Serum sphingolipids and incident diabetes in a US population with high diabetes burden: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL)

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ABSTRACT

Background: Genetic or pharmacological inhibition of de novo sphingolipid synthases prevented diabetes in animal studies.

Objectives: We sought to evaluate prospective associations of serum sphingolipids with incident diabetes in a population-based cohort.

Methods: We included 2010 participants of the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) aged 18–74 y and were free of diabetes and other major chronic diseases at baseline (2008–2011). Metabolomic profiling of fasting serum was performed using a global, untargeted approach. A total of 43 sphingolipids were quantified and, considering subclasses and chemical structures of individual species, 6 sphingolipid scores were constructed. Diabetes status was assessed using standard procedures including blood tests. Multivariable survey Poisson regressions were applied to estimate RR and 95% CI of incident diabetes associated with individual sphingolipids or sphingolipid scores.

Results: There were 224 incident cases of diabetes identified during, on average, 6 y of follow-up. After adjustment for socioeconomic and lifestyle factors, a ceramide score ($RR_{Q4\ versus\ Q1} = 2.40$; 95% CI: 1.24, 4.65; *P*-trend = 0.003) and a score of sphingomyelins with fully saturated sphingoid-fatty acid pairs ($RR_{Q4\ versus\ Q1} = 3.15$; 95% CI: 1.75, 5.67; *P*-trend <0.001) both were positively associated with risk of diabetes, whereas scores of glycosylceramides, lactosylceramides, or other unsaturated sphingomyelins (even if having an SFA base) were not associated with risk of diabetes. After additional adjustment for numerous traditional risk factors (especially triglycerides), both associations were attenuated and only the saturated-sphingomyelin score remained associated with risk of diabetes ($RR_{Q4\ versus\ Q1} = 1.98$; 95% CI: 1.09, 3.59; *P*-trend = 0.031).

Conclusions: Our findings suggest that a cluster of saturated sphingomyelins may be associated with elevated risk of diabetes beyond traditional risk factors, which needs to be verified in other population studies. This trial was registered at clinicaltrials.gov as NCT02060344. *Am J Clin Nutr* 2020;00:1–9.

Keywords: diabetes, Hispanic Americans, lipids, risk factors, sphingolipids

Introduction

Sphingolipids are a class of lipids having crucial roles in many biological functions (1–3). Ceramides, which structurally comprise a sphingoid backbone and a fatty acid group linked by an amide bond (**Supplementary Figure 1**), are the key precursor for these bioactive lipids (**Supplementary Figure 2**) (4, 5). Ceramides can be glycosylated to form glycosphingolipids or acquire a polar head group to form sphingomyelin (SM) (4, 5). Multiple lines of evidence suggest ceramides or their metabolites (in blood and/or tissue) as important drivers of metabolic dysfunction that may lead to cardiometabolic diseases (4, 5). In studies of animal models, genetic or pharmacological inhibition of de novo ceramide synthases improved glycemic control, reversed diet-induced obesity and insulin resistance, and prevented the development of diabetes (4–7). Similarly, accumulations of other sphingolipids such as SM have also been implicated to be metabolically detrimental (8, 9). Potential mechanistic actions of sphingolipids as toxic intermediators, such as downregulation of serine/threonine protein kinase B, have also been revealed (4, 5).

Remarkable technical advances in metabolomics afford many opportunities for deciphering metabolomic signatures of diabetes in humans (10). Although there is high biological plausibility for the unfavorable metabolic consequence of

sphingolipid accumulation, scant population data are available characterizing sphingolipid-associated metabolic risk in humans. Small cross-sectional studies have shown elevated concentrations of ceramides in obesity and diabetes (11, 12), whereas several prospective studies assessing the associations between sphingolipids and risk of diabetes yielded mixed findings (13–18). In this study, using lipidomic data quantified in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), we sought to better understand the prospective association between serum sphingolipids and risk of diabetes.

Methods

Study design and population

The HCHS/SOL is a prospective population-based study of 16,415 Hispanic/Latino adults aged 18–74 y at recruitment who were living in 4 US metropolitan areas (Bronx, NY; Chicago, IL; Miami, FL; and San Diego, CA). Participants were recruited by using a 2-stage probability sample design, as described previously (19, 20). A comprehensive battery of interviews and a clinical assessment with fasting blood draw were conducted by trained and certified staff at in-person clinic visits from 2008 to 2011 (visit 1). The second visit (visit 2) period started in October 2014 and concluded in December 2017. A total of 11,623 cohort members were re-examined in visit 2 to collect data predictive of cardiopulmonary outcomes and the onset of diabetes. The study was approved by the Institutional Review Boards at all participating institutions, and all participants gave written informed consent.

Sample collection and metabolomic profiling

At both study visits 1 and 2, participants were asked to fast for ≥8 h before the examination, consume only water and necessary medications, and to refrain from smoking or physical activity before undergoing the fasting examination procedures. Venous blood samples were collected, processed, and frozen (at −70°C) on-site toward the beginning of the visit.

A total of 3972 participants randomly selected from the whole study constituted the current subsample for metabolomic profiling in the HCHS/SOL. On the basis of discoveryHD4 platform at Metabolon Inc., quantification of serum metabolites was achieved by using an untargeted LC-MS-based metabolomic quantification protocol. More detailed experimental information on MS analysis, identification and classification of metabolites, and quality-control processes is reported in the **Supplementary Methods**. In total, 43 sphingolipids were captured by the platform and individual species were identified according to headgroups and total numbers of acyl carbon atoms and acyl double bonds. Of these, there were 3 ceramide, 5 glycosylceramide (GlyCer), 3 lactosylceramide (LacCer), and 32 SM species. For 21 of the 32 SMs, the platform identified 2 to 4 possible sphingoid-fatty acid pairs but was unable to unambiguously characterize the proportion of each pair (**Supplementary Table 1**). Nevertheless, previous results from the Lipid MAPS consortium which systematically fragmented each analyte provided the proportion for all these SMs (**Supplementary Table 2**) (21).

