational level were not found between both case and control groups [$\chi^2(2) = 3.2$; *p* = .21]. Additional details on baseline characteristics in both groups are shown in Table 1. Age group was a matching variable in the study design, but a higher mean age was found for cases (74.2 ± 5.9 vs 71.5 ± 3.8 in control group; *p* < .01) when considering age as a continuous variable.

Plasma concentrations of ceramide species among cases and controls are shown in Table 2. Significant differences between

Table 1. Baseline Characteristics (2015) in Cases (impaired lower-extremity function) and Control Group

	Cases (<i>n</i> = 43)	Control Group (<i>n</i> = 85)	<i>p</i> Value
Women, <i>n</i> (%)	25 (58.1)	49 (57.6)	1.00
Age, years: mean ± <i>SD</i>	74.2 ± 5.9	71.5 ± 3.8	<.01
Educational attainment, <i>n</i> (%)			
Primary education or less	24 (55.8)	35 (41.2)	.21
Secondary school	8 (18.6)	27 (31.8)	
University	11 (25.6)	23 (27.1)	
BMI, <i>n</i> (%)			
<25 kg/m ²	13 (30.2)	23 (27.0)	.08
25–30 kg/m ²	14 (32.6)	44 (51.8)	
≥30 kg/m ²	16 (37.2)	18 (21.2)	
Alcohol intake, g/day: mean ± <i>SD</i>	6.2 ± 11.6	7.6 ± 11.2	.52
Glucose level, mg/dL: mean ± <i>SD</i>	97.7 ± 20.7	94.2 ± 17.2	.31
Systolic blood pressure, mm Hg: mean ± <i>SD</i>	138.6 ± 16.2	138.5 ± 16.7	.98
Energy intake, kcal/day: mean ± <i>SD</i>	1915 ± 397	2014 ± 453	.23
Physical activity, METs h/w: mean ± <i>SD</i>	57.6 ± 33.1	68.9 ± 36.2	.09
Diabetes, <i>n</i> (%)	5 (11.6)	8 (9.4)	.70
Cardiovascular disease, <i>n</i> (%)	2 (4.7)	4 (4.7)	.99
Musculoskeletal disease, <i>n</i> (%)	26 (60.5)	37 (43.5)	.07

Notes: BMI = body mass index; METs = metabolic equivalents of task. Unpaired *t* test for continuous variables and chi-squared test for categorical variables were used for statistical comparison.

Table 2. Plasma Ceramides (nmol/mL) in Cases (impaired lower-extremity function) and Control Group

Ceramides	Geometric Mean (95% CI)		<i>p</i> Value
	Cases (<i>n</i> = 43)	Control Group (<i>n</i> = 85)	
C14:0 [§]	5.5 (5.0, 8.9)	4.5 (3.7, 5.3)	.09
C14:1 (n-5)	0.9 (0.5, 1.6)	1.1 (0.9, 1.3)	.25
C15:0 [§]	4.4 (3.5, 5.1)	3.5 (3.0, 4.0)	.25
C16:0	87.0 (79.9, 94.6)	80.0 (75.2, 85.2)	.21
C16:1 (n-7)	4.8 (4.2, 5.6)	4.1 (3.7, 4.7)	.18
C18:0	43.9 (41.1, 47.0)	42.7 (40.7, 44.7)	.52
C18:1 (n-7)	15.9 (15.0, 16.9)	15.9 (14.8, 17.0)	.50
C18:1 (n-9)	118.1 (108.6, 128.4)	111.3 (104.0, 119.0)	.37
C18:2 (n-6)	130.9 (120.3, 142.5)	131.2 (123.1, 139.7)	.98
C18:3 (n-3) [§]	9.7 (8.3, 11.4)	8.9 (8.0, 9.9)	.34
C18:3 (n-6)	1.3 (1.0, 1.5)	1.2 (1.1, 1.4)	.93
C19:0 [§]	4.6 (4.3, 5.0)	4.8 (4.2, 5.4)	.28
C20:0 [§]	8.0 (7.1, 9.1)	7.7 (6.8, 8.6)	.80
C20:1 [§]	10.6 (9.0, 12.5)	9.4 (8.4, 1.0)	.41
C20:2 [§]	9.2 (8.0, 10.5)	8.7 (7.8, 9.7)	.53
C20:3 (n-6)	8.4 (7.7, 9.3)	7.9 (7.3, 8.6)	.43
C20:4 (n-6)	28.9 (25.7, 32.6)	29.9 (27.5, 32.5)	.64
C20:5 (n-3)	1.1 (0.9, 1.4)	1.3 (1.1, 1.6)	.52
C21:0 [§]	3.3 (2.7, 4.0)	3.3 (2.9, 3.7)	.71
C22:0	1.5 (1.4, 1.7)	1.6 (1.5, 1.8)	.50
C22:1 [§]	2.9 (2.3, 3.7)	2.9 (2.5, 3.3)	.32
C22:2 (n-6) [§]	5.6 (4.5, 7.0)	5.9 (5.2, 6.7)	.51
C22:4 (n-6) [§]	8.2 (6.9, 9.6)	8.5 (7.4, 9.8)	.84
C22:5 (n-3)	1.3 (1.0, 1.7)	1.4 (1.2, 1.7)	.49
C22:6 (n-3)	4.6 (3.6, 5.9)	4.5 (3.6, 5.5)	.88
C24:0	1.3 (1.2, 1.5)	1.3 (1.2, 1.4)	.94
C24:1	2.2 (1.9, 2.6)	2.1 (1.8, 2.4)	.87

