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Plasma Metabolomic Profiles of Glycemic Index, Glycemic Load, and Carbohydrate Quality Index in the PREDIMED Study

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ABSTRACT

Background: The quality of carbohydrate consumed, assessed by the glycemic index (GI), glycemic load (GL), or carbohydrate quality index (CQI), affects the postprandial glycemic and insulinemic responses, which have been implicated in the etiology of several chronic diseases. However, it is unclear whether plasma metabolites involved in different biological pathways could provide functional insights into the role of carbohydrate quality indices in health.

Objectives: We aimed to identify plasma metabolomic profiles associated with dietary GI, GL, and CQI.

Methods: The present study is a cross-sectional analysis of 1833 participants with overweight/obesity (mean age = 67 y) from 2 case-cohort studies nested within the PREDIMED (Prevención con Dieta Mediterránea) trial. Data extracted from validated FFQs were used to estimate the GI, GL, and CQI. Plasma concentrations of 385 metabolites were profiled with LC coupled to MS and associations of these metabolites with those indices were assessed with elastic net regression analyses.

Results: A total of 58, 18, and 57 metabolites were selected for GI, GL, and CQI, respectively. Choline, cotinine, γ-butyrobetaine, and 36:3 phosphatidylserine plasmalogen were positively associated with GI and GL, whereas they were negatively associated with CQI. Fructose-glucose-galactose was negatively and positively associated with GI/GL and CQI, respectively. Consistent associations of 21 metabolites with both GI and CQI were found but in opposite directions. Negative associations of kynurenic acid, 22:1 sphingomyelin, and 38:6 phosphatidylethanolamine, as well as positive associations of 32:1 phosphatidylcholine with GI and GL were also observed. Pearson correlation coefficients between GI, GL, and CQI and the metabolomic profiles were 0.30, 0.22, and 0.27, respectively.

Conclusions: The GI, GL, and CQI were associated with specific metabolomic profiles in a Mediterranean population at high cardiovascular disease risk. Our findings may help in understanding the role of dietary carbohydrate indices in the development of cardiometabolic disorders. This trial was registered at isrctn.com as ISRCTN35739639. *J Nutr* 2020;00:1–9.

Keywords: glycemic index, glycemic load, carbohydrate quality index, metabolomics, PREDIMED

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Introduction

Sustaining a small postprandial increase in blood glucose and consequently insulin concentrations (1) may play a role in the prevention or management of several cardiometabolic disorders (2, 3), including obesity, type 2 diabetes (T2D), cardiovascular diseases (CVDs), and other chronic conditions such as cancer (3). Because carbohydrate is the main dietary component affecting postprandial glycemia (4), 2 indices, the glycemic index (GI) (5) and glycemic load (GL) (6), were introduced to quantify the glycemic response to carbohydrates in different foods and by food serving, respectively. According to previous meta-analyses of prospective studies, diets with high GI and/or high GL have been associated with increased risk of T2D, coronary heart disease, and some types of cancer (3, 7, 8). More recently, the carbohydrate quality index (CQI) was proposed as an index of dietary carbohydrate quality that includes the GI and intakes of total fiber, whole grains, and liquid or solid carbohydrates. A higher CQI has been associated with a lower risk of CVD (9) and lower risks of obesity (10) and hypertension (11).

Although these dietary carbohydrate indices have been related to health outcomes, the underlying mechanisms are not completely understood. The postprandial glycemic and insulinemic response may contribute to disease risk through modulation of several metabolic pathways (12). Consequently, a comprehensive metabolite profiling may provide a deeper understanding of the metabolic response to these indices. Prior studies have identified some circulating metabolites modulated after dietary interventions with differential levels of GI (13, 14) or GL (15, 16). However, to date, limited metabolomic analysis has been conducted using combinations of different metabolomic platforms to cover a wide range of metabolites and examine their association with dietary GI and GL, and none to our knowledge has assessed this issue in relation to the CQI. Identifying metabolites involved in different biological pathways related to these indices might provide new functional insight into their role in health.

Therefore, we used a multiplatform metabolomics approach to identify plasma metabolomic profiles associated with the dietary GI, GL, and CQI in the PREDIMED (Prevención con Dieta Mediterránea) study.

Methods

Study design

This study is a cross-sectional analysis of baseline data from 2 nested case-cohort studies on CVD (17) and T2D (18) within the PREDIMED study (ISRCTN35739639). The PREDIMED study is a multicenter trial examining the efficacy of 2 Mediterranean diet interventions over a control diet, for primary prevention of CVD (19). A detailed description of the PREDIMED trial can be found elsewhere (19, 20). The protocol of the PREDIMED trial was approved by the Research Ethics Committees of all participating centers.

Subject selection

For the present study, participants with available metabolomics data from 2 case-cohort studies (17, 18) were selected. Out of 1882 who completed a validated semiquantitative 137-item FFQ, 1871 participants were included (21). Participants ($n = 34$) who had extreme daily energy intakes (<500 or >3500 kcal/d for women and <800 or >4000 kcal/d for men) were excluded as well as those ($n = 4$) with ≥20% missing values in metabolites, leaving 1833 subjects for further analyses (**Supplemental Figure 1**).

