The Journal of Nutrition
Genomics, Proteomics, and Metabolomics

High Plasma Glutamate and a Low Glutamine-to-Glutamate Ratio Are Associated with Increased Risk of Heart Failure but Not Atrial Fibrillation in the Prevención con Dieta Mediterránea (PREDIMED) Study

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ABSTRACT

Background: Although the association between glutamate and glutamine in relation to cardiometabolic disorders has been evaluated, the role of these metabolites in the development of atrial fibrillation (AF) and heart failure (HF) remains unknown.

Objectives: We examined associations of glutamate, glutamine, and the glutamine-to-glutamate ratio with AF and HF incidence in a Mediterranean population at high cardiovascular disease (CVD) risk.

Methods: The present study used 2 nested case-control studies within the PREDIMED (Prevención con Dieta Mediterránea) study. During ~10 y of follow-up, there were 509 AF incident cases matched to 618 controls and 326 HF incident cases matched to 426 controls. Plasma concentrations of glutamate and glutamine were semiquantitatively profiled with LC–tandem MS. ORs were estimated with multivariable conditional logistic regression models.

Results: In fully adjusted models, per 1-SD increment, glutamate was associated with a 29% (95% CI: 1.08, 1.54) increased risk of HF and glutamine-to-glutamate ratio with a 20% (95% CI: 0.67, 0.94) decreased risk. Glutamine-to-glutamate ratio was also inversely associated with HF risk (OR per 1-SD increment: 0.80; 95% CI: 0.67, 0.94) when comparing extreme quartiles. Higher glutamate concentrations were associated with a worse cardiometabolic risk profile, whereas a higher glutamine-to-glutamate ratio was associated with a better cardiometabolic risk profile. No associations between the concentrations of these metabolites and AF were observed.

Conclusions: Our findings suggest that high plasma glutamate concentrations possibly resulting from alterations in the glutamate-glutamine cycle may contribute to the development of HF in Mediterranean individuals at high CVD risk. This trial was registered at www.isrctn.com as ISRCTN35739639. *J Nutr* 2020;150:2882–2889.

Keywords: glutamate, glutamine, atrial fibrillation, heart failure, PREDIMED

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Manuscript received May 24, 2020. Initial review completed June 26, 2020. Revision accepted August 13, 2020.
First published online September 16, 2020; doi: https://doi.org/10.1093/jn/nxaa273.

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Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide and the prevalence of heart failure (HF) is 2%–3% among adults (1). Both have emerged as relevant cardiac outcomes causing premature deaths and chronic disability (2). AF is characterized by rapid and disorganized electrical activity within atria resulting in the loss of contractile function and irregular ventricular contractions (3), whereas cardiac structural and functional abnormalities that impair contraction and/or relaxation of the myocardium characterize HF (4). Despite these differences, AF and HF often coincide (5, 6) partially owing to the presence of shared cardiometabolic risk factors (7). However, to better understand their common and/or different underlying pathophysiological processes there is need to examine these 2 outcomes with novel risk factors (8, 9). In this regard, some metabolic perturbations have been reported in AF and HF (10–12), and identifying putative markers reflecting metabolic pathways involved in their development could be a promising approach.

Alterations in the glutamate-glutamine cycle resulting in increases in glutamate and decreases in glutamine concentrations have been associated with an unfavorable cardiometabolic status (13). Our research group has prospectively demonstrated a positive association between systemic glutamate concentrations and the risk of type 2 diabetes (T2D) (14) and cardiovascular diseases (CVDs) (15) using 2 case-cohort studies in individuals at high CVD risk. The glutamine-to-glutamate ratio was, however, associated with a decreased risk of these chronic conditions.

Glutamate plays a role in apoptosis induction (16) and oxidative stress (17), whereas glutamine plays a role in myocardial metabolism (18) and exerts potent antioxidant and anti-inflammatory effects in the circulation by inducing the expression of heme oxygenase-1, heat shock proteins, and glutathione (19). Both of them are also involved in energy metabolism (20, 21), and dysregulations in myocardial energy metabolism have been associated with the development of AF and HF (22). However, studies examining these metabolites in relation to CVD subtypes such as AF and HF are limited to a small cross-sectional case-control study which also reported high glutamate and low glutamine plasma concentrations among HF patients (23). Therefore, the role of glutamate and glutamine in the development of AF and HF remains unexplored.