Notes: CI = confidence interval.

[§]10⁻¹.

cases and controls in ceramides concentrations were not found, although a slight trend toward higher ceramide levels was observed for those who developed ILEF. Mean values and standard deviations on raw ceramide values are shown in [Supplementary Table 1](#). When adjusting for sociodemographics, health behaviors, and chronic diseases, several ceramides showed an association with ILEF ([Table 3](#)). Specifically, higher levels of ceramides C14:0 [OR per *SD* = 1.66, 95% CI = (1.03, 2.68); *p* = .04], C16:0 [OR per *SD* = 1.61, 95% CI = (1.00, 2.59); *p* = .05], and C16:1 (n-7) [OR per *SD* = 1.64, 95% CI = (1.03, 2.60); *p* = .04] were associated with higher risk of ILEF. Similar estimates were observed when using the backward stepwise selection. The fully adjusted models showed an adequate fit, with Somers' *D* values ranging between 0.45 and 0.53 ([Table 3](#)).

Additionally, several ceramide ratios were considered, without significant results observed, although a slight trend toward significance was found between lower values in the C18:0/C16:0 ratio and the risk of ILEF [OR per *SD* = 0.63, 95% CI = (0.39, 1.01); *p* = .06] ([Table 4](#)).

When including age as a continuous variable in the fully adjusted model, similar trends were found for the ceramides which previously showed a significant relationship with ILEF: OR per *SD* = 1.51 [95% CI = (0.91, 2.50)] for C14:0, OR per *SD* = 1.48 [95% CI = (0.91, 2.40)] for C16:0, and OR per *SD* = 1.54 [95% CI = (0.95, 2.49)] for C16:1 (n-7), in the prospective relationship with incident ILEF. Interaction terms for sex and these ceramides were also included for the fully adjusted model and they were not significant: *p* = .91 in the

case of C14:0, *p* = .22 in the case of C16:0, and *p* = .51 in the case of C16:1 (n-7).

Lastly, these ceramide species which showed a significant association with ILEF were considered separately in models stratified by the median of cardiometabolic risk factors and energy and physical activity ([Table 5](#)). A strong relationship was observed between higher levels of ceramides C16:0 [OR per *SD* = 3.94, 95% CI = (1.24, 12.46); *p* = .02] and C16:1 (n-7) [OR per *SD* = 3.21, 95% CI = (1.21, 8.55); *p* = .02], and incidence of ILEF, in those with a higher BMI. In the specific case of C16:1 (n-7), this positive association was only observed among those with higher systolic blood pressure [OR per *SD* = 2.29, 95% CI = (1.08, 4.84); *p* = .03], showing in this case a significant differential relationship with the other stratified subgroup (below the median value) (*p* for interaction = .03).

Discussion

We have found an association between higher levels of plasma ceramides C14:0, C16:0, and C16:1 (n-7) and higher risk of ILEF. These results were observed after adjusting for potential confounders including sociodemographic variables, energy intake, health behaviors, and morbidity. Higher BMI and systolic blood pressure increased the strength of the associations found, suggesting that these ceramides might play a common role in the development of impaired physical function and cardiometabolic diseases. No effect of ratios of ceramides on the incidence of ILEF was found, although a marginal inverse effect was found for the C18:0/C16:0 ratio.