Calculation of nutrient and energy intakes and dietary GI, GL, and CQI

Nutrient and energy intakes were calculated using Spanish food composition tables (22). The validity and reproducibility of the FFQ for the measurements of the high-carbohydrate foods within it have been previously reported (21). The intraclass correlation coefficient between vegetables, potatoes, fruits, cereals, and pastries/cakes/sweets and repeated food records was 0.89, 0.75, 0.76, 0.72, and 0.84, respectively, whereas it was 0.83 for carbohydrates and 0.86 for fiber. GI values for each food were extracted from international GI and GL values (23) with glucose as the reference. For foods that were not in the tables, the mean was calculated for similar foods that were present in the FFQ. The total GL of each diet was determined by multiplying the total carbohydrates of a specified serving size of the food, the total number of food portions consumed per day, and its specific GI and then dividing their sum by 100. GI was calculated by dividing GL by total available carbohydrate intake and multiplying the result by 100 (6, 24). The CQI was defined comprising the following 4 criteria: dietary fiber intake (g/d; positively weighted), GI (negatively weighted), ratio of whole grains to total grains (positively weighted), and ratio of solid carbohydrate to solid carbohydrate + liquid carbohydrate (positively weighted) (25). For each of these 4 components, we categorized participants into quintiles and assigned a value (ranging from 1 to 5) according to each quintile (25). Finally, we constructed the CQI by summing all values. All criteria had the same weighting, and the CQI ranged from 4 to 20. After CQI estimation, 1829 subjects were available for analyses because the consumption of refined grains was 0 in 4 participants (Supplemental Figure 1).

Metabolomics

Fasting (for ≥8 h) plasma EDTA samples were collected from subjects and stored at −80°C. Pairs of samples for each participant were randomly ordered and analyzed using 2 LC–tandem MS methods to measure polar metabolites and lipids as described previously (26–28). Briefly, amino acids and other polar metabolites were profiled with a Shimadzu Nexera X2 U-HPLC (Shimadzu Corp.) coupled to a Q Exactive mass spectrometer (ThermoFisher Scientific). Metabolites

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Supplemental Tables 1–3 and Supplemental Figures 1–4 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/jn.
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Abbreviations used: AAMU, 5-acetylamino-6-amino-3-methyluracil; CQI, carbohydrate quality index; CV, cross-validation; CVD, cardiovascular disease; GI, glycemic index; GL, glycemic load; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PREDIMED, Prevención con Dieta Mediterránea; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol; T2D, type 2 diabetes.

were extracted from plasma (10 μL) using 90 μL 74.9:24.9:0.2 (by vol) acetonitrile/methanol/formic acid containing stable isotope–labeled internal standards [valine-d8 (Sigma-Aldrich) and phenylalanine-d8 (Cambridge Isotope Laboratories)]. The samples were centrifuged (10 min; 9000 × *g*; 4°C) and the supernatants were injected directly onto a 150 × 2-mm, 3-μm Atlantis HILIC column (Waters). The column was eluted isocratically at a flow rate of 250 μL/min with 5% mobile phase A (10 mmol ammonium formate/L and 0.1% formic acid in water) for 0.5 min followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 min. MS analyses were carried out using electrospray ionization in the positive-ion mode and full-scan spectra were acquired over 70–800 *m/z*. Lipids were profiled using a Shimadzu Nexera X2 U-HPLC (Shimadzu Corp.) coupled to an Exactive Plus orbitrap mass spectrometer (Thermo Fisher Scientific). Lipids were extracted from plasma (10 μL) using 190 μL isopropanol containing 1,2-didodecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids) as an internal standard. Lipid extracts (2 μL) were injected onto a 100 × 2.1-mm, 1.7-μm ACQUITY BEH C8 column (Waters). The column was eluted isocratically with 80% mobile phase A (95:5:0.1 10 mM ammonium acetate/methanol/formic acid, by vol) for 1 min followed by a linear gradient to 80% mobile phase B (99.9:0.1 methanol/formic acid, vol:vol) over 2 min, a linear gradient to 100% mobile phase B over 7 min, then 3 min at 100% mobile phase B. MS analyses were carried out using electrospray ionization in the positive-ion mode using full-scan analysis over 200–1100 *m/z*. Raw data were processed using Trace Finder versions 3.1 and 3.3 (Thermo Fisher Scientific) and Progenesis QI (Nonlinear Dynamics). Polar metabolite identities were confirmed using authentic reference standards and lipids were identified by head group and total acyl carbon number and total acyl double-bond content. In order to mitigate potential batch effects and temporal drift in LC-MS sensitivity over the analysis period, data were standardized to an external reference sample. To enable assessment of data quality and to facilitate data standardization across the analytical queue and sample batches, pairs of pooled plasma reference samples were analyzed at intervals of 20 study samples. One sample from each pair of pooled references served as a passive QC sample to evaluate the analytical reproducibility for measurement of each metabolite, whereas the other pooled sample was used to standardize using a "nearest neighbor" approach. Standardized values were calculated using the ratio of the value in each sample over the nearest pooled plasma reference multiplied by the median value measured across the pooled references. Plasma concentrations of 398 metabolites were analyzed. Missing values are those determinations that were below the limit of detection. From the 398 metabolites analyzed in the present study, 13 metabolites were removed owing to high numbers of missing values (i.e., >20%), leaving 385 metabolites for further analysis (**Supplemental Table 1**).

Assessment of other variables

Information about lifestyle variables, smoking status, medical history, and medication use was collected through a questionnaire. Physical activity was assessed using a validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire (29). Participants were considered to have T2D, dyslipidemia, or hypertension if they had previously been diagnosed and/or they were being treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, respectively. BMI was calculated (in kg/m^2). Participants' triacylglycerol and total and HDL-cholesterol concentrations were measured by using fasting plasma.

