Arginine catabolism metabolites and atrial fibrillation or heart failure risk: 2 case-control studies within the Prevención con Dieta Mediterránea (PREDIMED) trial

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ABSTRACT

Background: Arginine-derived metabolites are involved in oxidative and inflammatory processes related to endothelial functions and cardiovascular risks.

Objectives: We prospectively examined the associations of arginine catabolism metabolites with the risks of atrial fibrillation (AF) or heart failure (HF), and evaluated the potential modifications of these associations through Mediterranean diet (MedDiet) interventions in a large, primary-prevention trial.

Methods: Two nested, matched, case-control studies were designed within the Prevención con Dieta Mediterránea (PREDIMED) trial. We selected 509 incident cases and 547 matched controls for the AF case-control study and 326 cases and 402 matched controls for the HF case-control study using incidence density sampling. Fasting blood samples were collected at baseline and arginine catabolism metabolites were measured using LC-tandem MS. Multivariable conditional logistic regression models were applied to test the associations between the metabolites and incident AF or HF. Interactions between metabolites and intervention groups (MedDiet groups compared with control group) were analyzed with the likelihood ratio test.

Results: Inverse association with incident AF was observed for arginine (OR per 1 SD, 0.83; 95% CI: 0.73–0.94), whereas a positive association was found for N1-acetylspermidine (OR for Q4 compared with Q1 1.58; 95% CI: 1.13–2.25). For HF, inverse associations were found for arginine (OR per 1 SD, 0.82; 95% CI: 0.69–0.97) and homoarginine (OR per 1 SD, 0.81; 95% CI: 0.68–0.96), and positive associations were found for the asymmetric dimethylarginine (ADMA) and symmetric dimethlyarginine (SDMA) ratio (OR per 1 SD, 1.19; 95% CI: 1.02–1.41), N1-acetylspermidine (OR per 1 SD, 1.34; 95% CI: 1.12–1.60), and diacetylspermine (OR per 1 SD, 1.20; 95% CI: 1.02–1.41). In the stratified analysis according to the dietary intervention, the lower HF risk associated with arginine was restricted to participants in the MedDiet groups (P-interaction = 0.044).

Conclusions: Our results suggest that arginine catabolism metabolites could be involved in AF and HF. Interventions with the MedDiet may contribute to strengthen the inverse association between arginine and the risk of HF. This trial was registered at controlled-trials.com as ISRCTN35739639. *Am J Clin Nutr* 2022;116:653–662.

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Introduction

The increasing prevalences of atrial fibrillation (AF) and heart failure (HF) in the general population have led to substantial morbidity and mortality and constitute a significant public health burden (1, 2). Although AF and HF are different diseases, they share common risk factors (age, obesity, diabetes, hypertension, and unhealthy lifestyles) and pathophysiologies (endothelial dysfunction, oxidative stress, and inflammation) (3). However, the risks of developing AF and HF are not completely understood, and metabolomics could help to clarify the pathogenic pathways involved in both diseases (4).

L-arginine is a conditionally essential amino acid involved in different pathways (urea cycle, NO synthesis, polyamines synthesis) and in the production of a wide variety of bioactive components (5). Dietary and plasma arginine is the precursor of ornithine and citrulline and, alternatively, arginine is transformed by proteolysis in NG-monomethylarginine (NMMA), which in turn is converted into asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). Arginine is also a precursor of polyamine biosynthesis (putrescine, spermidine, spermine) and homoarginine.

One prospective study and 2 cross-sectional studies have suggested that arginine and its related metabolites are associated with AF or HF risks (6–8). Furthermore, an inverse association between serum homoarginine levels and cardiovascular events suggest similar effects on AF and HF risks (9). In contrast, ADMA and SDMA have been associated with higher risks of heart-related diseases, including AF and HF (6, 7, 10, 11). Previous cross-sectional and prospective metabolomic studies found positive associations between different polyamines and the risks of HF (8, 12, 13) or AF (14).

Although arginine is not an essential amino acid, it is well known that several food groups, including fish, nuts, legumes, and whole-grain cereals, are important dietary sources of arginine and other NO precursors, such as vitamin C, polyphenols, omega-3 fatty acids, and nitrate (15, 16). These food groups are several essential components of the Mediterranean diet (MedDiet), which has been associated with lower risks of AF and HF (17–19). In this context, the first aim of the present study was to prospectively examine the associations of metabolites implicated in different arginine catabolism pathways with the risks of incident AF or HF, in 2 case-control studies nested within the Prevención con Dieta Mediterránea (PREDIMED) intervention trial. The second aim was to evaluate the effects of the MedDiet on the associations between the arginine-related metabolites and incidences of AF or HF.