These results are in line with a previous cross-sectional study in 340 clinically normal participants older than 70 ([13](#)), where a set of 8 ceramides (C14:0, C16:0, C18:0, C20:0, C22:0, C23:0, C24:0, and C24:1) were assessed in relation to several gait parameters, based on 5 domains: pace, rhythm, phase, variability, and base of support ([28](#)). The authors found that higher levels of C16:0 were related to slower gait speed, decreased cadence, and increased double support time. Higher levels of C14:0 were only associated with increased double support time. Since we were able to assess incident cases of physical function impairment in a prospective cohort study, our results provide evidence for a longitudinal relationship between ceramide C16:0 and physical function, suggesting that this ceramide may be an early marker of functional decline. In addition, a narrative review of literature has suggested that higher levels of C16:0 might be a biomarker of mental health disorders including depression ([29](#)), Alzheimer's disease ([30](#)), and Parkinson disease ([31](#)). Since both physical function and mental function are closely related, we may hypothesize that ceramide C16:0 modulates the first stages in the pathological process of disability associated with aging.

Regarding the prospective relationship found between ceramides C14:0, C16:0, and C16:1 (n-7), and incident ILEF, the results are going in the same direction that findings related to other age-related conditions. A previous study showed that the intake of saturated fatty acids is related to higher risk of ILEF in older adults ([32](#)). Saturated fatty acids can lead to accumulation of ceramides, which could result in insulin resistance, a major metabolic defect in a health condition as type 2 diabetes ([33,34](#)).

We have not found significant relationships in the remaining ceramide species which were assessed, after adjusting for potential confounders. However, our results should be interpreted with caution since the relatively small sample size, which could be related to potential insufficient statistical power. For example, Murphy et al ([35](#)) found that ceramide C24:1 was inversely correlated with gait speed, one of the components used to assess ILEF in our study. On

Table 3. Conditional Logistic Regression Models for the Association Between Plasma Ceramides and 2-Year Incidence of Impaired Lower-Extremity Function