Covariates and other laboratory measurements

Structured questionnaires were administered by trained and bilingual interviewers to collect information on socioeconomic and demographic characteristics including Hispanic/Latino background, diet and lifestyle factors, and medical and family histories. Dietary total energy intake and the Alternative Healthy Eating Index (AHEI)-2010 score were computed using the National Cancer Institute methodology on the basis of data collected by 2 24-h dietary recalls with ∼1 mo apart in addition to a food propensity questionnaire (22). Physical activity was measured using the Global Physical Activity Questionnaire and data were summarized in metabolic equivalent-minutes/day (23). Blood pressure and anthropometric measurements were performed following standardized protocols (24). BMI was calculated as weight in kilograms divided by height in meters squared. Centralized laboratory tests were also performed to determine participants' fasting plasma glucose (FPG), fasting insulin, hemoglobin A1c (HbA1c), and fasting serum triglycerides, total and HDL cholesterol, and C-reactive protein (CRP). For participants without self-reported diabetes or with FPG ≤150 mg/dL, measurement of 2-h plasma glucose was also performed following a standard

The Hispanic Community Health Study/Study of Latinos is a collaborative study supported by contracts from the National Heart, Lung, and Blood Institute (NHLBI) to the University of North Carolina (HHSN268201300001I/N01-HC-65233), University of Miami (HHSN268201300004I/N01-HC-65234), Albert Einstein College of Medicine (HHSN268201300002I/N01-HC-65235), University of Illinois at Chicago (HHSN268201300003I/N01-HC-65236 Northwestern Univ), and San Diego State University (HHSN268201300005I/N01-HC-65237). The following Institutes/Centers/Offices have contributed to the HCHS/SOL through a transfer of funds to the NHLBI: National Institute on Minority Health and Health Disparities, National Institute on Deafness and Other Communication Disorders, National Institute of Dental and Craniofacial Research, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Neurological Disorders and Stroke, and NIH Institution-Office of Dietary Supplements . This work is supported by the NIDDK R01DK119268, and other funding sources for this study include UM1 HG008898 from the National Human Genome Research Institute; K01HL129892, R01HL060712, R01HL140976, and R01HL136266 from the NHLBI; and R01DK120870 and the New York Regional Center for Diabetes Translation Research (P30 DK111022) from the NIDDK.

Data described in the manuscript, code book, and analytic code will be made available upon request pending application.

Supplemental Methods, Supplemental Tables 1–4, and Supplemental Figures 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AHEI, Alternative Healthy Eating Index; CRP, C-reactive protein; FDR, false discovery rate; FHS, Framingham Heart study; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; GlyCer, glycosylceramide; HCHS/SOL, Hispanic Community Health Study/Study of Latinos; LacCer, lactosylceramide; OGTT, oral-glucose-tolerance test; SM, sphingomyelin.

Received November 13, 2019. Accepted for publication April 27, 2020.

First published online 0, 2020; doi: https://doi.org/10.1093/ajcn/nqaa114.

75-g 2-h oral-glucose-tolerance test (OGTT). HOMA-IR was derived using a common equation based on fasting glucose and insulin to evaluate for potentially impaired glucose tolerance (25).

Diabetes ascertainment

Diabetes cases were identified according to the following American Diabetes Association criteria applied to centrally measured laboratory tests: *1*) FPG ≥126 mg/dL; *2*) 2-h OGTT plasma glucose ≥200 mg/dL; and *3*) HbA1c ≥6.5%. In addition to laboratory test criteria, data on self-reported physician diagnosis or current use of antidiabetic medications were also used to identify additional diabetes cases. Based on these criteria, participants free of diabetes at visit 1 who were identified as having diabetes at visit 2 were deemed to be incident diabetes.

Sphingolipid score calculation

Considering subclasses and chemical structures of sphingolipids, we constructed 6 sphingolipid scores including a ceramide score, a GlyCer score, a LacCer score, and 3 SM scores. Each score was computed by summing the inverse normally transformed concentrations of the sphingolipids making up the score (26). The 3 SM scores took into account saturation of each SM species and included: *1*) an SM (0 db) score consisting of 5 SMs with fully saturated sphingoid-fatty acid pairs; *2*) an SM (1 db) score comprising 12 SMs with a single double bond, and for all but 1 the double bond belongs to the sphingoid base; and *3*) a SM (2+ db) score including 15 SMs, with each totaling 2–4 double bonds. For 12 of the 15 SMs constituting the SM (2+ db) score, the specific number of double bonds in the sphingoid or fatty acid base was uncertain despite confirmed total number of double bonds (Supplementary Table 1); nevertheless, according to results from the Lipid MAPS consortium (21), all leading species for these 12 SMs contain 2 sphingoid-base double bonds (Supplementary Table 2).

Statistical analysis

After excluding participants who had prevalent diabetes ($n = 804$) or cardiovascular disease or cancer ($n = 261$) at visit 1, those who died during follow-up or refused or were unable to attend V2 ($n = 895$), and those missing information on Hispanic background ($n = 2$), 2010 participants remained for the present analysis (**Supplementary Figure 3**). Except for 2 species with an undetectable rate of 20.05% and 19.15%, respectively, missing rates were <10% for the remainder and <0.5% for 35 sphingolipids (Supplementary Table 1). Before all analyses, missing/undetectable values were imputed using half of the lowest detected amount, and sphingolipid concentrations were inverse normally transformed to approximate a normal distribution (26). To account for oversampling of specific population subgroups and nonresponse to the follow-up visit, all analyses incorporated HCHS/SOL complex study design and sampling weights, as described previously (19, 20). Weights were trimmed and calibrated to 2010 US Census characteristics by age, sex, and Hispanic/Latino background in each field center's target population.

Age-adjusted descriptive characteristics of the study population were presented according to incident diabetes status as means (95% CI) or percentages (95% CI) where appropriate. The primary outcome for the current analysis was incident diabetes and the secondary outcomes were cardiometabolic traits measured at baseline. Partial Pearson correlations between individual sphingolipids and between sphingolipids and a number of cardiometabolic traits were computed, adjusting for age, sex, study field center, and Hispanic/Latino background. Multivariable survey Poisson regression models were used to compute RR with 95% CI of diabetes associated with sphingolipids, with each species being analyzed continuously as per SD increment. The yielded *P* values were corrected for the false discovery rate (FDR) using the Benjamini–Hochberg procedure (27). Potential confounders included in the multivariable model were: age, sex, study field center, Hispanic/Latino background, US native status, socioeconomic status (education and annual household income), diet and other lifestyle factors (AHEI-2010 score, dietary total energy intake, smoking status, drinking status, and physical activity), medication use (use of hypertensive or lipid-lowering drugs), fasting time, and family history of diabetes. In addition to multivariable adjustment, exploratory analyses were performed to further adjust for a number of diabetes risk factors (BMI, waist circumference, systolic and diastolic blood pressure, CRP, triglycerides, and HDL cholesterol), both individually and concordantly.