Statistical analyses

Baseline characteristics of study participants were expressed as means ± SDs for quantitative traits and percentages for categorical variables. Missing values of individual metabolites were imputed (in those metabolites with <20% values missing) using the random forest imputation approach ("missForest" function from the "randomForest" R package). The concentrations of metabolites were normalized and scaled to multiples of 1 SD with the rank-based inverse normal transformation. Owing to the high dimensionality and collinear nature of the data, linear regression with elastic net penalty was implemented in the "glmnet" R package ($\alpha = 0.5$) to build a multimetabolite model for GI, GL, or CQI. We performed 10-fold cross-validation (CV) to find the optimal value of the tuning parameter that resulted in a mean squared error within 1 SD of the minimum (30). The performance of the model was examined based on parameters of lambda.min. The multimetabolite model was computed as the weighted sum of the selected metabolites with weights equal to regression coefficients from the model.

In the training set, we applied a 10-fold CV approach to obtain the performance of the model without overfitting: we split the data into a 90% set and 10% set. Within the 90% set, we used the same elastic net procedure we used to build the model. Another 10-fold CV was used to tune the model parameters. Then, we used the other 10% set to evaluate the model fit at the previous step. This procedure ensures that the other 10% set is completely separated from the model-building procedure, so that the performance estimated in this step is unbiased. We then repeated all these steps 10 times and averaged their performance in the 10% set. Because each of them was an unbiased estimate of performance, the average was also unbiased. Pearson correlations were calculated to evaluate the performance of the multimetabolite model in assessing GI, GL, or CQI. For reproducibility purposes, regression coefficients were reported using 9–10 iterations of the 10-CV elastic regression approach in the whole data set. To address potential sources of reverse causation bias in relation to the association between metabolites and the 3 dietary carbohydrate indices, we conducted a sensitivity analysis by omitting individuals with prevalent T2D (31). To test the robustness of the findings we conducted 2 sensitivity analyses: *1*) using an elastic net logistic regression and using extreme tertiles (tertile 3 compared with tertile 1) of the carbohydrate quality indices instead of treating them as continuous variables; and *2*) adding covariates in the elastic net regression model such as age, sex, BMI, smoking status, alcohol, physical activity, coffee, or dietary factors not related to carbohydrate quality (i.e., dairy, meat, eggs, olive oil) or blood lipids, or all the aforementioned covariates together. All analyses were performed using R statistical package 3.4.3 (www.r-project.org) (R Development Core Team, 2012).

Results

Table 1 summarizes general characteristics by the GI/GL and CQI data sets used for analyses including 1833 participants. The mean age of participants at baseline was 67.2 y and the mean BMI was 29.9 in the GI/GL data set and 29.8 in the CQI data set. The mean GI, GL, and CQI was 47.4, 114.9, and 6.4, respectively. Pearson correlation analyses revealed that GI was moderately correlated with GL ($r = 0.55$) and CQI ($r = -0.42$), whereas CQI was weakly correlated with GL ($r = -0.12$).

Plasma metabolites associated with GI, GL, and CQI

Of the 385 metabolites used in the analyses, the elastic net regression model selected 58, 18, and 57 metabolites for GI, GL, and CQI, respectively, while remaining robust to the effects of collinearity between metabolites (**Figures 1–3**). The selected metabolites shown in the respective Figures 1–3 were ranked from the highest to the lowest elastic net positive and negative regression coefficients.

Metabolomic profile of GI

Twenty-nine metabolites were positively associated with GI: 32:1 phosphatidylcholine (PC), metronidazole, 36:4 PC, 5-acetylamino-6-amino-3-methyluracil (AAMU), indoxylsulfate, γ-butyrobetaine, betaine, cotinine, choline, 24:1 ceramide d18:1, piperine, uric acid, 2 plasmalogens [36:3 phosphatidylserine (PS), 36:1 PS], N1-acetylspermidine, and proline were among those metabolites with high regression coefficients. Among the 29 metabolites negatively associated with GI, the highest

TABLE 1 Characteristics of the study subjects and according to extreme tertiles (T1 and T3) of GI, GL, and CQI[1]