	Odds Ratio (95% CI) per 1-SD Increase	<i>p</i> Value	Somers' <i>D</i> ** (95% CI)
C14:0			
Model 1	1.45 (0.99, 2.12)	.05	0.14 (−0.07, 0.35)
Model 2	1.68 (1.04, 2.71)	.04*	0.45 (0.26, 0.63)
Model 3	1.46 (0.99, 2.14)	.05	0.21 (0.01, 0.41)
C14:1 (n-5)			
Model 1	0.76 (0.48, 1.22)	.26	0.06 (−0.15, 0.26)
Model 2	0.81 (0.48, 1.38)	.43	0.48 (0.30, 0.66)
Model 3	0.79 (0.49, 1.28)	.34	0.12 (−0.09, 0.33)
C15:0			
Model 1	1.24 (0.86, 1.78)	.24	0.04 (−0.17, 0.25)
Model 2	1.34 (0.87, 2.05)	.19	0.50 (0.32, 0.68)
Model 3	1.27 (0.88, 1.84)	.21	0.17 (−0.04, 0.37)
C16:0			
Model 1	1.35 (0.82, 1.85)	.32	0.06 (−0.15, 0.27)
Model 2	1.64 (1.01, 2.66)	.05*	0.53 (0.35, 0.70)
Model 3	1.34 (0.91, 1.98)	.13	0.17 (−0.04, 0.37)
C16:1 (n-7)			
Model 1	1.22 (0.85, 1.75)	.27	0.04 (−0.17, 0.25)
Model 2	1.64 (1.03, 2.62)	.04*	0.51 (0.34, 0.69)
Model 3	1.25 (0.86, 1.81)	.24	0.13 (−0.08, 0.33)
C18:0			
Model 1	1.17 (0.79, 1.73)	.44	0.03 (−0.19, 0.24)
Model 2	1.12 (0.69, 1.82)	.65	0.48 (0.30, 0.67)
Model 3	1.12 (0.75, 1.68)	.58	0.13 (−0.08, 0.33)
C18:1 (n-7)			
Model 1	0.88 (0.52, 1.48)	.63	−0.05 (−0.26, 0.17)
Model 2	0.82 (0.48, 1.40)	.47	0.48 (0.30, 0.67)
Model 3	0.85 (0.49, 1.46)	.55	0.05 (−0.16, 0.27)
C18:1 (n-9)			
Model 1	1.22 (0.85, 1.77)	.28	0.04 (−0.18, 0.25)
Model 2	1.39 (0.89, 2.17)	.15	0.51 (0.33, 0.70)
Model 3	1.20 (0.82, 1.74)	.35	0.17 (−0.04, 0.38)
C18:2 (n-6)			
Model 1	0.98 (0.67, 1.44)	.93	−0.01 (−0.22, 0.21)
Model 2	1.11 (0.69, 1.81)	.66	0.48 (0.30, 0.66)
Model 3	0.94 (0.63, 1.40)	.75	0.06 (−0.16, 0.28)
C18:3 (n-3)			
Model 1	1.17 (0.82, 1.66)	.40	0.08 (−0.13, 0.28)
Model 2	1.45 (0.96, 2.20)	.08	0.53 (0.35, 0.70)
Model 3	1.26 (0.85, 1.85)	.24	0.21 (−0.00, 0.42)
C18:3 (n-6)			
Model 1	1.02 (0.69, 1.49)	.94	−0.01 (−0.22, 0.21)
Model 2	1.01 (0.64, 1.59)	.96	0.48 (0.29, 0.66)
Model 3	0.98 (0.66, 1.46)	.92	0.08 (−0.13, 0.29)
C19:0			
Model 1	0.61 (0.27, 1.35)	.22	0.07 (−0.15, 0.28)
Model 2	0.49 (0.19, 1.30)	.15	0.51 (0.33, 0.69)
Model 3	0.50 (0.21, 1.20)	.12	0.26 (0.05, 0.47)
C20:0			
Model 1	0.92 (0.64, 1.33)	.67	−0.01 (−0.23, 0.21)
Model 2	0.92 (0.60, 1.40)	.69	0.47 (0.29, 0.66)
Model 3	0.94 (0.65, 1.35)	.72	0.10 (−0.12, 0.31)
C20:1			
Model 1	1.21 (0.84, 1.74)	.30	0.03 (−0.18, 0.25)
Model 2	1.15 (0.74, 1.79)	.54	0.50 (0.32, 0.68)
Model 3	1.17 (0.81, 1.69)	.42	0.11 (−0.10, 0.32)
C20:2			
Model 1	1.10 (0.77, 1.58)	.60	0.02 (−0.20, 0.23)
Model 2	1.40 (0.88, 2.22)	.16	0.49 (0.31, 0.68)
Model 3	1.09 (0.75, 1.59)	.64	0.09 (−0.13, 0.30)