The current study tests the hypothesis that increased and decreased plasma concentrations of glutamate and glutamine, respectively, and a decreased glutamine-to-glutamate ratio are associated with the risk of AF and HF, in 2 new prospective case-control studies nested within the PREDIMED (Prevención con Dieta Mediterránea) study. Secondarily, this study evaluates the hypothesis that these metabolites and the ratio are associated with cardiometabolic risk factors.

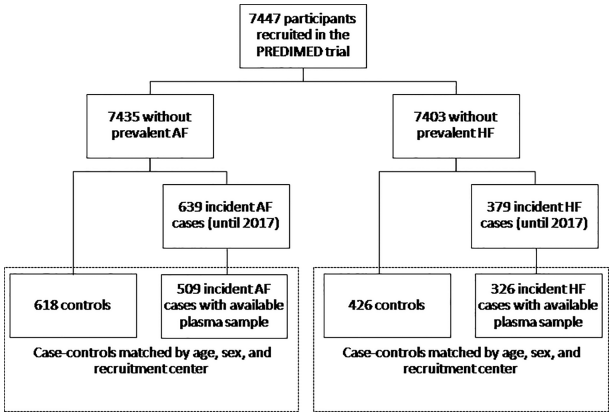

FIGURE 1 Flowchart of study participants. AF, atrial fibrillation; HF, heart failure; PREDIMED, Prevención con Dieta Mediterránea.

Methods

Study design and participants

This study used 2 case-control studies (AF and HF) nested within the PREDIMED trial (www.predimed.es; ISRCTN35739639), a primary prevention CVD study utilizing the Mediterranean diet (MedDiet). The protocol and design of the PREDIMED study have been described in detail elsewhere (24). In brief, 7447 older adults at high CVD risk were allocated to a MedDiet supplemented with extra-virgin olive oil (EVOO), a MedDiet supplemented with mixed nuts, or a control diet consisting of advice to reduce fat intake. The study had a period from 25 June, 2003 to 1 December, 2010 (median follow-up of 4.8 y) where information about CVD-related outcomes was collected and analyzed (25) and an extended follow-up until December 2017. Five hundred and nine incident AF events and 326 incident HF events were ascertained (**Figure 1**). We selected respective AF and HF matched controls using the risk-set sampling strategy described by Prentice and Breslow (26). The controls were randomly selected among those who were free of AF or HF at the time of diagnosis of the cases, and matched with AF or HF cases on age at recruitment (±5 y), sex, and center. In 1 of the PREDIMED centers, no cases and controls were selected between 2015 and 2017 because of lack of event information during this extended follow-up period. Following the aforementioned strategy, 2 or 3 controls were selected for overlapping cases (between AF and HF cases) with different event dates to match time at risk for each pair. The number of controls was 618 for AF and 426 for HF cases (Figure 1). There were 108 overlapping cases of AF and HF. There were also 135 controls included in both case-control subsets for AF and HF. The protocol of the PREDIMED trial was approved by the research ethics committees of all participating centers.

Supported by NIH grant R01HL118264 (to FBH); Spanish Ministry of Health (Instituto de Salud Carlos III) and Ministerio de Economía y Competitividad-Fondo Europeo de Desarrollo Regional projects CNIC-06/2007, RTIC G03/140, CIBER 06/03, PI06-1326, PI07-0954, PI11/02505, SAF2016-80532, SAF2009-12304, and AGL2010-22319-C03-03; and Generalitat Valenciana PROMETEO 17/2017, ACOMP2010-181, AP111/10, AP-042/11, ACOM2011/145, ACOMP/2012/190, ACOMP/2013/159, and ACOMP/213/165. CP was the recipient of Instituto de Salud Carlos III Miguel Servet fellowship grant CP 19/00189. PH-A was supported by Juan de la Cierva-Formación postdoctoral fellowship FJCI-2017-32205. MG-F was supported by American Diabetes Association grant #1-18-PMF-029.

Author disclosures: JS-S is a nonpaid member of the Scientific Committee of the International Nut and Dried Fruit Foundation, has received grants/research support from the American Pistachio Growers and International Nut and Dried Fruit Foundation through his institution, has received honoraria from Nuts for Life, Danone, and Eroski, reports personal fees from Danone, and is a member of the executive committee of Instituto Danone Spain. All other authors report no conflicts of interest.

Supplemental Methods, Supplemental Tables 1–6, and Supplemental Figure 1 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: AF, atrial fibrillation; CVD, cardiovascular disease; ECG, electrocardiogram; EVOO, extra-virgin olive oil; HF, heart failure; MedDiet, Mediterranean diet; PREDIMED, Prevención con Dieta Mediterránea; TG, triacylglycerol; T2D, type 2 diabetes.