Methods

Study design and population

For the present study, we designed 2 nested, case-control studies within the PREDIMED trial, a multicenter, parallel-group, randomized controlled trial aiming to evaluate the effect of the MedDiet on the primary prevention of cardiovascular disease (CVD) (20, 21). A total of 7447 participants (males aged 55–80 years and females aged 60–80 years) at high CVD risk but free of CVD at baseline were randomly assigned to 1 of 3 dietary intervention groups: *1*) the MedDiet enriched with extra virgin olive oil (EVOO); *2*) the MedDiet enriched with mixed nuts; and *3*) a low-fat diet (control group). The study had 2 periods: an intervention period from June 2003 to December 2010 (median follow-up of 4.8 years) and an extended follow-up until December 2017. The methods and design of the study have been described elsewhere (20).

A variable number of controls per case was randomly selected from all participants at risk at the time of the occurrence of the incident case (incidence density sampling with replacement). Thus, selected controls could be selected again as a control for another index case and, later, they could become a case (22). The matching factors were the recruitment center, year of birth (±5 years), and sex. Per case, 1 to 3 controls (depending on the availability of samples) were randomly selected from matched, disease-free (at the time of endpoint diagnosis in the matched case) study participants. The inclusion criterion of the case-control studies was the availability of preintervention EDTA plasma samples; participants with prevalent AF or HF at baseline were excluded (**Figures 1** and **2**).

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Supplemental Tables 1 and 2 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: ADMA, asymmetric dimethylarginine; AF, atrial fibrillation; CVD, cardiovascular disease; ECG, electrocardiogram; EVOO, extra virgin olive oil; FDR, false discovery rate; HF, heart failure; MedDiet, Mediterranean diet; MET, metabolic equivalent task; NMMA, NG-monomethylarginine; NOS, NO synthase; PREDIMED, Prevención con Dieta Mediterránea; SDMA, symmetric dimethylarginine.

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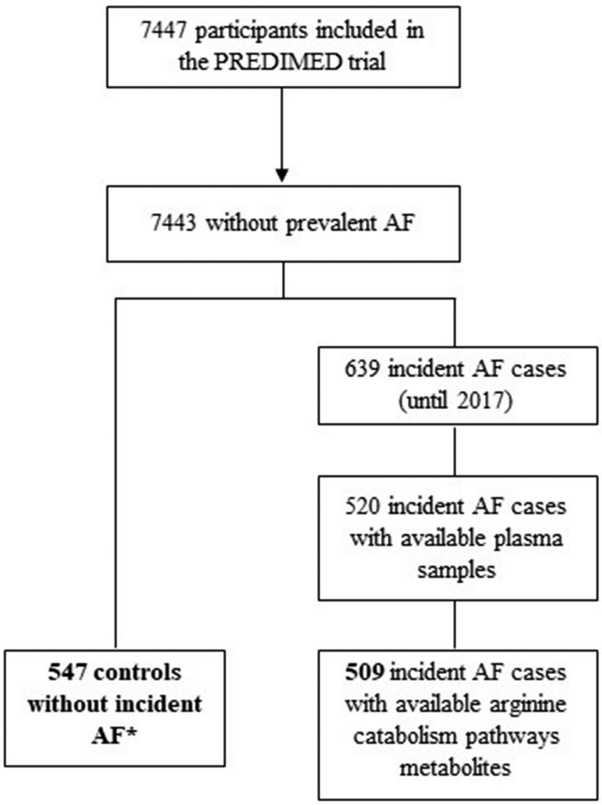

FIGURE 1 Flowchart of the case-control design for incident AF within the PREDIMED trial. *Incidence density sampling with replacement was used as the control sampling method. Abbreviations: AF, atrial fibrillation; PREDIMED, Prevención con Dieta Mediterránea.