		GI		GL		CQI		
Characteristic	All subjects	T1	T2	T1	T2	All subjects	T1	T2
n	1833	614	611	611	611	1829	646	404
Age, y	67.2 ± 6.0	67.6 ± 5.7	66.8 ± 6.2	67.3 ± 5.8	67.2 ± 6.2	67.2 ± 6.0	67.2 ± 6.3	66.8 ± 5.9
Female sex	1055 (57.6)	412 (67.1)	284 (46.5)	420 (68.7)	292 (47.8)	1051 (57.5)	299 (46.3)	291 (72.0)
BMI, kg/m^2	29.9 ± 3.6	30.3 ± 3.8	29.6 ± 3.4	30.3 ± 3.7	29.5 ± 3.4	29.9 ± 3.6	29.7 ± 3.5	30.2 ± 3.6
Cholesterol in plasma, mg/dL	211 ± 35.6	212 ± 35.6	211 ± 34.7	213 ± 35.3	211 ± 35.2	211 ± 35.7	211 ± 34.8	210 ± 36.9
Triglycerides in plasma, mg/dL	134 ± 74.4	134 ± 75.1	134 ± 67.7	133 ± 75.9	135 ± 75.4	134 ± 74.5	138 ± 80.8	130 ± 63.4
HDL-C in plasma, mg/dL	51.7 ± 11.4	51.7 ± 11.1	51.5 ± 11.3	52.7 ± 11.8	51.5 ± 11.7	51.7 ± 11.4	51.4 ± 11.6	51.9 ± 11.4
Type 2 diabetes	492 (26.8)	223 (36.3)	103 (16.9)	205 (33.6)	116 (19.0)	490 (26.8)	112 (17.3)	119 (29.5)
Dyslipidemia	1408 (76.8)	471 (76.7)	468 (76.6)	467 (76.4)	479 (78.4)	1405 (76.8)	488 (75.5)	311 (77.0)
Hypertension	1599 (87.2)	526 (85.7)	548 (89.7)	534 (87.4)	541 (88.5)	1597 (87.3)	581 (89.9)	350 (86.6)
Family history of CVD	451 (24.6)	159 (25.9)	148 (24.2)	170 (27.8)	130 (21.3)	450 (24.6)	154 (23.8)	115 (28.5)
Smoking								
Current	287 (15.7)	74 (12.1)	129 (21.1)	76 (12.4)	120 (19.6)	287 (15.7)	125 (19.3)	52 (12.9)
Former	454 (24.8)	131 (21.3)	170 (27.8)	125 (20.5)	174 (28.5)	454 (24.8)	199 (30.8)	80 (19.8)
Never	1092 (59.6)	409 (66.6)	312 (51.1)	410 (67.1)	317 (51.9)	1088 (59.5)	322 (49.8)	272 (67.3)
Physical activity, MET-min/wk	244 ± 236	243 ± 233	249 ± 256	231 ± 209	258 ± 257	245 ± 237	250 ± 261	230 ± 209
GI	47.4 ± 4.5	42.6 ± 2.4	52.3 ± 2.6	44.5 ± 3.8	50.0 ± 3.9	47.5 ± 4.5	50.8 ± 3.3	45.8 ± 3.9
GL	115 ± 40.2	91.4 ± 29.8	139 ± 42.2	74.9 ± 13.6	159 ± 31.6	115 ± 40.2	134 ± 40.5	103 ± 35.5
CQI	4.67 ± 2.4	6.16 ± 1.9	3.10 ± 2.1	5.75 ± 2.2	3.75 ± 2.3	4.67 ± 2.4	2.15 ± 0.9	8.19 ± 1.1
Total carbohydrate intake, g/d	240 ± 73.4	213.6 ± 66.5	265.1 ± 76.5	169 ± 30.5	319 ± 57.4	240 ± 73.3	264 ± 72.8	225 ± 70.1
Total protein intake, g/d	92.5 ± 21.1	92.1 ± 22.4	92.4 ± 20.7	81.2 ± 17.9	104.0 ± 20.3	92.5 ± 21.0	92.5 ± 20.6	93.5 ± 22.5
Fat, g/d	98.5 ± 27.7	96.4 ± 28.3	98.8 ± 27.1	87.3 ± 24.5	109 ± 28.8	98.6 ± 27.8	101 ± 27.3	95.5 ± 28.5
MUFAs, g/d	48.9 ± 15.1	47.3 ± 15.1	49.5 ± 14.9	44.3 ± 13.8	52.7 ± 15.6	48.9 ± 15.1	50.5 ± 14.9	46.8 ± 15.4
SFAs, g/d	25.4 ± 8.3	24.9 ± 8.4	25.4 ± 8.4	22.1 ± 6.8	28.5 ± 9.0	25.4 ± 8.4	26.4 ± 8.5	24.1 ± 8.3
PUFAs, g/d	15.7 ± 6.4	15.4 ± 6.8	15.6 ± 5.9	13.4 ± 5.7	17.8 ± 6.7	15.7 ± 6.4	15.9 ± 5.9	15.6 ± 6.9
Total energy intake, kcal/d	2283 ± 544	2140 ± 515	2406 ± 558	1837 ± 353	2755 ± 474	2284 ± 544	2423 ± 541	2176 ± 525
Vegetable intake, g/d	332 ± 150	350 ± 164	306 ± 137	311 ± 136	351 ± 163	332 ± 150	295 ± 124	378 ± 170
Legume intake, g/d	20.3 ± 12.7	22.9 ± 17.3	18.3 ± 8.8	18.4 ± 13.0	22.2 ± 12.9	20.3 ± 12.7	18.5 ± 8.8	22.2 ± 15.8
Fruit intake, g/d	361 ± 197	379 ± 195	327 ± 192	313 ± 169	415 ± 223	361 ± 197	330 ± 191	395 ± 212
Meat intake, g/d	134 ± 56.3	132 ± 58.2	136 ± 58.3	126 ± 54.1	139 ± 60.4	134 ± 56.2	138 ± 58.3	127 ± 57.3
Fish intake, g/d	101 ± 53.0	104 ± 64.3	96.6 ± 44.3	98.3 ± 47.5	102 ± 48.1	101 ± 53.1	96.4 ± 44.8	109.0 ± 70.3
Nuts intake, g/d	10.9 ± 13.6	10.3 ± 13.7	10.9 ± 13.3	9.3 ± 12.8	12.0 ± 13.7	10.8 ± 13.6	10.9 ± 13.6	11.6 ± 14.5
Egg intake, g/d	20.1 ± 11.0	20.4 ± 11.9	20.3 ± 10.9	18.9 ± 10.7	20.9 ± 10.7	20.1 ± 11.0	20.5 ± 10.8	19.7 ± 12.0
Dairy intake, g/d	375 ± 221	450 ± 235	313 ± 200	345 ± 197	413 ± 242	375 ± 221	329 ± 205	408 ± 231
Fiber, g/d	25.3 ± 8.5	24.7 ± 8.6	25.4 ± 8.7	20.8 ± 5.7	29.9 ± 9.6	25.3 ± 8.5	22.7 ± 6.2	30.8 ± 10.0
Alcohol intake, g/d	9.53 ± 15.5	7.09 ± 12.8	12.4 ± 18.4	7.28 ± 13.2	12.3 ± 18.2	9.55 ± 15.5	12.15 ± 18.0	6.14 ± 11.6
Cereal intake, g/d	231 ± 101	174 ± 74.8	283 ± 111	152 ± 51.9	315 ± 103	231 ± 101	268 ± 107	210 ± 91.2
Sugar intake, g/d	6.79 ± 11.6	1.50 ± 4.15	14.3 ± 15.3	2.03 ± 5.32	12.1 ± 15.0	6.81 ± 11.6	13.1 ± 14.6	2.21 ± 5.04
Beverage intake, g/d	18.8 ± 61.3	12.8 ± 42.9	24.3 ± 71.1	9.80 ± 31.5	28.6 ± 84.7	18.8 ± 61.3	32.7 ± 87.3	10.5 ± 37.9