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Measurement of glutamate and glutamine

Fasting plasma samples were collected using EDTA-coated tubes and stored at −80°C. Glutamate and glutamine concentrations were measured at the Broad Institute (Boston, MA) using LC-tandem MS techniques (27). A system composed of a Shimadzu Nexera X2 U-HPLC (Shimadzu Corp.) coupled to a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific) was used. Metabolite identities were confirmed using authentic reference standards. Raw data were processed via TraceFinder software (Thermo Fisher Scientific).

AF and HF ascertainment

During the first study period (2003–2010), information on AF and HF was collected from contacts with participants and primary health care physicians, annual follow-up visits, and yearly ad hoc reviews of outpatient and inpatient medical charts. During the extended follow-up period up to 2017, information on AF and HF was collected by reviewing the outpatient and inpatient medical charts of the participants. Study physicians collected this information. If a clinical diagnosis of CVD was made, all relevant documentation, including clinical records of hospital discharge, outpatient clinics, and family physicians' records, was obtained. The medical charts were labeled only with the study identification number and were sent anonymously to the Clinical End-Point Adjudication Committee. The End-Point Adjudication Committee, chaired by a cardiologist, adjudicated the events according to prespecified criteria. Two cardiologists independently evaluated the documentation and if they did not agree on the classification of the event, a third cardiologist (the committee's chair) intervened. In some cases, more relevant information was requested to complete the ascertainment. The diagnostic criteria and procedures have been reported in detail elsewhere (28, 29).

AF was initially identified from an annual review of all medical records of each participant and yearly electrocardiograms (ECGs) performed during follow-up examinations. If AF was mentioned anywhere in the medical record or AF was present in the ECG, all relevant documentation was submitted to the Clinical End-Point Adjudication Committee following the aforementioned procedure.

HF was defined according to the 2005 (time of study design) guidelines on the diagnosis and treatment of acute and chronic HF (30, 31). The **Supplemental Methods** specify the diagnostic criteria.

Covariate assessment

Lifestyle variables, smoking status, medical history, and medication use were collected using a questionnaire at baseline. Physical activity was assessed using the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire (32); its intensity was measured with metabolic equivalents. Habitual diet was assessed by a 137-item validated semiquantitative FFQ (33, 34). Energy and nutrients from food consumption were computed using food composition tables (35). 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 as weight divided by height squared (kg/m^2).

Statistical analyses

Circulating concentrations of glutamate and glutamine were transformed using a natural logarithmic approach to approximate a normal distribution. We also examined glutamine-to-glutamate ratio as a metabolite trait by dividing the raw values and then taking ln transformations.

Baseline characteristics of AF and HF cases and matched controls are described as means ± SDs for quantitative variables and percentages for categorical variables. Baseline characteristics were compared between cases and controls using Student's *t* test for continuous variables and χ^2 tests for categorical variables.

To investigate the association of glutamate, glutamine, and glutamine-to-glutamate ratio with AF and HF, we conducted conditional logistic regression, where the outcome was the case/control status for AF or HF. A crude model and 2 multivariable-adjusted conditional logistic regression models were fitted as follows: *1*) multivariable model 1 adjusted for potential confounders including smoking (never, current, or former), family history of CVD (yes or no), physical activity (metabolic equivalent tasks; min/d), alcohol intake (g/d), BMI, intervention group assignments (MedDiet + EVOO, MedDiet + nuts, or control interventions), hypertension (yes or no), dyslipidemia (yes or no), and T2D (yes or no); and *2*) multivariable model 2 in addition adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medications). Metabolites were analyzed both as continuous variables (1-SD increments in their ln-transformed concentrations were calculated among controls and then applied to the entire sample) and by using quartiles (with cutoffs defined among controls). To evaluate the linear trend across quartiles, the median metabolite concentration within each quartile was included in the conditional logistic regression models as a continuous variable. We fitted cubic splines to a conditional logistic regression model (36) in order to examine the possibly nonlinear relation between metabolites and AF and HF risk. Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms.

We also conducted stratified analyses by age group (<65 y compared with ≥65 y), sex (male, female), T2D (yes, no), and obesity status (BMI <30.0, ≥30.0). Potential effect modification was examined by adding a multiplicative term (1 df) between stratifying variables and metabolites (continuous), and ratio (continuous) into a multivariable unconditional logistic regression to test for interactions by using the likelihood ratio tests. Because this prospective study was conducted in the framework of dietary interventions, possible interactions of each metabolite and ratio with the intervention groups (MedDiet + EVOO and MedDiet + nuts compared with control group) were evaluated using the likelihood ratio test.