The associations of sphingolipid scores with the risk of diabetes were examined using the aforementioned survey Poisson regressions with time between the 2 visits as offset. The scores were analyzed categorically as quartiles and continuously as per SD increment. *P* values for linear trend were computed by assigning the median for each score quartile as a continuous variable. Potential nonlinearity for the associations was assessed using restricted cubic splines with 3 knots at percentiles 10%, 50%, and 90% of the distribution. A *P* value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero. Additional exploratory analyses adjusting for the aforementioned traditional risk factors for diabetes were performed to evaluate any independent associations. To exclude the impacts of lipid-lowering treatments on the examined associations, sensitivity analyses that excluded participants who were lipid-lowering medication users at baseline were also performed. We tested the potential interaction of the sphingolipid scores with Hispanic/Latino background. We performed additional analyses by limiting to individuals who had prediabetes at baseline. All statistical analyses were performed using R (version 3.3.2; R Foundation) and Stata (version 15.1; StataCorp).

Results

Population characteristics

Age-adjusted baseline population characteristics according to quartile of the SM (0 db) score are reported in **Table 1**. Individuals with a higher SM (0 db) score had a cluster of diabetes risk factors including higher levels of BMI, waist circumference, DBP, triglycerides, and CRP.

TABLE 1 Age-adjusted baseline population characteristics according to quartile of SM (0 db) score in the Hispanic Community Health Study/Study of Latinos[1]

	Quartile for SM (0 db) score				
	Q1	Q2	Q3	Q4	*P* value
Age, y	34.0 (32.3, 35.8)	39.2 (37.2, 41.1)	40.6 (38.6, 42.3)	40.9 (39.4, 42.4)	<0.001
Male, %	62.5 (56.8, 67.8)	50.1 (44.5, 55.8)	47.5 (41.4, 53.7)	41.8 (36.0, 48.0)	<0.001
Study field center					
Bronx	37.4 (31.0, 44.3)	28.2 (23.4, 33.5)	27.4 (22.0, 33.6)	28.1 (22.6, 34.3)	0.035
Chicago	12.7 (9.9, 16.3)	14.6 (11.5, 18.5)	11.4 (8.3, 15.5)	12.6 (9.4, 16.7)	
Miami	33.4 (27.2, 40.3)	31.8 (25.7, 38.5)	33.5 (27.7, 40.0)	29.9 (24.4, 36.1)	
San Diego	16.5 (12.0, 22.1)	25.4 (20.2, 31.6)	27.6 (22.2, 33.9)	29.4 (23.3, 36.3)	
Hispanic background, %					
Dominican	14.6 (10.1, 20.7)	10.9 (8.0, 14.6)	9.2 (6.6, 12.8)	10.1 (7.0, 14.3)	0.15
Central/South American	15.1 (11.9, 19.1)	13.0 (9.6, 17.2)	9.6 (7.3, 12.6)	12.1 (9.0, 16.1)	
Cuban	20.9 (16.1, 26.7)	23.1 (18.2, 28.9)	24.5 (19.3, 30.5)	21.3 (16.5, 27.0)	
Mexican	24.7 (20.1, 30.1)	34.1 (28.5, 40.2)	36.8 (31.2, 42.9)	34.4 (28.6, 40.7)	
Puerto-Rican	20.2 (15.6, 25.8)	16.7 (13.4, 20.7)	14.9 (11.3, 19.5)	18.2 (13.5, 24.1)	
Other/>1 heritage	4.4 (2.7, 7.0)	2.2 (1.1, 4.4)	4.9 (2.3, 10.3)	3.9 (1.9, 8.0)	
US native, %	29.1 (24.1, 34.6)	32.1 (27.1, 37.5)	24.5 (19.4, 30.5)	35.8 (29.7, 42.3)	0.037
Above high school education, %	38.1 (32.7, 43.9)	43.1 (37.5, 48.8)	44.5 (38.0, 51.1)	49.9 (43.9, 55.8)	0.34
Family yearly income, %					
<$30,000	61.3 (55.5, 66.9)	49.1 (43.4, 54.8)	58.7 (52.9, 64.3)	58.1 (51.5, 64.4)	0.040
≥$30,000	32.4 (27.4, 37.9)	43.4 (37.9, 49.0)	37.6 (32.0, 43.5)	35.4 (29.4, 41.8)	
Not reported	6.2 (4.1, 9.4)	7.5 (4.6, 11.8)	3.7 (2.1, 6.3)	6.6 (4.2, 10.2)	
Current smoker, %	24.1 (19.2, 29.7)	25.3 (20.5, 30.8)	16.7 (12.8, 21.5)	17.3 (13.3, 22.2)	0.12
Current drinker, %	54.7 (49.1, 60.2)	56.8 (51.0, 62.4)	55.3 (48.9, 61.5)	54.4 (48.2, 60.6)	0.87
Total energy intake, kcal/d	2101 (2029, 2173)	2058 (1972, 2145)	1965 (1891, 2040)	2016 (1930, 2104)	0.048
AHEI-2010 score	46.2 (45.4, 47.0)	46.7 (45.9, 47.5)	47.1 (46.2, 48.1)	47.0 (46.0, 48.0)	0.16
Physical activity, MET-min/d	11.9 (9.3, 14.4)	11.6 (9.5, 13.6)	12.7 (10.8, 14.7)	14.3 (11.0, 17.5)	0.22
Use of antihypertensive drugs, %	5.5 (3.6, 8.4)	9.5 (6.8, 13.1)	11.5 (8.4, 15.6)	12.5 (9.6, 16.0)	<0.001
Use of lipid-lowering drugs, %	6.4 (3.9, 10.3)	6.7 (4.4, 9.9)	5.3 (3.5, 8.1)	5.6 (3.5, 8.8)	0.66
Family history of diabetes, %	38.9 (33.7, 44.3)	35.0 (29.6, 40.8)	38.2 (32.5, 44.2)	40.8 (34.8, 47.1)	0.27
Fasting time, hours	14.4 (13.9, 14.9)	14.4 (14.1, 14.8)	14.2 (13.8, 14.6)	14.4 (14.0, 14.8)	0.84
Risk factors					
BMI, kg/m^2	26.6 (26.0, 27.2)	28.2 (27.5, 28.9)	30.1 (29.4, 30.9)	31.0 (30.1, 31.9)	<0.001
Waist circumference, cm	90.7 (89.1, 92.2)	94.6 (93.1, 96.2)	98.9 (97.2, 100.7)	101.0 (99.1, 102.9)	<0.001
SBP, mmHg	118.1 (116.4, 119.7)	115.4 (113.7, 117.1)	119.6 (117.7, 121.6)	118.3 (116.6, 120.1)	0.20
DBP, mmHg	70.0 (68.8, 71.2)	70.1 (68.9, 71.2)	74.5 (73.1, 75.8)	73.6 (72.1, 75.0)	<0.001
Triglycerides, mg/dL	106.3 (98.4, 114.2)	123.8 (113.7, 133.9)	125.8 (117.9, 133.6)	136.6 (126.1, 147.0)	<0.001
HDL cholesterol, mg/dL	48.4 (46.9, 50.0)	49.2 (47.6, 50.9)	50.6 (49.1, 52.2)	49.2 (47.5, 50.9)	0.31
CRP, mg/L	2.5 (2.0, 2.9)	4.3 (2.9, 5.8)	3.5 (3.0, 3.9)	4.1 (3.5, 4.7)	0.004