[1] Values are means ± SDs or *n* (%). CQI, carbohydrate quality index; CVD, cardiovascular disease; GI, glycemic index; GL, glycemic load.

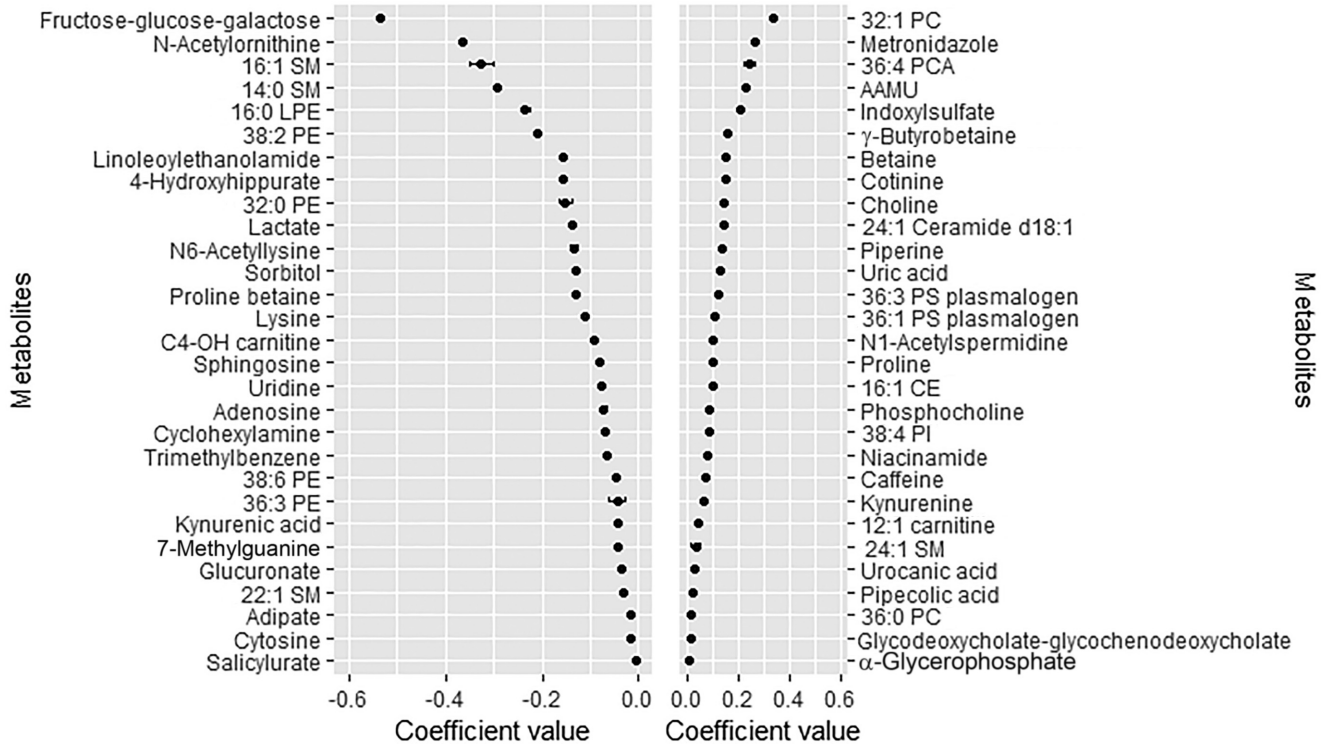

FIGURE 1 Coefficients for the 58 metabolites selected 9–10 times in the 10 times iterated 10-fold cross-validation of the elastic regression procedure (using lambda.min) using the whole data set of subjects ($n = 1833$) and associated with glycemic index (continuous). Metabolites with negative coefficients ($m = 29$) are plotted in the left part, whereas those with positive coefficients ($m = 29$) are shown in the right part. AAMU, 5-acetylamino-6-amino-3-methyluracil; CE, ceramide; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

regression coefficients were found for fructose-glucose-galactose followed by N-acetylornithine, sphingomyelins (SMs) (16:1, 14:0), 16:0 lysophosphatidylethanolamine (LPE), phosphatidylethanolamines (PEs) (38:2, 32:0), linoleoylethanolamide, 4-hydroxyhippurate, lactate, N6-acetyllysine, sorbitol, proline betaine, lysine, C4-OH carnitine, and sphingosine. **Supplemental Figure 2** shows the 39 metabolites selected for GI after excluding participants with T2D.

Metabolomic profile of GL

Out of the 18 metabolites associated with GL, 9 had positive and 9 negative regression coefficients. The 9 metabolites with positive coefficients were 32:1 PC, cotinine, C26 carnitine, methionine, 36:3 PS plasmalogen, choline, dimethylglycine, γ-butyrobetaine, and 16:1 lysophosphatidylcholine (LPC). The highest negative regression coefficient was found for fructose-glucose-galactose followed by γ-aminobutyric acid, SMs (18:1, 22:1, 18:2), 38:6 PE, 58:10 triacylglycerol (TAG), succinate, and kynurenic acid. After excluding T2D prevalent cases, 6 metabolites were selected by elastic net regression (**Supplemental Figure 3**).