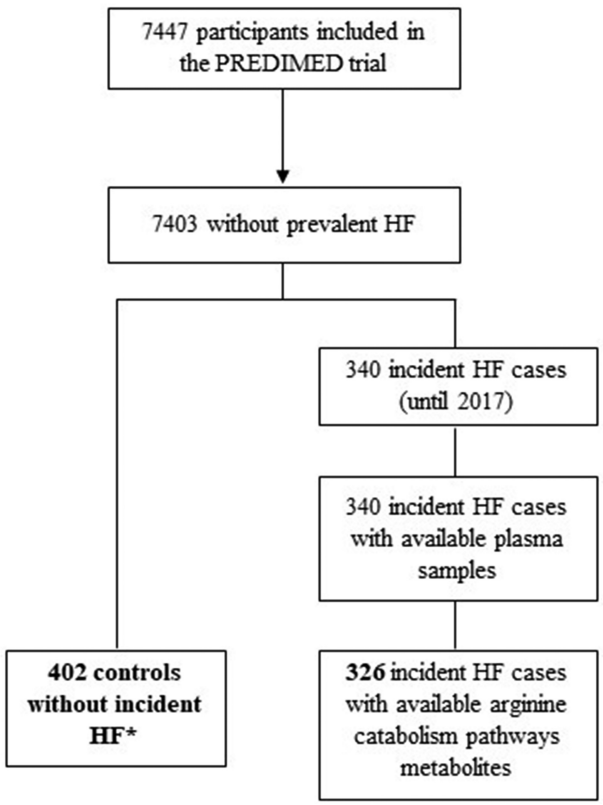

FIGURE 2 Flowchart of the case-control design for incident HF within the PREDIMED trial. *Incidence density sampling with replacement was used as the control sampling method. Abbreviations: HF, heart failure; PREDIMED, Prevención con Dieta Mediterránea.

The protocol of the PREDIMED trial was approved by the Research Ethics Committees of each of the recruitment centers, and all participants provided written informed consent before inclusion in the study to authorize the use of samples for biochemical measurements and genetic studies. The extended follow-up was approved by the Research Ethics Committee of the University of Barcelona-Hospital Clinic, as the coordinating center.

Ascertainment of AF and HF cases

The PREDIMED protocol included AF and HF as prespecified secondary endpoints (17, 18). During the first period of 2003 to 2010, information on both outcomes was collected from continuous contact with participants and primary health-care physicians; annual follow-up visits; and yearly, ad hoc reviews of medical charts. Meanwhile, during the extended follow-up period up to 2017, information on AF and HF was updated by yearly reviews of the medical charts of participants. If a clinical diagnosis of AF or HF was found, all relevant documentation, including clinical records of hospital discharge, outpatient clinics, and family physicians' records, were obtained and codified to send it to the Clinical End-Point Committee. This documentation was independently reviewed by 2 cardiologists and, if they did not agree on the diagnosis of the event, consensus was reached by asking a third cardiologist. The Clinical End-Point Committee adjudicated the events according to published criteria in a "Manual of Operations." The physicians who collected the events and the members of the Clinical End-Point Committee were blinded to the intervention group. The mean times from baseline to AF or HF diagnosis were 6.7 years (SD, 3.3 years) and 7.3 years (SD, 3.3 years), respectively.

The diagnostic criteria and procedures for adjudicating confirmed cases of AF and HF have been reported in detail elsewhere (17, 18). Briefly, AF was initially identified from an annual review of all medical records of each subject and yearly electrocardiograms (ECGs) performed in the health-care centers during follow-up examinations. A diagnosis of AF was made only if both AF was present in an ECG tracing and an explicit medical diagnosis of AF was identified by a physician. AF events related to myocardial infarction of cardiac surgery were not included. The diagnosis criteria of HF were defined according to the 2005 (time of study design) guidelines of the European Society of Cardiology (23). The presence of HF was determined as having symptoms and/or signs of HF (more frequently breathlessness of fatigue at rest or during exertion, or ankle swelling) attributable to objective evidence of cardiac dysfunction at rest (preferably by echocardiography).

Sample collection and metabolite profiling

At baseline, fasting plasma samples were collected after at least an 8-hour fast using EDTA-coated tubes and stored at −80°C until their analysis. Pairs of case-control samples were

shipped and assayed in the same analytical run, randomly sorted to reduce bias and inter-assay variability.

Metabolomic analyses were carried out at the Broad Institute of MIT and Harvard using LC-tandem MS. Hydrophilic interaction LC coupled with high-resolution, positive ion–mode MS detection was used to analyze arginine, ornithine, citrulline, NMMA, the ADMA/SDMA ratio, acetylputrescine, N1-acetylspermidine, and diacetylspermine, as described previously (24). We monitored internal standard peak areas for quality control and to ensure system performance throughout analyses. Moreover, pooled plasma reference samples were interleaved every 20 samples as an additional quality control (25). Raw data were processed with the use of TraceFinder (Thermo Fisher Scientific) and Progenesis QI (Nonlinear Dynamics) software to integrate chromatographic peaks, and the data were visually inspected to ensure the quality of signal integration. Inter-assay reproducibility was assessed with CVs from 82 duplicate plasma samples and pooled quality-control plasma samples inserted every 20 samples. The metabolite CVs ranged from 2.4% to 8.1%.

Covariates assessment

Information about sociodemographic variables, lifestyle habits, prevalent and family histories of diseases, and medication use was collected using different questionnaires at the baseline visit. Trained personnel measured height and body weight, and the BMI was calculated as kg/m^2. Physical activity was measured using the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire (26, 27). Three missing values for physical activity variable were imputed using the command impute from Stata and using age, sex, BMI, total energy intake, and smoking as covariates.