AHEI, Alternative Healthy Eating Index; CRP, C-reactive protein; DBP, diastolic blood pressure; MET, metabolic equivalent; Q, quartile; SBP, systolic blood pressure; SM, sphingomyelin.

[1] All data are age-adjusted mean (95% CI) for continuous variables or percentage (95% CI) for categorical variables.

During, on average, 6 y of follow-up, 224 incident diabetes cases were identified from the 2012 analytic samples. **Supplementary Table 3** presents age-adjusted baseline population characteristics according to incident diabetes status at the follow-up visit.

Sphingolipids and cardiometabolic traits

Supplementary Figure 4 depicts distinct intercorrelations between individual sphingolipids after the adjustment for age, sex, study field center, and Hispanic/Latino background. Most sphingolipid species were positively associated with each other. Sphingolipids in 1 subclass showed weak to moderate correlations with those in another subclass, whereas there were moderate to substantial correlations between sphingolipids within a certain subclass.

The associations between individual sphingolipids and cardiometabolic traits are shown in **Supplementary Figure 5**. Some SM species, especially 3 saturated SMs including SM (36:0), SM (38:0), and SM (d18:0/22:0) showed moderate and positive correlations with obesity and several glycemic traits including FPG and HOMA IR ($r \sim 0.30$ to 0.40), and the strongest correlations were observed between the 3 ceramide species and triglycerides ($r \sim 0.40$ to 0.50). Correlations between the 6 sphingolipid scores and cardiometabolic traits are presented in **Figure 1**, with the strongest correlation observed between the ceramide score and triglycerides ($r = 0.52$).

Sphingolipids and risk of diabetes

After adjustment for potential confounders and correction for multiple testing, significant associations with diabetes risk

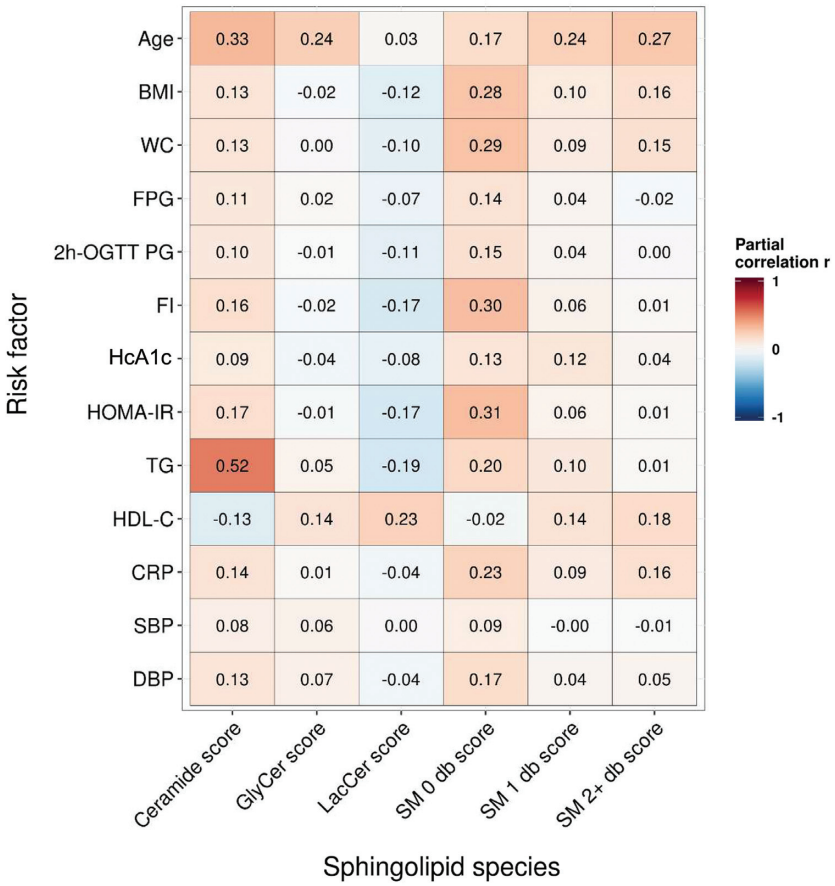

FIGURE 1 Correlations between sphingolipid scores and cardiometabolic traits. CRP, C-reactive protein; db, ; DBP, diastolic blood pressure; FI, ; FPG, fasting plasma glucose; GlyCer, glycosylceramide; HbA1c, hemoglobin A1c; LacCer, lactosylceramide; OGTT, oral-glucose-tolerance test; SBP, systolic blood pressure; SM, sphingomyelin; WC, waist circumference; TG, triglycerides. Data are partial Pearson correlation coefficients with adjustment for age, sex, study field center, and Hispanic/Latino background.

were observed for 2 ceramides (d18:1/16:0 and d18:1/18:0), GlyCer (d18:1/24:1[2OH]), 3 saturated SMs (36:0, 38:0, and d18:0/22:0), and 2 SMs (d18:1/22:0 and SM d18:1/24:0) carrying a single sphingoid-base double bond (**Figure 2**). Of these 8 sphingolipids, the associations for ceramide (d18:1/18:0) and for 3 saturated SMs were highly significant (FDR-adjusted P <0.001). After additional adjustment for other diabetes risk factors including general and central adiposity, blood pressure, CRP, HDL cholesterol, and triglycerides, the saturated SMs 36:0 ($RR_{\text{per SD}} = 1.41$; 95% CI: 1.17, 1.69), 38:0 ($RR_{\text{per SD}} = 1.32$; 95% CI: 1.11, 1.56), and d18:0/22:0 ($RR_{\text{per SD}} = 1.45$, 95% CI: 1.24, 1.71) remained significantly associated with risk of diabetes (FDR-adjusted P <0.02).