Metabolomic profile of CQI

Twenty-nine metabolites were positively associated with CQI, whereas 28 were negatively associated. The highest positive regression coefficient was observed for lysine followed by uridine, indole-3-propionate, linoleoylethanolamide, 4-pyridoxate, hypoxanthine, proline betaine, 42:11 PE plasmalogen, 20:4 carnitine, N-acetylornithine, fructose-glucose-galactose, 40:10 PC, 51:3 TAG, 38:2 PE, 36:3 PE, hippurate, acetylcholine, and 34:3 PC plasmalogen. High negative regression coefficients were obtained for 24:1 ceramide d18:1, γ-butyrobetaine, phenylacetylglutamine, caffeine, 12:1 carnitine, 54:1 TAG, N1-acetylspermidine, 36:5 PC plasmalogen B, uric acid, arginine, 36:3 PS plasmalogen, phosphocreatine, β-alanine, 36:4 PC plasmalogen, choline, and 58:6 TAG. Fifty metabolites were associated with CQI after the exclusion of participants with T2D (**Supplemental Figure 4**).

Pearson correlations between metabolomic profiles and the 3 indices

In the training set, the unbiased metabolomic profiles acquired using the 10-fold CV approach were significantly correlated with GI ($r = 0.30$), GL ($r = 0.22$), and CQI ($r = 0.27$) (Table 2).

Overlapping metabolites among the 3 indices

Consistent associations between some metabolites (choline, cotinine, fructose-glucose-galactose, γ-butyrobetaine, and 36:3 PS plasmalogen) and all 3 indices were observed (**Supplemental Table 2**). 4-Hydroxyhippurate, acetylamino-6-amino-3-methyluracil, caffeine, proline betaine, uric acid, uridine, indoxylsulfate, linoleoylethanolamide, lysine, N-acetylornithine, N1-acetylspermidine, piperine, sorbitol, urocanic acid, 12:1 carnitine, and lipid species including 14:0 SM, 16:0 LPE, 24:1 ceramide d18:1, 36:1 PS plasmalogen, and PE (36:3, 38:2) were associated with both GI and CQI (**Supplemental Table 3**). Finally, associations of kynurenic acid, 22:1 SM, 32:1 PC, and 38:6 PE with GI and GL were found (Supplemental Table 3).

The sensitivity analysis using extreme tertiles of GI, GL, and CQI in the elastic net logistic regression showed comparable

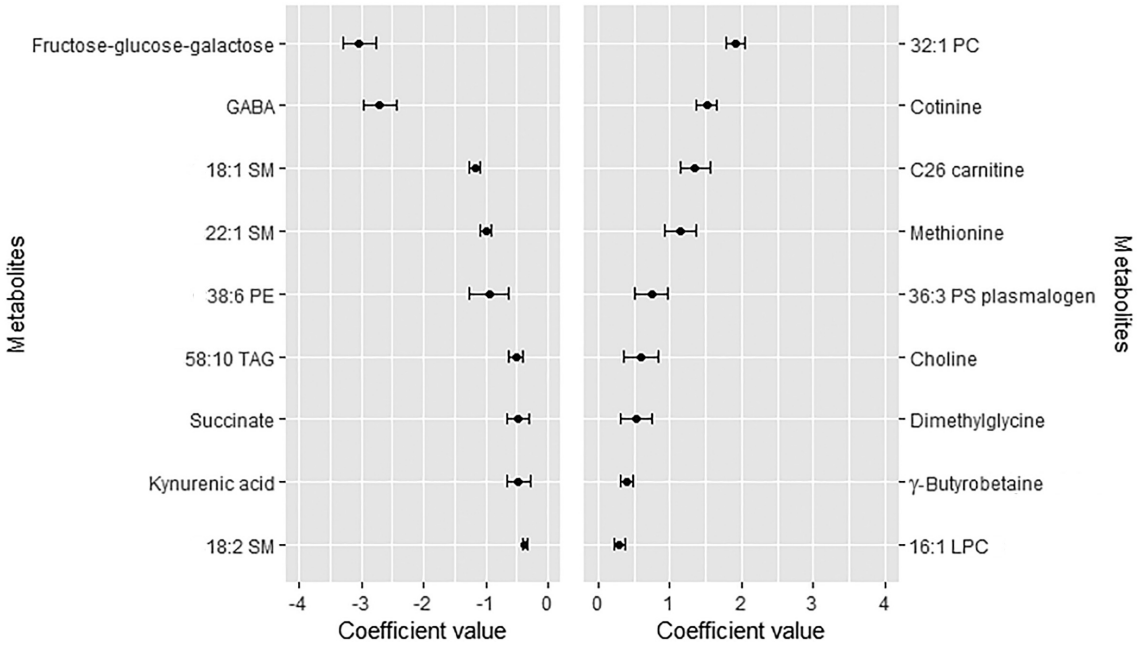

FIGURE 2 Coefficients for the 18 metabolites selected 9–10 times in the 10 times iterated 10-fold cross-validation of the elastic regression procedure (using lambda.min) using the whole data set of subjects (n = 1833) and associated with glycemic load (continuous). Metabolites with negative coefficients (m = 9) are plotted in the left part, whereas those with positive coefficients (m = 9) are shown in the right part. GABA, γ-aminobutyric acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol.

results in terms of the metabolites selected (data not shown). Analyses adjusting for potential confounders also yielded consistent results for the 3 carbohydrate quality indices.