Table 3. Continued

	Odds Ratio (95% CI) per 1-SD Increase	<i>p</i> Value	Somers' <i>D</i> ** (95% CI)
C20:3 (n-6)			
Model 1	1.14 (0.78, 1.66)	.50	−0.01 (−0.23, 0.21)
Model 2	1.25 (0.76, 2.05)	.38	0.49 (0.31, 0.67)
Model 3	1.10 (0.74, 1.63)	.63	0.08 (−0.13, 0.30)
C20:4 (n-6)			
Model 1	0.92 (0.62, 1.35)	.66	0.04 (−0.17, 0.25)
Model 2	0.97 (0.62, 1.52)	.89	0.48 (0.29, 0.66)
Model 3	0.88 (0.58, 1.31)	.52	0.13 (−0.08, 0.34)
C20:5 (n-3)			
Model 1	0.80 (0.55, 1.18)	.27	0.03 (−0.18, 0.25)
Model 2	0.86 (0.54, 1.35)	.51	0.48 (0.29, 0.67)
Model 3	0.78 (0.53, 1.16)	.23	0.14 (−0.07, 0.35)
C21:0			
Model 1	1.02 (0.71, 1.47)	.92	−0.01 (−0.23, 0.20)
Model 2	0.88 (0.57, 1.36)	.58	0.49 (0.30, 0.67)
Model 3	1.07 (0.74, 1.55)	.74	0.07 (−0.14, 0.29)
C22:0			
Model 1	0.84 (0.54, 1.30)	.43	0.03 (−0.18, 0.24)
Model 2	0.83 (0.55, 1.26)	.39	0.47 (0.28, 0.66)
Model 3	0.84 (0.55, 1.28)	.41	0.08 (−0.13, 0.30)
C22:1			
Model 1	0.83 (0.55, 1.25)	.37	0.01 (−0.20, 0.23)
Model 2	0.71 (0.45, 1.13)	.15	0.50 (0.32, 0.68)
Model 3	0.76 (0.50, 1.15)	.19	0.18 (−0.02, 0.38)
C22:2 (n-6)			
Model 1	1.17 (0.82, 1.68)	.39	0.03 (−0.18, 0.24)
Model 2	1.12 (0.73, 1.72)	.60	0.47 (0.29, 0.66)
Model 3	1.15 (0.80, 1.65)	.46	0.13 (−0.08, 0.33)
C22:4 (n-6)			
Model 1	0.88 (0.60, 1.31)	.53	0.03 (−0.18, 0.25)
Model 2	0.83 (0.53, 1.31)	.42	0.48 (0.30, 0.67)
Model 3	0.91 (0.61, 1.36)	.65	0.11 (−0.11, 0.32)
C22:5 (n-3)			
Model 1	0.91 (0.63, 1.32)	.63	0.02 (−0.20, 0.23)
Model 2	1.04 (0.66, 1.63)	.87	0.48 (0.29, 0.66)
Model 3	0.97 (0.66, 1.41)	.86	0.09 (−0.12, 0.30)
C22:6 (n-3)			
Model 1	0.98 (0.67, 1.42)	.90	−0.01 (−0.23, 0.21)
Model 2	1.01 (0.66, 1.56)	.95	0.48 (0.30, 0.66)
Model 3	0.92 (0.63, 1.35)	.67	0.10 (−0.11, 0.32)
C24:0			
Model 1	0.93 (0.63, 1.36)	.70	−0.01 (−0.23, 0.21)
Model 2	0.93 (0.60, 1.45)	.75	0.48 (0.29, 0.66)
Model 3	0.94 (0.64, 1.36)	.73	0.06 (−0.15, 0.28)
C24:1			
Model 1	1.06 (0.73, 1.55)	.75	−0.02 (−0.23, 0.21)
Model 2	1.29 (0.80, 2.09)	.29	0.49 (0.31, 0.68)
Model 3	0.98 (0.66, 1.46)	.94	0.07 (−0.15, 0.28)

Notes: CI = confidence interval. Model 1 was a crude model, without adjusting for any covariate. Model 2 was adjusted for level of education (primary education or less, secondary school, university), body mass index (<25, 25–30, ≥30 kg/m²), alcohol intake (tertiles), energy intake (tertiles), presence of chronic conditions (including diabetes, cardiovascular disease, and musculoskeletal disease) (yes/no), and physical activity (tertiles). Model 3 was adjusted using backward stepwise selection over the full list of covariates considered in Model 2. After running the backward stepwise selection, presence of musculoskeletal disease was included in the final model in all cases; level of education was included in the Model 3 for C18:3 (n-3) and C19:0, while physical activity and alcohol intake were included in the Model 3 for C19:0 and C22:1, respectively.

**The Somers' *D* was used as an indicator of model fit in each case, providing an estimate of the rank correlation of the predicted probabilities and the incidence of physical impairment.

**p* < .05.

Table 4. Conditional Logistic Regression Models for the Association Between Ratios of Selected Plasma Ceramides and 2-Year Incidence of Impaired Lower-Extremity Function

	Odds Ratio (95% CI) per 1-SD Increase	p Value
C16:0/C24:0		
Model 1	1.08 (0.76, 1.54)	.67
Model 2	1.09 (0.71, 1.68)	.69
Model 3	1.08 (0.75, 1.56)	.66
C18:0/C16:0		
Model 1	0.75 (0.51, 1.11)	.15
Model 2	0.63 (0.39, 1.01)	.06
Model 3	0.74 (0.49, 1.10)	.14
C18:0/C24:0		
Model 1	0.96 (0.66, 1.41)	.84
Model 2	0.84 (0.50, 1.40)	.51
Model 3	0.96 (0.64, 1.43)	.83
C22:0/C24:0		
Model 1	0.74 (0.42, 1.30)	.29
Model 2	0.72 (0.37, 1.39)	.32
Model 3	0.72 (0.39, 1.33)	.29
C24:1/C24:0		
Model 1	0.98 (0.68, 1.42)	.91
Model 2	1.05 (0.66, 1.65)	.85
Model 3	0.92 (0.61, 1.39)	.68