When sphingolipid scores were analyzed in the multivariable models, higher ceramide score (P-trend = 0.003) and SM (0 db) score (P-trend <0.001) were both significantly associated with increased risk of diabetes (**Table 2**, model 1). When comparing the highest with the lowest score quartiles, RRs of diabetes were 2.40 (95% CI: 1.24, 4.65) for the ceramide score and 3.15 (95% CI: 1.75, 5.67) for the SM (0 db) score. There were no significant associations between GlyCer, LacCer, SM (1 db) (all component SMs contain an SFA base), or SM (2+ db) scores and risk of diabetes (P-trend >0.05)(Table 2).

With additional adjustment for traditional risk factors for diabetes, the association of ceramide score with risk of diabetes was attenuated to be nonsignificant (P-trend = 0.78) (Table 2). In further analyses in which these diabetes risk factors were individually included in the multivariable model, adjustment for triglycerides was found largely responsible for the attenuation of the association for the ceramide score (**Supplementary Figure 6**) and for individual ceramides (data not shown). The positive association of SM (0 db) score with risk of diabetes, despite being attenuated, remained significant after the additional adjustment (P-trend = 0.031), with RRs of 1.98 (95% CI: 1.09, 3.59) comparing extreme quartiles and 1.31 (95% CI: 1.12, 1.53) for each 1 additional SD increment (Table 2 and Supplementary Figure 6). Irrespective of additional adjustment for other diabetes risk factors or not, there was no evidence for nonlinear relations between the 6 sphingolipid scores and risk of diabetes (**Supplementary Figure 7**). The observed associations between sphingolipid scores and risk of diabetes did not differ substantially according to Hispanic/Latino backgrounds (P-interaction >0.05 based on model 2), and they were not altered materially after excluding individuals who were lipid-lowering medication users at the baseline visit (**Supplementary Table 4**).

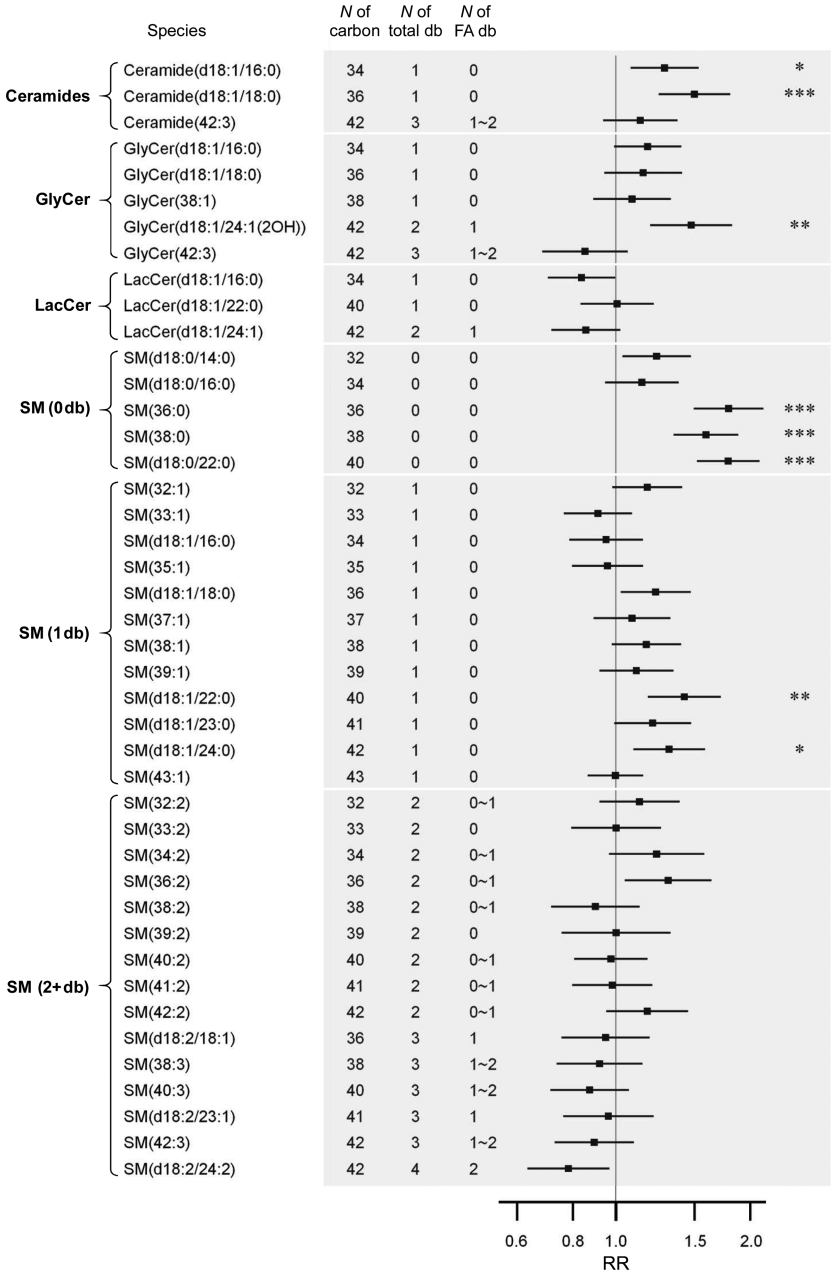

FIGURE 2 Association of individual sphingolipids with incident diabetes. Results were from multivariable survey Poisson regression models with adjustment for field center, age, sex, Hispanic background, US native, education, annual household income, AHEI-2010 score, total energy intake, smoking status, drinking status, physical activity, use of hypertensive or lipid-lowering drugs, fasting time for blood drawn, and family history of diabetes. * $0.05 < P \leq 0.01$, ** $0.05 < P \leq 0.01$, *** $P < 0.001$ after correction for multiple testing. AHEI, Alternative Healthy Eating Index; db, double bonds; FA, fatty acids; GlyCer, glycosylceramide; LacCer, lactosylceramide; SM, sphingomyelin.