Discussion

Using baseline data from 2 nested case-cohort studies within the PREDIMED study and performing a comprehensive metabolite profiling, we identified several metabolites that were associated with dietary GI, GL, and CQI.

Previous dietary intervention studies have identified certain metabolites modulated by their GI or GL content. In the GLYNDIET, a 6-mo randomized, parallel, controlled, clinical trial conducted among 102 overweight/obese adults with available metabolites, the low-GI diet intervention was associated with increased serine concentrations, and with decreased concentrations of leucine, valine, and several lipid species including 2 SMs, 2 LPCs, and 6 PCs as compared with the high-GI diet (13). In our study, also some amino acids (lysine, proline) and lipid species (SMs, PCs, PEs, LPE) were associated with the GI. Furthermore, a previous randomized, controlled, crossover feeding trial of two 28-d diet periods of high- and low-GL diets found significantly higher plasma kynurenic acid concentrations during the low-GL diet period (16), which is in the same direction as the association observed between this metabolite and GL in our study. Recently, the same group evaluated the effects on metabolic profiles of a low-GL compared with a high-GL diet in a larger sample and found 18 metabolites involved in inflammation and energy metabolism pathways that were significantly different between diets (15). Another 28-d crossover design study with 21 obese adults identified a cluster of 152 metabolites that discriminated 1 diet from the other 2 (low-fat, low-GI, or very-low carbohydrate diet) (14). Cytosine, hippurate, and pipecolic acid differentiated the low-GI diet from the other 2 diets (14), these metabolites also being associated with GI in our study. On the other hand, no previous study has examined the association of metabolites with dietary CQI and we identified for the first time, to our knowledge, a related metabolic profile.

The majority of the metabolites identified by elastic net regression for the 3 dietary carbohydrate indices are involved in several metabolic pathways but some of them may originate from food and food additives, be formed through microbial activity in the gastrointestinal tract, or be produced endogenously in response to postprandial glycemia and insulinemia. Choline was positively associated with dietary GI and GL, negatively associated with CQI, and total choline can be found in beef/chicken liver, eggs, wheat germ, bacon, and soybeans (32); moreover, elevated circulating concentrations have been associated with components of metabolic syndrome (33) and CVD (33–35). Notably, its downstream metabolite, betaine, which is also found in wheat bran and wheat germ (32), was associated with increased GI and elevated plasma betaine concentrations have been associated with CVD outcomes (34, 36). Its derivative dimethylglycine, which has been associated with incident acute myocardial infarction (37), was associated with increased GL in our study. γ-Butyrobetaine, which is produced as a gut microbial intermediate in the metabolism of L-carnitine to trimethylamine and reported to exert atherogenic effects on mice (38), was also positively associated with GI and GL and negatively with CQI, supporting the relation between dietary carbohydrate indices and cardiometabolic diseases. Hippurate, another gut microbial metabolite of polyphenol metabolism and associated with the consumption of polyphenol-rich foods and beverages (39), was associated with increased CQI. However, the hippurate derivative, 4-hydroxyhippuric acid, was negatively associated with GI and positively with CQI, and high concentrations in plasma may indicate an increased consumption of polyphenol-rich red wine