To test the robustness of the associations of the metabolites and ratio with the risk of AF and HF, we conducted 2 sensitivity analyses: *1*) further adjusting multivariable model 2 for food groups including vegetables, fruits, meat, fish, dairy products, cereals, and legumes; and *2*) adding into the multivariable model 2 covariates such as lipids and glucose. Missing values of these covariates were replaced with a mean.

We applied multiple linear regression analyses to examine cross-sectional relations of metabolites and glutamine-to-glutamate ratio with lipids [total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols (TGs)], blood glucose, and BMI. Models were adjusted for age, sex, center, smoking, physical activity, alcohol intake, BMI, dyslipidemia, hypertension, and T2D. Lipid-modifying medication or antidiabetic medication was also included as a confounder for lipids or blood glucose, respectively. Cholesterol, LDL cholesterol, HDL cholesterol, TG, and glucose concentrations were log transformed to normalize their distributions. To make the presentation of results more interpretable with respect to the lipids and glucose results we exponentiated the β coefficients. Statistical analyses were performed using Stata 14.1 (Stata Corp.). A 2-sided P value < 0.05 was considered significant.

Results

Compared with controls, participants who developed AF and HF were more likely to have a higher BMI and prevalent hypertension (**Table 1**). Furthermore, those with incident AF were also more likely to use antihypertensive medication, whereas those participants with HF were also more likely to have a higher prevalence of T2D and use oral antidiabetic agents. Higher glutamine-to-glutamate ratio was associated with age and higher physical activity, but with lower T2D prevalence in the AF case-control study and lower BMI in both case-control studies (**Supplemental Tables 1, 2**). Participants from both case-control studies with a higher glutamine-to-glutamate ratio were also likely to use oral antidiabetic agents less often.

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TABLE 1 Baseline characteristics of the study population[1]

	AF cases	Controls	HF cases	Controls
N	509	618	326	426
Age, y	68.2 ± 6.1	68.5 ± 6.1	70.3 ± 5.8	70.4 ± 5.9
Sex, % women	49.7	49.2	58.3	54.2
BMI, kg/m^2	30.7 ± 3.8	29.8 ± 3.8*	31.1 ± 3.8	29.4 ± 3.6*
Physical activity, MET-min/wk	226 ± 209	232 ± 217	217 ± 196	215 ± 217
Intervention group, %				
MedDiet + EVOO	31.4	36.4	30.9	37.6
MedDiet + Nuts	31.4	28.6	32.5	26.5
Control group	37.0	34.9	36.5	35.9
Alcohol intake, g/d	8.9 ± 13.3	9.7 ± 15.0	7.9 ± 14.6	8.1 ± 12.1
Family history of CVD, %	19.1	20.1	19.3	19.2
Type 2 diabetes, %	47.9	49.8	59.5	52.1*
Hypertension, %	88.4	82.8*	87.4	82.2*
Dyslipidemia, %	65.2	68.4	64.1	69.0
Antihypertensive medication, %	79.5	72.5*	76.4	75.1
Oral antidiabetic agents, %	30.8	31.4	40.5	32.6*
Insulin medication, %	7.3	7.4	10.4	7.9
Lipid-lowering medication, %	36.4	35.4	37.1	38.5
Smoking, %				
Never	58.7	57.9	59.8	61.3
Former	26.9	28.8	25.8	27.5
Current	14.3	13.3	14.4	11.3

[1]Values are means ± SDs or percentages. The χ^2 test was used for comparison of categorical variables and Student's *t* test was used for comparison of continuous variables. AF, atrial fibrillation; CVD, cardiovascular disease; EVOO, extra-virgin olive oil; HF, heart failure; MedDiet, Mediterranean diet; MET, metabolic equivalent.

*P value < 0.05 comparing cases and controls. There were 108 overlapping cases of AF and HF.