Notes: CI = confidence interval. Model 1 was a crude model, without adjusting for any covariate. Model 2 was adjusted for level of education (primary education or less, secondary school, university), body mass index (<25, 25–30, ≥30 kg/m²), alcohol intake (tertiles), energy intake (tertiles), presence of chronic conditions (including diabetes, cardiovascular disease, and musculoskeletal disease) (yes/no), and physical activity (tertiles). Model 3 was adjusted using backward stepwise selection over the full list of covariates considered in Model 2. After running the backward stepwise selection, presence of musculoskeletal disease was included in the final model in all cases.

the other hand, we have adjusted several models in our study to check whether the trends found were similar beyond the potential confounders included in each model. Sensitivity analyses by age and sex were provided for those ceramides which showed a significant relationship, as described below.

Age group and sex were the matching variables in this case-control design and therefore they were not included as covariates in the conditional logistic regression models proposed. A previous study found higher intraindividual increases in ceramides with age for women compared to men (36). Since sex and age could influence longitudinal changes in circulating ceramides (37), sensitivity analyses for the effect of age and sex on the relationship studied were conducted. Our results suggest that there was not a differential effect of C14:0, C16:0, and C16:1 (n-7) on incident ILEF based on sex. On the other hand, a similar trend in the results obtained was observed when adding age as a covariate into the fully adjusted model considered in this research.

The present study also explored the prospective relationship between some ratios of ceramides and incident ILEF. A marginal inverse effect on the incidence of ILEF was found for the C18:0/C16:0 ratio, although in general terms no significant relationships were found for the ratios of ceramides assessed. Some plasma ceramide ratios have been previously found as predictors of dementia risk (38) and cardiovascular death (27). Further studies are needed to assess the potential relationship between ratios of ceramides and functional decline.

The strongest associations between levels of ceramides C16:0 and C16:1 (n-7) and physical impairment were found in those participants with higher BMI and higher systolic blood pressure. The result obtained when stratifying by BMI could be due to the potential role of ceramides modulating the relationship between obesity and diabetes; specifically, C16:0 seems to be an important mediator of obesity-derived insulin resistance and lower levels could regulate the development of obesity and type 2 diabetes (39). In addition, circulating ceramides could have a similar effect than cholesterol on cardiovascular disease (27,40), and the role of diet has just been explored on the relationship between ceramides and cardiovascular risk. For example, Wang et al (5) showed that the association between a higher level of ceramides and risk of cardiovascular disease could be mitigated by a Mediterranean diet, while a Nordic diet could lead to decreased ceramide levels (41). Further research should assess the role of healthy dietary patterns on the association between ceramides and physical function.

A strength of this study was the use of incident cases from a prospective cohort study, as well as the objective assessment of ILEF, based on the Short Physical Performance Battery test. The use of incident cases as persons who develop the health outcome during the observation period is a widely employed approach in this type of study designs (42). In fact, it allows for keeping the temporal association between the ceramide exposure and the ILEF outcome in longitudinal studies (eg, it avoids bias associated with the use of prevalent cases) while making an efficient use of the stored plasma samples. The relatively small sample size should be highlighted as one of the main limitations of the present study, although our sample size is in line with previous metabolomics research carried out with small-to-moderate sample sizes (13,41,43), because of the high cost of analyzing metabolomic profiles. Moreover, ceramide measurement has been conducted by liquid chromatography-tandem mass spectrometry, which is a specific and sensitive method (44). As an additional limitation, multiple comparisons were conducted, considering different models for each ceramide. For this reason, crude and fully adjusted models, a model adjusted using stepwise selection, and additional sensitivity analyses by sex and age (the matching variables in this study), were run in order to confirm the trends found in the results obtained, beyond the statistical significance. For adjusted models, 2 different approaches were considered, obtaining similar results: while stepwise selection is based on statistical significance of covariates to increase statistical power, the fully adjusted model includes a fixed set of covariates which are potential confounders in the relationship studied and allows for potential comparisons across models, since the same set of covariates is being considered. This said, further longitudinal studies across a similar set of ceramide species should be conducted to confirm the prospective relationships found.