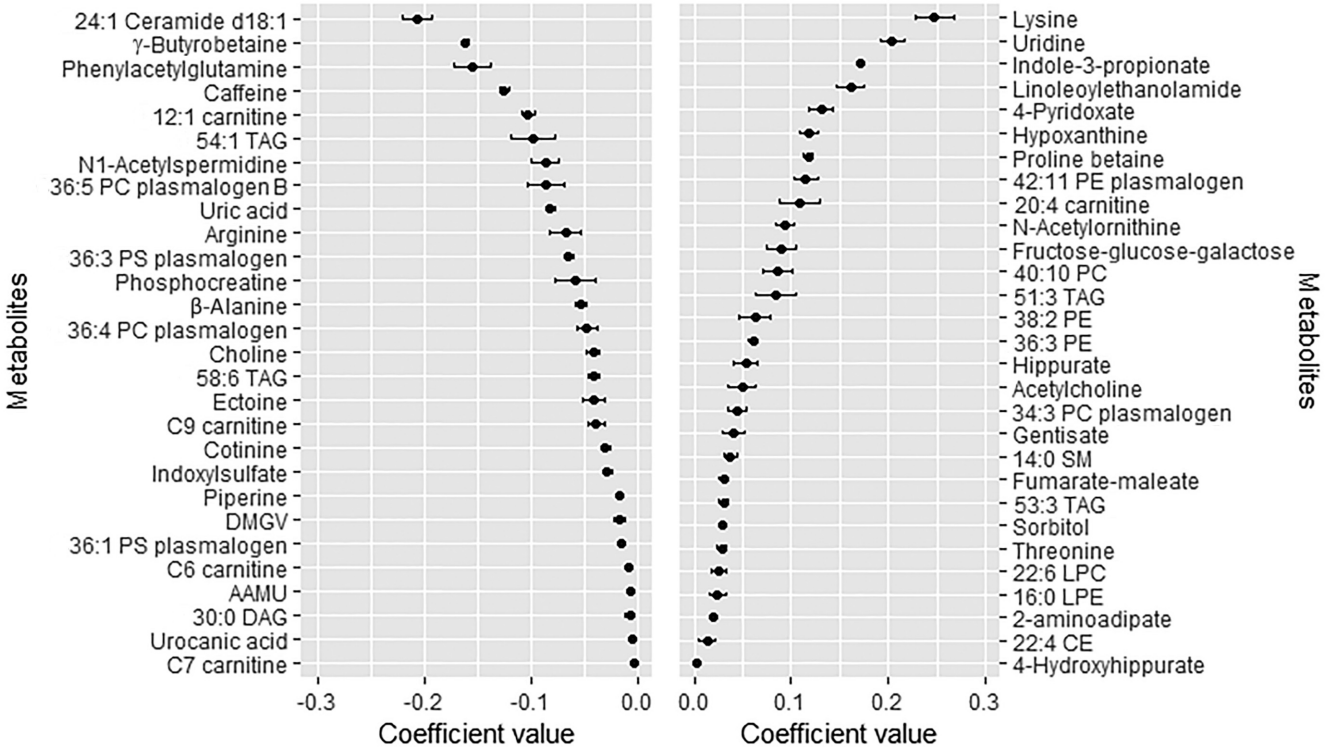

FIGURE 3 Coefficients for the 57 metabolites selected 9–10 times in the 10 times iterated 10-fold-cross validation of the elastic regression procedure (using lambda.min) using the whole data set of subjects ($n = 1829$) and associated with carbohydrate quality index (continuous). Metabolites with negative coefficients ($m = 28$) are plotted in the left part, whereas those with positive coefficients ($m = 29$) are shown in the right part. AAMU, 5-acetylamino-6-amino-3-methyluracil; CE, ceramide; DAG, diacylglycerol; DMGV, dimethylguanidino valeric acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol.

and red grape juice (40). Kynurenic acid, which exerts anti-inflammatory effects (15), was found to be inversely associated with GI and GL. On the other hand, uric acid (a marker of oxidative stress) was associated with increased GI and decreased CQI. Epidemiological evidence suggests that high uric acid concentrations are associated with circulating inflammatory markers (41) and increased risk of cardiometabolic diseases (42, 43). Similarly, we found indoxyl sulphate, a protein-bound uremic solute that induces oxidative stress and endothelial dysfunction in animal models (44), to be associated with increased GI and decreased CQI. Among metabolites involved in energy metabolism, α-glycerophosphate and lactate were selected for GI, succinate for GL, and fumarate for CQI. Our results pointing to positive and negative associations of cotinine, a metabolite of nicotine (45), with GI/GL and CQI, respectively, may be explained by a residual effect, namely the higher the GI/GL the higher the prevalence of smoking. Similarly, the positive association of caffeine and its metabolite, AAMU, with GI and their negative association with CQI suggest a higher and a lower coffee consumption in those individuals with an increased GI and CQI, respectively. Our findings in relation to sorbitol suggest that lower GI and higher CQI are related to increased artificial sweeteners consumption. One possible explanation for this finding is reverse causation, considering the high prevalence of T2D in our population. Reverse causation also emerges as a prevailing explanation for the association between the metabolite defined as fructose-glucose-galactose and the 3 indices. This potential explanation is further supported by the fact that when we excluded T2D cases sorbitol was no longer associated with GI/CQI and fructose-glucose-galactose with any of these 3 indices. The LC-MS method did not distinguish glucose, fructose, and galactose from one another. However, circulating plasma fructose and galactose concentrations are generally very low and therefore we can assume that glucose mainly accounted for this chromatographic peak. In addition to these metabolites, several plasma phospholipids were associated with the 3 dietary carbohydrate indices. In this regard, increased concentrations of 32:1 PC have been positively associated with T2D (46) and, in our study, with high GI and GL. Concerning LPC species, positive associations between 16:1 LPC and insulin resistance have been recently reported from our group (47) and, in the current study, 16:1 LPC was associated with higher GL. Similarly, 16:0 LPE was associated with higher CQI and this phospholipid has been previously associated with lower risk of T2D (18). Considering ceramides, our group has previously reported positive associations between baseline plasma concentrations and incident CVD in the PREDIMED cohort (17) and the 24:1 ceramide was associated with

TABLE 2 Ten-fold cross-validated Pearson correlations between the multimetabolite model and GI, GL, and CQI[1]

Outcome	Pearson's *r*	95% CI
GI	0.30	0.26, 0.35
GL	0.22	0.17, 0.27
CQI	0.27	0.23, 0.31

[1] CQI, carbohydrate quality index; GI, glycemic index; GL, glycemic load.

higher GI and lower CQI. We also observed that long-chain acylcarnitines were associated with higher GL, whereas short- and medium-chain acylcarnitines were associated with lower CQI. Elevated concentrations of short-, medium-, and long-chain acylcarnitines may be indicative of dysregulated fatty acid oxidation and mitochondrial function and, in the PREDIMED cohort, have been related to a higher risk of CVD (48).

This study has some limitations that need to be mentioned. Firstly, although we used a validated FFQ across a relatively large sample size, measurement errors are inevitable. Secondly, participants were older adults at high CVD risk from a Mediterranean region and the generalizability of the findings to other age groups or populations may be limited. Thirdly, owing to its cross-sectional design, causation of the observed associations cannot be inferred. Fourthly, although the metabolomic profiles of GI and GL differed, there was an overlapping for several metabolites identified. Further studies are needed to understand the differences in the metabolites selected for these 2 carbohydrate quality indices, which were moderately correlated in our study ($r = 0.55$).

In conclusion, our findings suggest that a lower GI or GL and a higher CQI are associated with a metabolomic profile that is related to a potential favorable cardiometabolic risk in an older Mediterranean population at high risk of CVD. These associations cannot be viewed as causal and further studies are needed to assess whether these metabolic profiles are associated with chronic disease risk, so as to improve our understanding of biological mechanisms through which carbohydrate quality indices affect health.

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