Association of baseline metabolites with risk of incident AF and HF

Table 2 and Figure 2 present associations of plasma concentrations of glutamate and glutamine and the glutamine-to-glutamate ratio at baseline with risk of incident AF and HF. Overall, no significant associations between the metabolites under study and AF were observed. Sensitivity analyses did not change these results (**Supplemental Tables 3**, **5**). On the other hand, in the fully adjusted model, the estimated OR for incident HF reached significance only in the highest quartile, compared with the lowest, of the glutamine-to-glutamate ratio (0.57; 95% CI: 0.35, 0.94; *P*-trend = 0.039). In multivariable model 2, baseline glutamate was associated with increased HF risk (OR per 1-SD increment: 1.29; 95% CI: 1.08, 1.54; $P = 0.004$), whereas the glutamine-to-glutamate ratio was associated with a decreased risk (OR per 1-SD increment: 0.80; 95% CI: 0.67, 0.94; $P = 0.008$). In the unadjusted model, baseline concentrations of glutamine were also significantly associated with decreased risk of HF (OR per 1-SD increment: 0.87; 95% CI: 0.76, 0.99), but these associations were no longer significant after adjustment for potential confounders. Spline analysis (**Supplemental Figure 1**) suggested nonlinear associations of glutamate and the glutamine-to-glutamate ratio with HF risk (*P* value for nonlinearity with HF risk of glutamate was 0.016, and that of the glutamine-to-glutamate ratio was 0.030). In both sensitivity analyses, glutamate concentrations in the highest quartile were significantly associated with higher HF risk than in the lowest quartile (**Supplemental Tables 4**, **6**). A 1-SD increment in concentrations of glutamate was also significantly associated with higher risk of HF incidence (Supplemental Tables 4, 6). Both sensitivity analyses also showed that a high glutamine-to-glutamate ratio was associated with a decreased risk of HF (Supplemental Tables 4, 6).

We did not observe any interaction between baseline glutamate, glutamine, or glutamine-to-glutamate ratio and age, sex, T2D, or obesity status on AF and HF (*P* values for interaction > 0.05). Similarly, the interactions between the intervention groups (MedDiet + EVOO and MedDiet + nuts compared with control group) and the metabolites were not significant.

Metabolites in relation to lipids, glucose, and BMI

High plasma glutamate concentrations were associated with lower concentrations of HDL cholesterol, higher concentrations of plasma TGs and glucose, and higher BMI. On the other hand, the glutamine-to-glutamate ratio was positively associated with HDL cholesterol and inversely associated with TGs, glucose, and BMI (Table 3). Glutamine was positively associated with HDL cholesterol and negatively associated with plasma glucose concentrations.

Discussion

In our study, involving 2 prospective case-control studies nested within the PREDIMED study cohort, baseline plasma glutamate concentrations (per 1-SD increment) were associated with a 29% increased risk of HF but not AF. In contrast, a higher glutamine-to-glutamate ratio (per 1-SD increment) was associated with a 20% decreased risk of HF, suggesting that an imbalance between plasma glutamine and glutamate concentrations may contribute to the development of this pathology. These associations were nonlinear; the associations with HF were more pronounced among individuals with higher concentrations of glutamate and higher values of the glutamate-to-glutamine ratio.

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TABLE 2 Associations of baseline plasma glutamine and glutamate concentrations and the glutamine-to-glutamate ratio by quartiles with risk of incident AF and HF in a nested case-control study of the PREDIMED study[1]