Correlations were 0.60 between SM (0 db) and SM (1 db) scores, 0.40 between SM (0 db) and SM (2+ db) scores, and 0.77 between SM (1 db) and SM (2+ db) scores. When the 3 SM scores were simultaneously added to model 2, the RR (per SD) for the SM (0 db) score was slightly strengthened to be 1.42 (95% CI: 1.10, 1.82), whereas those for SM (1 db) ($RR_{\text{per SD}} = 1.07$; 95% CI: 0.74, 1.54) and SM (2+ db) scores ($RR_{\text{per SD}} = 0.72$; 95% CI: 0.50, 1.04) remained nonsignificant.

The SM (0 db) score remained associated with increased risk of diabetes among individuals with prediabetes ($RR_{\text{per SD}}$ based on model 2: 1.33; 95% CI: 1.12, 1.57; 194 incident diabetes cases among 996 participants), but not among those without prediabetes at baseline ($RR_{\text{per SD}} = 0.88$; 95% CI: 0.58, 1.34; 30 incident diabetes cases among 1014 participants). However, the latter was based on a small number of cases and the difference in the association according to prediabetes status at baseline was not significant (P-interaction = 0.35).

The SM (0 db) score was similarly associated with a higher risk of diabetes after excluding 14 incident cases of diabetes that were identified solely by a self-report of physician's diagnosis

TABLE 2 Sphingolipid score and risk of diabetes in the Hispanic Community Health Study/Study of Latinos

	Quartile for sphingolipid score					
	Q1	Q2	Q3	Q4	*P* for trend	Per 1 SD increment
Ceramide score						
Case/participants	30/503	53/502	61/502	80/503		
Model 1 (RR [95% CI])	1.00 (reference)	2.01 (1.00, 2.33)	2.33 (1.15, 4.71)	2.40 (1.24, 4.65)	0.003	1.34 (1.12, 1.61)
Model 2 (RR [95% CI])	1.00 (reference)	1.40 (0.74, 2.68)	1.31 (0.69, 2.49)	1.18 (0.66, 2.10)	0.78	0.99 (0.83, 1.18)
GlyCer score						
Case/participants	45/503	54/502	54/502	71/503		
Model 1 (RR [95% CI])	1.00 (reference)	1.25 (0.67, 2.33)	1.05 (0.58, 1.91)	1.72 (0.97, 3.04)	0.088	1.18 (0.94, 1.47)
Model 2 (RR [95% CI])	1.00 (reference)	1.16 (0.65, 2.10)	1.09 (0.61, 1.95)	1.53 (0.90, 2.59)	0.14	1.14 (0.92, 1.41)
LacCer score						
Case/participants	70/503	42/502	65/502	47/503		
Model 1 (RR [95% CI])	1.00 (reference)	0.47 (0.29, 0.75)	1.07 (0.69, 1.67)	0.74 (0.45, 1.21)	0.67	0.89 (0.75, 1.06)
Model 2 (RR [95% CI])	1.00 (reference)	0.53 (0.33, 0.85)	1.23 (0.79, 1.92)	0.95 (0.58, 1.54)	0.62	0.98 (0.83, 1.17)
SM (0 db) score						
Case/participants	28/503	53/502	54/502	89/503		
Model 1 (RR [95% CI])	1.00 (reference)	2.29 (1.24, 4.22)	2.75 (1.46, 5.19)	3.15 (1.75, 5.67)	<0.001	1.55 (1.32, 1.81)
Model 2 (RR [95% CI])	1.00 (reference)	1.84 (0.98, 3.47)	1.97 (1.06, 3.66)	1.98 (1.09, 3.59)	0.031	1.31 (1.12, 1.53)
SM (1 db) score						
Case/participants	43/503	65/502	51/502	65/503		
Model 1 (RR [95% CI])	1.00 (reference)	1.47 (0.87, 2.49)	1.49 (0.85, 2.60)	1.58 (0.86, 2.90)	0.14	1.14 (0.96, 1.36)
Model 2 (RR [95% CI])	1.00 (reference)	1.25 (0.73, 2.12)	1.23 (0.73, 2.07)	1.41 (0.78, 2.54)	0.28	1.08 (0.89, 1.30)
SM (2+ db) score						
Case/participants	50/503	54/502	68/502	52/503		
Model 1 (RR [95% CI])	1.00 (reference)	0.90 (0.49, 1.62)	1.38 (0.77, 2.48)	0.95 (0.48, 1.89)	0.73	1.00 (0.79, 1.25)
Model 2 (RR [95% CI])	1.00 (reference)	0.87 (0.48, 1.56)	1.10 (0.64, 1.90)	0.72 (0.36, 1.44)	0.57	0.90 (0.71, 1.14)

Results were from multivariable survey Poisson regression models.

Model 1 was adjusted for field center, age, sex, Hispanic background, US native, education, annual household income, AHEI-2010 score, total energy intake, smoking status, drinking status, physical activity, use of hypertensive or lipid-lowering drugs, fasting time for blood drawn, and family history of diabetes.

Model 2 was adjusted for covariates in model 1 and further adjusted for BMI, waist circumference, systolic and diastolic blood pressure, C-reactive protein (log), HDL cholesterol, and triglycerides (log).

AHEI, Alternative Healthy Eating Index; db, double bond (on both sphingoid bases and fatty acids chain of sphingolipids); GlyCer, glycosylceramide; LacCer, lactosylceramide; SM, sphingomyelin.

($RR_{\text{per SD}} = 1.31$; 95% CI: 1.12, 1.55). In addition, the association of the SM (0 db) score with risk of diabetes was attenuated, but not eliminated after further adjustment for fasting plasma insulin ($RR_{\text{per SD}} = 1.23$; 95% CI: 1.04, 1.45) or HOMA-IR ($RR_{\text{per SD}} = 1.20$; 95% CI: 1.02, 1.43).

Discussion

Using unbiased lipidomic data from a population-based prospective study of US Hispanic/Latino adults with, on average, 6 y of follow-up, our analyses systematically assessed lists of sphingolipids and different a priori sphingolipid scores for associations with numerous cardiometabolic biomarkers and risk of diabetes. Distinct correlations between individual sphingolipids and cardiometabolic traits were observed. A ceramide score and a score of saturated SMs were both found to be positively associated with diabetes risk after adjustment for socioeconomic and lifestyle factors. With additional adjustment for numerous traditional risk factors, both associations were attenuated and only the association for the SM score remained significant.

