Full title: High plasma glutamate and low glutamine-to-glutamate ratio are associated with increased risk of heart failure but not atrial fibrillation in the PREDIMED study

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Abbreviations: AF, atrial fibrillation; BMI, body mass index; CVD, cardiovascular diseases; ECGs, electrocardiograms; EVOO, extra-virgin olive oil; HF, heart failure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MedDiet, Mediterranean diet; PREDIMED, Prevención con Dieta Mediterránea; TAG, triacylglycerols; T2D, type 2 diabetes;

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.

Objective We examined associations of glutamate, glutamine and glutamine-to-glutamate ratio with AF and HF incidence in a Mediterranean population at high cardiovascular risk.

Methods The present study used two nested case-control studies within the PREDIMED study (Prevención con Dieta Mediterránea). During ≈10 years of follow-up, there were 509 AF incident cases matched to 618 controls and 326 HF incident cases matched to 426 controls. Plasma levels of glutamate and glutamine were semi-quantitatively profiled with liquid chromatography tandem mass spectrometry. Odds ratios were estimated with multivariable conditional logistic regression models.

Results In fully adjusted models, per 1-SD, 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: 0.80; 95% CI: 0.67, 0.94) when comparing extreme quartiles. Higher glutamate levels were associated with a worse, while higher glutamine-to-glutamate ratio with a better cardiometabolic risk profile. No associations between the levels of these metabolites with AF were observed.

Conclusion Our findings suggest that high plasma glutamate levels possibly resulting from alterations in the glutamate-glutamine cycle may contribute to the development of HF in Mediterranean individuals at high cardiovascular risk.

Keywords: Glutamate; glutamine; heart failure; PREDIMED

Trial registration: ID: ISRCTN35739639. URL: http://www.isrctn.com.

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 characterised 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 of these differences, AF and HF often coincide (5, 6) partially due 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 two 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 levels have been associated with an unfavourable cardiometabolic status (13). Our research group has prospectively demonstrated a positive association between systemic glutamate levels and the risk of type 2 diabetes (T2D) (14) and cardiovascular diseases (CVD) (15) using two case-cohort studies in individuals at high cardiovascular 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 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 levels 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 levels of glutamate and glutamine, respectively, and a decreased glutamine-to-glutamate ratio are associated with the risk of AF, and HF in two new prospective case-control studies, nested within the PREDIMED study. Secondarily, this study evaluates the hypothesis that these metabolites and the ratio are associated with cardiometabolic risk factors.

Methods

Study design and participants

This study used two case-control studies (AF and HF) nested within the PREDIMED trial (www.predimed.es, ISRCTN35739639), a primary prevention CVD study with Mediterranean diet (MedDiet). The protocol and design of the PREDIMED study have been described in details 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 October 1, 2003 to December 1, 2010 (median follow-up of 4.8 years) where information about CVD-related

outcomes was collected and analyzed (25) and an extended follow-up till December 2017. Five hundred 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 years), sex and center. In one 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 strategy described above, 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.

Measurement of Glutamate and Glutamine

Fasting plasma samples were collected using EDTA tubes and stored at −80 °C. Glutamate and glutamine levels were measured at the Broad Institute (Boston, Massachusets, USA) using liquid chromatography-tandem mass spectrometry (LC-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 to 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 were obtained. The medical charts were labelled 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 pre-specified 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).

a) 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 Committee following the procedure explained above.

b) 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 diagnostic criteria are specified in the **Supplemental methods**.

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), while its intensity was measured with metabolic equivalents. Habitual diet was assessed by a 137-item validated semiquantitative food frequency questionnaire (33, 34). Energy and 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. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Statistical analyses

Circulating levels 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 natural logarithmic 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 x^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 in minutes per day), alcohol intake (g/day), BMI (kg/m^2), 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 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medications). Metabolites were analyzed as both continuous variables [1-standard deviation (SD) (1-SD) increment in their ln-transformed levels calculated among controls and then applied to all sample] and by using quartiles (with cut-points defined among controls). To appraise 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 additionally conducted stratified analyses by age group (<65 yrs vs. ≥65 yrs), sex (male, female), T2D (yes, no) and obesity status (<30.0, ≥30.0 kg/m^2). 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. Since 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 vs. control group) was 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 the 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, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triacylglycerols (TAG)], 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 anti-diabetic medication was also included as confounder for lipids or blood glucose, respectively. Cholesterol, LDL-C, HDL-C, TAG and glucose levels were log-transformed to normalize their distributions. To make more informative the presentation of results of lipids and glucose we exponentiated the beta coefficients. Statistical analyses were performed using Stata 14.1 (Stata Corp.). A 2-sided *P* value less than 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, while 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, (**Supplementary tables 1 and 2**). Participants from the both case-control studies with a higher glutamine-to-glutamate ratio were also likely to use less often oral antidiabetic agents.