	Q1	Q2	Q3	Q4	*P*-trend
AF					
Glutamate					
Cases, *n*	130	139	105	135	
Crude model	Ref.	1.07 (0.78, 1.47)	0.79 (0.56, 1.10)	0.99 (0.70, 1.42)	0.693
Multivariable model 1	Ref.	1.01 (0.73, 1.39)	0.75 (0.53, 1.07)	0.86 (0.60, 1.25)	0.270
Multivariable model 2	Ref.	1.02 (0.73, 1.41)	0.75 (0.53, 1.06)	0.86 (0.59, 1.25)	0.254
Glutamine					
Cases, *n*	132	102	134	141	
Crude model	Ref.	0.79 (0.56, 1.11)	1.07 (0.76, 1.49)	1.13 (0.81, 1.58)	0.253
Multivariable model 1	Ref.	0.78 (0.55, 1.11)	1.08 (0.76, 1.53)	1.20 (0.84, 1.71)	0.157
Multivariable model 2	Ref.	0.76 (0.53, 1.09)	1.09 (0.77, 1.55)	1.23 (0.86, 1.75)	0.118
Glutamine-to-glutamate ratio					
Cases, *n*	131	115	131	132	
Crude model	Ref.	0.89 (0.63, 1.28)	1.04 (0.73, 1.47)	1.05 (0.74, 1.48)	0.572
Multivariable model 1	Ref.	0.97 (0.68, 1.41)	1.15 (0.80, 1.65)	1.22 (0.84, 1.75)	0.201
Multivariable model 2	Ref.	1.00 (0.69, 1.45)	1.18 (0.82, 1.71)	1.25 (0.86, 1.80)	0.173
HF					
Glutamate					
Cases, *n*	65	67	83	111	
Crude model	Ref.	1.01 (0.65, 1.56)	1.30 (0.83, 2.02)	1.81 (1.18, 2.77)	0.002
Multivariable model 1	Ref.	1.05 (0.66, 1.67)	1.08 (0.66, 1.75)	1.47 (0.93, 2.33)	0.068
Multivariable model 2	Ref.	1.09 (0.68, 1.75)	1.08 (0.66, 1.77)	1.56 (0.98, 2.49)	0.045
Glutamine					
Cases, *n*	97	83	80	66	
Crude model	Ref.	0.74 (0.49, 1.13)	0.78 (0.53, 1.16)	0.64 (0.42, 0.99)	0.061
Multivariable model 1	Ref.	0.94 (0.59, 1.49)	0.98 (0.64, 1.51)	0.94 (0.58, 1.53)	0.876
Multivariable model 2	Ref.	0.90 (0.56, 1.43)	0.93 (0.60, 1.45)	0.96 (0.59, 1.57)	0.917
Glutamine-to-glutamate ratio					
Cases, *n*	115	79	73	59	
Crude model	Ref.	0.67 (0.45, 1.01)	0.57 (0.38, 0.86)	0.47 (0.30, 0.74)	0.001
Multivariable model 1	Ref.	0.71 (0.46, 1.10)	0.71 (0.46, 1.11)	0.60 (0.37, 0.97)	0.051
Multivariable model 2	Ref.	0.70 (0.45, 1.09)	0.72 (0.46, 1.12)	0.57 (0.35, 0.94)	0.039

[1]Values are ORs (95% CIs). Ln transformation was applied to the raw values of individual metabolites. In the case of ratio of metabolites its raw value underwent ln transformation. Conditional logistic regression analysis was used. Multivariable model 1 adjusted for smoking, family history of cardiovascular disease, physical activity, alcohol intake, BMI (kg/m^2), intervention group (MedDiet + extra-virgin olive oil or MedDiet + nuts), dyslipidemia, hypertension, and type 2 diabetes. Multivariable model 2 in addition adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication). Case and control subjects were matched on age, sex, and recruitment center. AF, atrial fibrillation; HF, heart failure; MedDiet, Mediterranean diet; PREDIMED, Prevención con Dieta Mediterránea; Ref., reference.

To the best of our knowledge, the present study is the first to examine the role of glutamate/glutamine metabolism on the development of AF and HF. To date only a small cross-sectional study exists, reporting positive associations of high plasma glutamate and low plasma glutamine concentrations with HF (23). Emerging studies have related circulating glutamate and glutamine to cardiometabolic health including coronary artery disease (37), and more recently overall CVD (15) and T2D (14). In the present study, a positive association between glutamate and specific cardiometabolic risk factors such as TGs, glucose, and BMI, and negative associations with HDL cholesterol, were also demonstrated. These results support the evidence that glutamine and the glutamine-to-glutamate ratio exhibit contrary associations with these cardiometabolic risk factors. Such findings are in agreement with the results of a previous study conducted in the North American and Swedish populations (13). These associations further support the hypothesis that glutamate/glutamine metabolism could be involved in the development of CVDs.

Caspase activation is most likely the predominant mechanism in the induction of apoptosis, and can be induced by glutamate (16). Caspase-activated apoptosis affects contractility in failing myocardium (38), therefore a similar mechanism of glutamate-induced mitochondrial dysfunction can be hypothesized for myocardial cells. Because glutamate is associated with oxidative stress not only in neuronal cells but also in the heart conducting system (17), it is possible that elevated plasma glutamate concentrations could also induce mitochondrial oxidative stress affecting the function of cardiomyocytes. Previous research has also shown that glutamate receptors regulate glutamate signaling in cardiac tissues of humans and may play a role in the physiology of the heart (39, 40). For example, ionotropic glutamate receptors may modulate cardiac contractility by increasing the frequency of intracellular Ca^{2+} oscillations (41). Whether increased plasma glutamate results in overactivation of these receptors leading to calcium overload inside the cardiomyocytes, which in turn can result in apoptosis (42), is a hypothesis that deserves further investigation. In contrast, glutamine cardioprotection has been associated with improved myocardial metabolism, ATP availability, and enhanced myocardial glutathione content (18, 19). A recent study suggested that myocardial injury may play a role in HF onset (43), and glutamine supplementation has been shown to protect against myocardial injury in animal