In conclusion, our findings support the hypothesis that several ceramides influence physical function impairment in older adults. Higher levels of ceramide species have been previously identified as early markers of cardiometabolic and neurodegenerative diseases, and the results obtained in the present study suggest that ceramides C14:0, C16:0, and C16:1 (n-7) can be used as predictive biomarkers of ILEF. Since the use of incident cases of ILEF as cases in the case-control design, our results provide evidence for a higher risk of developing ILEF associated with higher levels in the ceramides abovementioned. This research explores for the first time the prospective relationship between ceramides and ILEF in older adults and, although the results should be interpreted with caution since the relatively small sample size, our findings could help to disentangle

Table 5. Conditional Logistic Regression Models for the Association Between Significant Ceramide Species and 2-Year Incidence of Impaired Lower-Extremity Function, Stratified by the Median Value of Cardiometabolic Risk Factors and Health Behaviors

	C14:0 Odds Ratio (95% CI) per 1-SD Increase	C16:0 Odds Ratio (95% CI) per 1-SD Increase	C16:1 (n=7) Odds Ratio (95% CI) per 1-SD Increase
Body mass index			
<26.92 kg/m ²	1.44 (0.84, 2.47)	1.20 (0.72, 2.01)	1.19 (0.70, 2.01)
≥26.92 kg/m ²	1.88 (0.75, 4.68)	3.94* (1.24, 12.46)	3.21* (1.21, 8.55)
<i>p</i> Value for interaction	.27	.09	.21
Glucose level			
<91 mg/dL	1.24 (0.57, 2.71)	1.19 (0.51, 2.78)	1.20 (0.54, 2.67)
≥91 mg/dL	1.53 (0.85, 2.78)	1.86 (0.90, 3.86)	1.62 (0.84, 3.12)
<i>p</i> Value for interaction	.28	.18	.20
Systolic blood pressure			
<139.25 mm Hg	0.77 (0.34, 1.75)	0.82 (0.37, 1.80)	0.61 (0.28, 1.36)
≥139.25 mm Hg	1.76 (0.87, 3.56)	1.74 (0.79, 3.83)	2.29* (1.08, 4.84)
<i>p</i> Value for interaction	.20	.13	.03*
Energy intake			
<1924 kcal/day	1.53 (0.81, 2.88)	1.81 (0.86, 3.78)	1.26 (0.71, 2.24)
≥1924 kcal/day	1.45 (0.74, 2.83)	1.24 (0.69, 2.24)	1.27 (0.62, 2.60)
<i>p</i> Value for interaction	.85	.46	.99
Physical activity			
<59 METs h/w	1.41 (0.84, 2.36)	1.43 (0.81, 2.53)	1.34 (0.77, 2.32)
≥59 METs h/w	2.27 (0.94, 5.51)	1.52 (0.71, 3.27)	1.17 (0.58, 2.34)
<i>p</i> Value for interaction	.33	.33	.38

Notes: CI = confidence interval; METs = metabolic equivalents of task.

Significant ceramides considered were those which shown a significant relationship with the incidence of impaired lower-extremity function in the previous regression models. Stratified conditional logistic regression models were adjusted for level of education (primary education or less, secondary school, university), body mass index (<25, 25–30, ≥30 kg/m²), alcohol intake (tertiles), energy intake (tertiles), diabetes, cardiovascular disease, musculoskeletal disease (yes/no), and physical activity (tertiles). The stratification variable was not considered as a covariate in the corresponding cases.

**p* < .05.

the role of ceramides in functional decline and other determinants of unhealthy aging.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Author Contributions

The authors' responsibilities were as follows: E.L.-G. created the study concept and design; F.F.C. provided statistical expertise and drafted the manuscript; E.L.-G. supervised the work; E.L.-G. obtained funding; E.A.S., A.B., and F.R.-A. provided input and critical feedback after reviewing the first draft. All authors have read and approved the manuscript as submitted and are prepared to take public responsibility for the work. The funding agencies had no role in study design, data analysis, interpretation of results, manuscript preparation, or in the decision to submit this manuscript for publication.

Conflict of Interest

None declared.

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