Association of baseline metabolites with risk of incident AF and HF

Associations between plasma levels of glutamate, glutamine and glutamine-to-glutamate ratio at baseline and risk of incident AF and HF are presented in **Table 2** and **Figure 2**. Overall, no significant associations between the metabolites under study and AF were observed. Sensitivity analyses did not change these results (**Supplementary tables 3 and 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* for trend=0.039). In the multivariable model 2, baseline glutamate was associated with increased HF risk (OR per 1 SD: 1.29; 95% CI: 1.08, 1.54; *P*=0.004), while the glutamine-to-glutamate ratio was associated with a decreased risk (OR per 1 SD: 0.80; 95% CI: 0.67, 0.94; *P*=0.008). In the unadjusted model, baseline levels of glutamine were also significantly associated with decreased risk of HF (OR per 1 SD: 0.87; 95% CI: 0.76, 0.99), but these associations were no longer significant after adjustment for potential confounders. Spline analysis (**Supplementary figure 1**) suggested nonlinear associations of glutamate and glutamine-to-glutamate ratio with HF risk (*P* value for nonlinearity with HF risk of glutamate was 0.016, and that of glutamine-to-glutamate ratio was 0.030). In both sensitivity analyses, glutamate levels

192 in the highest quartile were significantly associated with higher HF risk compared
193 with the lowest quartile (**Supplementary tables 4 and 6**). One SD increment in levels
194 of glutamate was also significantly associated with higher risk of HF incidence
195 (**Supplementary tables 4 and 6**). Both sensitivity analyses also showed that a high
196 glutamine-to-glutamate ratio was associated with a decreased risk of HF
197 (**Supplementary tables 4 and 6**).

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

203 **Metabolites in relation to lipids, glucose and BMI**

204 High plasma glutamate levels were associated with lower levels of HDL cholesterol,
205 and higher levels of plasma TAG, glucose and BMI. On the other hand, the
206 glutamine-to-glutamate ratio was positively associated with HDL cholesterol and
207 inversely associated with TAG, glucose and BMI (**Table 3**). Glutamine was positively
208 associated with HDL cholesterol and negatively associated with plasma glucose
209 levels.

210 **Discussion**

211 In our study, involving two prospective case-control studies nested within the
212 PREDIMED study cohort, baseline plasma glutamate levels were associated with a
213 29% increased risk of HF but not AF. In contrast, a higher glutamine-to-glutamate
214 ratio was associated with a 20% decreased risk of HF suggesting that an imbalance

between glutamine and glutamate plasma levels may contribute to the development of this pathology. These associations were nonlinear, the associations with HF were more pronounced among individuals with higher levels of glutamate and higher values of glutamate-to-glutamine.

To the best of our knowledge, the present study is the first to examine the role of the glutamate/glutamine metabolism on the development of AF and HF. To date only a small cross-sectional study exists, reporting positive associations of high glutamate and low glutamine plasma levels with HF (23). Emerging studies have related circulating glutamate and glutamine to cardiometabolic health including coronary heart 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 TAG, 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. Since 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 levels could also induce mitochondrial oxidative stress affecting the function of

cardiomyocytes. Previous research has also shown that glutamate receptors regulate glutamate signalling 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 over-activation 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, adenosine triphosphate (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 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 two metabolites in relation to HF risk. As 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 levels of glutamine and glutamate may reflect alterations in myocardial metabolism and a decreased myocardial ATP and other phosphorylation substrates availability (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). As 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 glutamate generation 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/glutamate ratio were not associated with AF due 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 cardiovascular 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 levels, 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 for the first time significant prospective associations between baseline plasma glutamate levels 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

288 are needed to confirm these findings along with experimental studies to investigate
289 potential mechanisms linking these metabolites with HF.