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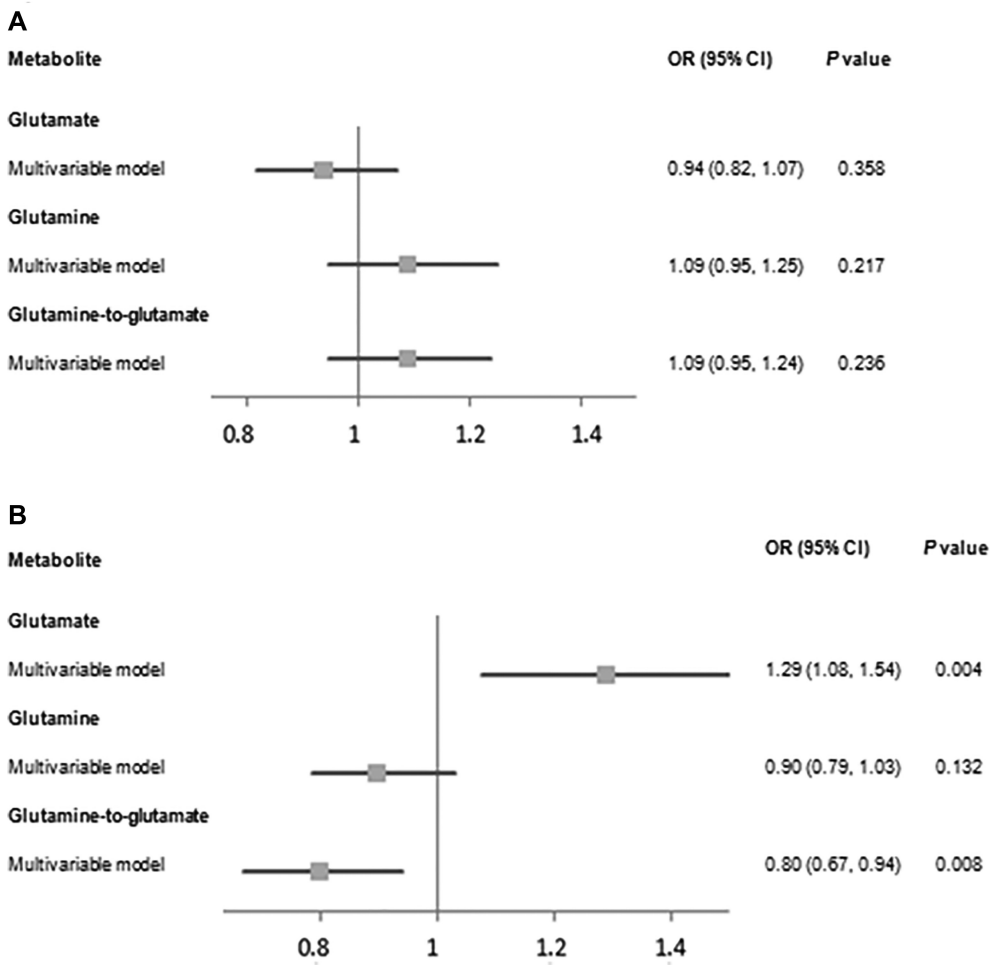

FIGURE 2 Associations of baseline plasma metabolite concentrations (per 1-SD increment) with risk of atrial fibrillation in a nested case-control study (509 cases, 618 controls) (A) or risk of heart failure in a nested case-control study (326 cases, 426 controls) (B) of the PREDIMED study. Ln transformation was applied to the raw values of individual metabolites. In the case of ratio of metabolites its raw value underwent ln transformation. Conditional logistic regression analysis was used. The multivariable model adjusted for smoking, family history of CVD, physical activity, alcohol intake, BMI (kg/m^2), intervention group (MedDiet + extra-virgin olive oil or MedDiet + nuts), dyslipidemia, hypertension, type 2 diabetes, and medication use (lipid-modifying, antihypertensive, and antidiabetic medication).

models (44, 45) as well as in patients undergoing elective cardiac surgery (46). Our findings regarding the glutamine-to-glutamate ratio highlight the importance of the balance between these 2 metabolites in relation to HF risk. Because the metabolism of glutamate and glutamine is related to energy production (20, 21), the glutamine-to-glutamate ratio may reflect the mitochondrial status of energy metabolism in cardiomyocytes. Therefore, we hypothesize that an imbalance between circulating