As the precursors for a variety of sphingolipids, ceramides are widely implicated in the metabolic pathways modulating insulin sensitivity and development of cardiometabolic disease (5). Evidence is notable that ceramides with long-chain (e.g. C16:0 and C18:0) rather than very-long-chain fatty acids (e.g. C24:0 or C24:1) might be metabolically toxic (28, 29). In the current study, we initially found both ceramides C16:0 and C18:0 as well as a priori score of 3 ceramides to be significantly associated with elevated risk of diabetes. However, all associations were attenuated to be nonsignificant after further adjustment for various traditional risk factors for diabetes (mainly due to the adjustment for triglycerides). The attenuation of the associations was not surprising given the moderate to high correlation between ceramides and triglycerides and the strong association between triglycerides and risk of diabetes in our study population (multivariable-adjusted RR = 5.19 comparing extreme quartiles, 95% CI: 2.50, 10.79).

Our nontargeted platform quantified only 3 ceramide species and failed to identify other ceramides such as those carrying very-long-chain fatty acids. Notwithstanding, distinct ceramide species have been shown to be substantially correlated with each other (e.g. $r > 0.70$ between ceramides C16:0 and C24:0 [30, 31]), and thus our findings may still provide highly relevant biological insights into the association between ceramide accumulation and risk of diabetes. In line with our findings, other studies (13, 16) in which a larger number of plasma ceramides were quantified by lipidomic platforms have shown no association between numerous ceramide species and risk of diabetes. Although a

positive association between a (serum or plasma) ceramide ratio (d18:1/18:0 to d18:1/16:0) and risk of diabetes was identified and validated in another study of a North European population (15), potential confounding by triglycerides was not addressed.

Notably, ours and other population studies have measured ceramides in serum or plasma where major lipids exist predominantly within lipoprotein particles (1, 2). Thus, the measurements may reflect not only the production or accumulation of ceramides, but also the concentration and metabolism of lipoproteins. The substantial attenuation of the ceramides-diabetes association after the full adjustment in our study may highlight the importance of accounting for other major lipids, especially triglycerides, when assessing the association of circulating ceramides with metabolic risk. Furthermore, given the potentially different role of circulating versus adipose sphingolipid metabolism in obesity (32), future population studies that measure sphingolipids in relevant (e.g. insulin-sensitive) tissues may provide additional insights into the metabolic consequence of sphingolipid accumulation.

GlyCer and LacCer are sphingolipids with an addition of a sugar head to a ceramide scaffold, and have been suggested to be involved in a number of biological processes including regulation of insulin sensitivity (1, 2, 33). In our study, these species were weakly correlated with glycemic traits in the cross-sectional analyses and were not independently associated with incident diabetes.

One novel finding of our study is that only SMs consisting of saturated sphingoid-fatty acid pairs (e.g. d18:0/22:0), but not desaturated SMs (even if containing SFAs, e.g. d18:1/18:0), were independently associated with risk of diabetes. The associations of SMs with diabetes risk reported in several previous studies have been mixed (13, 14, 17, 18). Positive associations were reported for 2 SMs (d16:1/18:0 and d18:1/18:0) in the study of Singapore Chinese (13), whereas the Framingham Heart study (FHS) (18) and a German cohort (14) each identified a specific SM species (d18:1/22:0 in the FHS and d18:1/16:1 in the German cohort) inversely associated with risk of diabetes. In an analysis of at-risk participants included in the PREDIMED trial (17), there was a strong inverse association between an SM score that comprised 11 SMs (d18:1) and risk of diabetes. A direct comparison of findings from these studies may be difficult given the divergent background characteristics of the study population (e.g. general [13, 14, 18] versus at-risk individuals adherent to the Mediterranean diet [17]) and the varying lipidomic approaches, both may have led to differences in types and concentrations of the sphingolipid species identified. It should be noted that these previous studies did not evaluate SMs carrying saturated sphingoid bases (d18:0), which may be due to the relatively small amount of these SMs in human blood (21).

The exact biological mechanisms driving the observed detrimental association between fully saturated SMs and risk of diabetes remain unclear. However, our cross-sectional analyses of baseline data showed that the 3 SMs independently associated with diabetes risk were also moderately and positively correlated with FPG, HOMA IR, and CRP (Supplementary Figure 4). Furthermore, the association between the saturated SM score and risk of diabetes was moderately attenuated after further adjustment for HOMA-IR. These observations may provide some mechanistic supports for a role of SMs in modulating metabolic risk (e.g. by inducing insulin resistance [4–7]).

Strengths of our study include the prospective and population-based design, the collection and measurements of a wide range of covariates, a broad spectrum of serum sphingolipid profiling, and the ascertainment of diabetes according to multiple standard procedures. Some limitations should also be acknowledged when interpreting our findings. Due to the observational nature, our study cannot make causal inference. The global, nontargeted platform we used for metabolomic profiling was unable to unambiguously capture the proportion of sphingoid-fatty acid pairs for some sphingolipids especially SMs (though the proportions have been reported by studies of other populations [21]), neither did it distinguish between glucosylceramides and galactosylceramides (both were referred to as GlyCer). Finally, it is unclear to what extent the association of sphingolipids with diabetes risk could be ethnically specific. Because our analyses were based on US Hispanics/Latinos who have poorer metabolic features and higher diabetes burden than other ethnic groups (34), caution is needed with regards to population-level generalization of our findings.

In summary, in a population-based prospective study of US Hispanics/Latinos, our findings identified SMs with a saturated sphingoid-fatty acid pair to be correlated with numerous cardiometabolic traits and be independently associated with increased risk of diabetes. Additional studies are needed to replicate these associations and to better understand the underlying mechanisms.

We thank the staff and participants of HCHS/SOL for their important contributions. A complete list of HCHS/SOL staff and investigators can be found in (19) or at http://sites.cscc.unc.edu/hchs/.

The authors' contributions were as follows—GCC, JCC, and QQ: designed the study; GCC and JCC: performed statistical analysis; GCC: drafted the manuscript; GAM: contributed to the measurement of metabolites and prepared the methodological descriptions of metabolomic profiling; QQ: had primary responsibility for the final content; and all authors: contributed to interpretation of the results, edited and reviewed the manuscript, and read and approved the final manuscript. GAM is an employee of Metabolon, Inc. and, as such, has affiliations with or financial involvement with Metabolon, Inc. The other authors report no conflicts of interest.