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292 MR-C, JL, MG-F, ET, CC, LL, DC, RE, MC, MF, CR, FA, MFIOL, JMS-L, LS-M,
293 LL, MM-G, FBH, JS-S conducted research; DC, RE, MF, FA, MFIOL, LS-M, MM-
294 G, JS-S were the coordinators of subject recruitment at the outpatient clinics; CP and
295 PHA analyzed the data; MB, CP, PHA, FBH, JS-S interpreted statistical analysis and
296 data; CC acquired and processed metabolomics data; CP drafted the paper; FBH and
297 JS-S supervised the study and MB, CP, JS-S had full access to all of the data in the
298 study and took responsibility for the integrity of the data and the accuracy of the data
299 analysis. All authors revised the manuscript for important intellectual content, read
300 and approved the final manuscript.

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Table 1. Baseline characteristics of the study population.

	AF Cases	Controls	HF Cases	Controls
n	509	618	326	426
Age (years)	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
Body mass index, 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/day)	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

Data are presented as mean (SD) or percentage. The x^2 test was used for comparison of categorical variables and Student's t-test was used for comparison of continuous variables. Abbreviations: 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.

Table 2. Associations of baseline plasma glutamine and glutamate levels and the glutamine-to-glutamate ratio by quartiles with risk of incident atrial fibrillation and heart failure in a nested case-control study of the PREDIMED study[1].

	Q1	Q2	Q3	Q4	*P* trend
Atrial fibrillation					
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
Heart failure					
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 OR (95% CI). A natural logarithmic transformation was applied to the raw values of individual metabolites. In the case of ratio of metabolites its raw value underwent natural logarithmic transformation. Conditional logistic regression analysis was used. Multivariable model 1 adjusted for smoking, family history of CVD, physical activity, alcohol intake, BMI (kg/m^2), intervention group (MedDiet + EVOO or MedDiet + nuts), dyslipidemia, hypertension and type 2 diabetes; Multivariable model 2 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication). Abbreviations: Ref, reference.

Case and control subjects were matched on age, sex and recruitment center.

Table 3 Multiple linear regression coefficients for plasma metabolite (per 1 SD increment) in relation to lipids, glucose and body mass index in the HF case-control study.

Parameters	Glutamate	Glutamine	Glutamine-to-glutamate ratio
logChol, mg/dL[1]	-1.006 ± 0.03	1.005 ± 0.003	1.006 ± 0.003
logHDL-C, mg/dL[1]	-1.02 ± 0.003*	1.007 ± 0.003*	1.01 ± 0.03*
logLDL-C, mg/dL[1]	-1.008 ± 0.005	1.004 ± 0.004	1.007 ± 0.004
logTAG, mg/dL[1]	1.03 ± 0.07*	-1.01 ± 0.006	-1.02 ± 0.006*
logGlucose, mg/dL[2]	1.01 ± 0.004*	-1.01 ± 0.003*	-1.01 ± 0.004*
BMI, kg/m[2] [3]	0.74 ± 0.13*	-0.19 ± 0.11	-0.58 ± 0.12*

[1]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.

[2]Multiple linear regression analysis for glucose was adjusted for age, sex, center, smoking, physical activity, alcohol intake, BMI, dyslipidemia, hypertension and anti-diabetic medication.

[3]Multiple linear regression analysis for BMI was adjusted for age, sex, center, smoking, physical activity, alcohol intake, dyslipidemia, hypertension and type 2 diabetes.

Values are expressed as β ± SE, where β has been exponentiated for lipids.

*P value <0.05.

Abbreviations: BMI, body mass index; Chol, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TAG, triacylglycerols.

Legends

Figure 1. Flow-chart of study participants.

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. A natural logarithmic transformation was applied to the raw values of individual metabolites. In the case of ratio of metabolites its raw value underwent natural logarithmic transformation. Conditional logistic regression analysis was used. Multivariable model adjusted for smoking, family history of CVD, physical activity, alcohol intake, BMI (kg/m^2), intervention group (MedDiet + EVOO or MedDiet + nuts), dyslipidemia, hypertension, type 2 diabetes and medication use (lipid-modifying, antihypertensive, and antidiabetic medication).