TABLE 3 Multiple linear regression coefficients for plasma metabolites (per 1-SD increment) in relation to lipids, glucose, and BMI in the heart failure case-control study[1]

Parameters	Glutamate	Glutamine	Glutamine-to-glutamate ratio
logChol,[2] mg/dL	−1.006 ± 0.03	1.005 ± 0.003	1.006 ± 0.003
logHDL-C,[2] mg/dL	−1.02 ± 0.003*	1.007 ± 0.003*	1.01 ± 0.03*
logLDL-C,[2] mg/dL	−1.008 ± 0.005	1.004 ± 0.004	1.007 ± 0.004
logTG,[2] mg/dL	1.03 ± 0.07*	−1.01 ± 0.006	−1.02 ± 0.006*
logGlucose,[3] mg/dL	1.01 ± 0.004*	−1.01 ± 0.003*	−1.01 ± 0.004*
BMI,[4] kg/m^2	0.74 ± 0.13*	−0.19 ± 0.11	−0.58 ± 0.12*

[1] Values are $\beta \pm$ SE, where β has been exponentiated for lipids. $*P$ value < 0.05. Chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triacylglycerol.
[2] Multiple linear regression analysis for lipids was adjusted for age, sex, center, smoking, physical activity, alcohol intake, BMI, dyslipidemia, hypertension, type 2 diabetes, and use of lipid-modifying medication.
[3] Multiple linear regression analysis for glucose was adjusted for age, sex, center, smoking, physical activity, alcohol intake, BMI, dyslipidemia, hypertension, and antidiabetic medication.
[4] Multiple linear regression analysis for BMI was adjusted for age, sex, center, smoking, physical activity, alcohol intake, dyslipidemia, hypertension, and type 2 diabetes.

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concentrations of glutamine and glutamate may reflect alterations in myocardial metabolism and a decreased availability of myocardial ATP and other phosphorylation substrates (47), thus affecting cardiomyocyte contractility. Furthermore, the importance of the antioxidant/oxidant balance for myocardial contractility is known (48), and glutathione seems to protect the cardiac myocyte from free-radical damage (49). Because glutathione is directly produced from glutamine (50), disruption of the glutamate-glutamine cycle may decrease glutathione stores in the cardiomyocytes, thus increasing their susceptibility to damage from reactive oxygen species. A decrease in the glutamine-to-glutamate ratio may also reflect higher glutaminase activity, which is responsible for the generation of glutamate from glutamine (51), and this activity is accompanied by increased generation of reactive oxygen species (51).

We cannot exclude the possibility that glutamate and the glutamine-to-glutamate ratio were not associated with AF owing to the very diverse genetic background that characterizes this disease. The genetics of AF is complex with both rare and common variants that increase susceptibility to AF being described (52). Further genetic research in the study population is necessary, in order to test this hypothesis.

The strengths of this study include the prospective design and the long follow-up period. Several limitations should also be noted. First, the elderly participants at high CVD risk from a Mediterranean region might limit the generalizability of our findings to other populations with low CVD risk. Second, although we carefully adjusted for many potential confounders, residual confounding cannot be ruled out (53). Third, information on blood urea nitrogen concentrations, which would help to better describe the role of glutamine/glutamate in the pathway of nitrogen metabolism, is not available in the study database. Fourth, the selected metabolites were drawn from a larger LC-MS panel of metabolites that may play a role in the development of AF and HF and potentially confound the observed associations.

In conclusion, our study has documented, to our knowledge for the first time, significant prospective associations of baseline plasma glutamate concentrations and the glutamine-to-glutamate ratio with HF risk but not AF. These findings underscore the potential role of the glutamate–glutamine pathway in the pathogenesis of HF. Further prospective studies are needed to confirm these findings along with experimental studies to investigate potential mechanisms linking these metabolites with HF.

Acknowledgments

The authors' responsibilities were as follows—FBH, JS-S, and MAM-G: designed the research; DC, RE, M Fitó, FA, M Fiol, LS-M, MAM-G, and JS-S: were the coordinators of subject recruitment at the outpatient clinics; CP and PH-A: analyzed the data; MB, CP, PH-A, FBH, and JS-S: interpreted the statistical analysis and data; CC: acquired and processed the metabolomics data; CP: drafted the paper; FBH and JS-S: supervised the study; MB, CP, and JS-S: had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; and all authors: conducted the research, revised the manuscript for important intellectual content, and read and approved the final manuscript.

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