References

1. Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. Nat Rev Endocrinol 2017;13(2):79–91.
2. Iqbal J, Walsh MT, Hammad SM, Hussain MM. Sphingolipids and lipoproteins in health and metabolic disorders. Trends Endocrinol Metab 2017;28(7):506–18.
3. Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. Nat Rev Mol Cell Biol 2018;19(3):175–91.
4. Chaurasia B, Summers SA. Ceramides – lipotoxic inducers of metabolic disorders. Trends Endocrinol Metab 2015;26(10):538–50.
5. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. Cell Metab 2012;15(5):585–94.
6. Bikman BT, Guan Y, Shui G, Siddique MM, Holland WL, Kim JY, Fabrias G, Wenk MR, Summers SA. Fenretinide prevents lipid-induced insulin resistance by blocking ceramide biosynthesis. J Biol Chem 2012;287(21):17426–37.
7. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 2007;5(3):167–79.
8. Claus RA, Dorer MJ, Bunck AC, Deigner HP. Inhibition of sphingomyelin hydrolysis: targeting the lipid mediator ceramide as a key regulator of cellular fate. CMC 2009;16(16):1978–2000.

9. Li Z, Zhang H, Liu J, Liang CP, Li Y, Li Y, Teitelman G, Beyer T, Bui HH, Peake DA, et al. Reducing plasma membrane sphingomyelin increases insulin sensitivity. Mol Cell Biol 2011;31(20):4205–18.
10. Guasch-Ferre M, Hruby A, Toledo E, Clish CB, Martinez-Gonzalez MA, Salas-Salvado J, Hu FB. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Dia Care 2016;39(5):833–46.
11. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, Sanders P, Siddall P, Kuo MS, Perreault L. Serum sphingolipids: relationships to insulin sensitivity and changes with exercise in humans. Am J Physiol Endocrinol Metab 2015;309(4):E398–408.
12. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, Defronzo RA, Kirwan JP. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes 2009;58(2):337–43.
13. Chew WS, Torta F, Ji S, Choi H, Begum H, Sim X, Khoo CM, Khoo EYH, Ong WY, Van Dam RM, et al. Large-scale lipidomics identifies associations between plasma sphingolipids and T2DM incidence. JCI Insight 2019;5:e126925.
14. Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost HG, Fritsche A, Haring HU, Hrabe de Angelis M, Peters A, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 2013;62(2):639–48.
15. Hilvo M, Salonurmi T, Havulinna AS, Kauhanen D, Pedersen ER, Tell GS, Meyer K, Teeriniemi AM, Laatikainen T, Jousilahti P, et al. Ceramide stearic to palmitic acid ratio predicts incident diabetes. Diabetologia 2018;61(6):1424–34.
16. Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, Castro-Perez J, Kozlitina J, Scherer PE. Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the Dallas Heart Study. Diabetologia 2018;61(12):2570–9.
17. Razquin C, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, Papandreou C, Ros E, Estruch R, Guasch-Ferre M, et al. Plasma lipidomic profiling and risk of type 2 diabetes in the PREDIMED trial. Dia Care 2018;41(12):2617–24.
18. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, et al. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. J Clin Invest 2011;121(4):1402–11.
19. Lavange LM, Kalsbeek WD, Sorlie PD, Aviles-Santa LM, Kaplan RC, Barnhart J, Liu K, Giachello A, Lee DJ, Ryan J, et al. Sample design and cohort selection in the Hispanic Community Health Study/Study of Latinos. Ann Epidemiol 2010;20(8):642–9.
20. Sorlie PD, Aviles-Santa LM, Wassertheil-Smoller S, Kaplan RC, Daviglus ML, Giachello AL, Schneiderman N, Raij L, Talavera G, Allison M, et al. Design and implementation of the Hispanic Community Health Study/Study of Latinos. Ann Epidemiol 2010;20(8):629–41.
21. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res 2010;51(11):3299–305.
22. Siega-Riz AM, Sotres-Alvarez D, Ayala GX, Ginsberg M, Himes JH, Liu K, Loria CM, Mossavar-Rahmani Y, Rock CL, Rodriguez B, et al. Food-group and nutrient-density intakes by Hispanic and Latino backgrounds in the Hispanic Community Health Study/Study of Latinos. Am J Clin Nutr 2014;99(6):1487–98.
23. Arredondo EM, Sotres-Alvarez D, Stoutenberg M, Davis SM, Crespo NC, Carnethon MR, Castaneda SF, Isasi CR, Espinoza RA, Daviglus ML, et al. Physical activity levels in U.S. Latino/Hispanic adults: results from the Hispanic community health study/study of Latinos. Am J Prev Med 2016;50(4):500–8.
24. Daviglus ML, Talavera GA, Aviles-Santa ML, Allison M, Cai J, Criqui MH, Gellman M, Giachello AL, Gouskova N, Kaplan RC, et al. Prevalence of major cardiovascular risk factors and cardiovascular diseases among Hispanic/Latino individuals of diverse backgrounds in the United States. JAMA 2012;308(17):1775–84.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.
26. Papandreou C, Bullo M, Ruiz-Canela M, Dennis C, Deik A, Wang D, Guasch-Ferre M, Yu E, Razquin C, Corella D, et al. Plasma metabolites predict both insulin resistance and incident type 2 diabetes: a metabolomics approach within the Prevencion con Dieta Mediterranea (PREDIMED) study. Am J Clin Nutr 2019;109(3):626–34.
27. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Statist 2001;29(4):1165–88.
28. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, Dogra S, Ohman MK, Takeda K, Sugii S, et al. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. Cell Metab 2014;20(4):687–95.
29. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, Mauer J, Xu E, Hammerschmidt P, Bronneke HS, et al. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. Cell Metab 2014;20(4):678–86.
30. Zhao W, Wang X, Deik AA, Hanna DB, Wang T, Haberlen SA, Shah SJ, Lazar JM, Hodis HN, Landay AL, et al. Elevated plasma ceramides are associated with antiretroviral therapy use and progression of carotid artery atherosclerosis in HIV infection. Circulation 2019;139(17):2003–11.
31. Lemaitre RN, Yu C, Hoofnagle A, Hari N, Jensen PN, Fretts AM, Umans JG, Howard BV, Sitlani CM, Siscovick DS, et al. Circulating sphingolipids, insulin, HOMA-IR, and HOMA-B: The Strong Heart Family Study. Diabetes 2018;67(8):1663–72.
32. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. Diabetes 2006;55(9):2579–87.
33. Merrill AH Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chem Rev 2011;111(10):6387–422.
34. Rodriguez CJ, Allison M, Daviglus ML, Isasi CR, Keller C, Leira EC, Palaniappan L, Pina IL, Ramirez SM, Rodriguez B, et al. Status of cardiovascular disease and stroke in Hispanics/Latinos in the United States: a science advisory from the American Heart Association. Circulation 2014;130(7):593–625.