Statistical analysis

Means and SDs were used to describe quantitative variables and percentages were used to describe categorical variables. Individual circulating arginine catabolism metabolite values were normalized and scaled to multiples of 1 SD using Blom's inverse normal transformation.

We used conditional logistic regression models to estimate the associations between metabolites and incident AF or HF. Matched ORs and their 95% CIs for AF or HF were estimated, with the metabolites categorized into quartiles (using the first quartile as the reference category). For the categorization of the subjects into quartiles, first we used the controls, and then we applied the cutoffs to cases. To analyze linear trends across the quartiles of arginine catabolism pathway metabolites and AF or HF, we assigned the median value of each category and modeled the values as continuous. In comparison, we calculated the matched ORs for the SDs of each metabolite as continuous variables. In addition, we conducted additional conditional, logistic regression models with each metabolite as a quadratic term to explore potential nonlinear associations between the metabolites and the AF or HF risk. *P* values were adjusted with the use of the Simes false discovery rate (FDR) procedure to account for multiple testing (28). A multivariable model adjusted for potential confounders was fitted. Model 1 was adjusted for age, intervention group (MedDiet + EVOO, MedDiet + nuts, or control), smoking status (never, current, or former), BMI (kg/m^2), leisure-time physical activity [metabolic equivalent task (MET) min/day], and prevalent chronic conditions at baseline (hypertension, type 2 diabetes, and dyslipidemia). In addition, 3 sensitivity analyses were performed: *1*) conditional logistic regression models were adjusted for variables included in model 1 + changes during the intervention period on BMI, glucose, triglycerides, total cholesterol, systolic and diastolic blood pressure, and physical activity; *2*) restricting the follow-up time until 1 December 2010, when the intervention period finished; and *3*) excluding those subjects with major CVD (stroke, myocardial infarction) and non-CVD (cancer, diabetes, neurodegenerative diseases) before the date of the AF or HF event.

Potential interactions between the intervention (MedDiet groups merged together compared with the control group) and arginine and N1-acetylspermidine as continuous variables (per 1-SD increase) were tested using conditional logistic models including a multiplicative interaction term [MedDiet (yes/no) × Metabolite]. The *P* values for the interactions were calculated using the likelihood ratio test. Conditional logistic regression models were adjusted for age, sex, recruitment center, smoking status (never, current, or former), BMI (kg/m^2), leisure-time physical activity (MET min/day), prevalent chronic conditions at baseline, (hypertension, type 2 diabetes, and dyslipidemia), and propensity scores that used 30 baseline variables to estimate the probability of assignment to each of the intervention groups (21). Statistical analyses were performed using STATA/SE version 12.0 (Stata Corp.).

Results

Table 1 shows the baseline characteristics of participants in the 2 case-control studies nested within the PREDIMED trial, separated by AF or HF incidence. The numbers of incident cases of AF and HF were 509 and 326, respectively. Participants who developed AF were more likely to show hypertension at baseline than those participants who did not develop the disease. Meanwhile, we observed that incident HF cases were more likely to smoke and present with hypertension, type 2 diabetes, and AF than their controls.

The associations between arginine catabolism metabolites at baseline and the risk of AF are shown in **Table 2**. In the adjusted model and after correcting for multiple testing, arginine was associated with a lower risk of AF across quartiles (OR for Q4 compared with Q1, 0.55; 95% CI: 0.38–0.80; FDR-corrected *P*-trend = 0.018), but ornithine, citrulline, or homoarginine were not associated with the AF risk. When we considered the metabolites as continuous variables, a 1-SD increase in baseline arginine was associated with a 17% (OR for 1-SD increase, 0.83; 95% CI: 0.73–0.94; FDR-corrected $P = 0.044$) lower risk of AF. Regarding polyamines, N1-acetylspermidine was longitudinally associated with the AF risk when viewed by quartiles (OR for Q4 compared with Q1, 1.58; 95% CI: 1.13–2.25; FDR-corrected *P*-trend = 0.049), but not as a continuous variable. Similar results were observed when we added a quadratic term of the metabolites to assess a potential nonlinear association between arginine metabolites and polyamines and AF (data not shown). Results from sensitivity analyses did not substantially

TABLE 1 Baseline characteristics of AF and HF cases and controls[1]

Characteristics	Case-control sets for AF		Case-control sets for HF	
	Controls	Cases	Controls	Cases
n	547	509	402	326
Age, years	68.4 (6.2)	68.3 (6.1)	70.2 (5.9)	70.3 (5.8)
Sex, female, %	49.6	49.8	55.0	58.3
Intervention group, %				
MedDiet + EVOO	37.1	31.6	38.1	31.0
MedDiet + nuts	28.5	31.6	25.6	32.5
Control	34.4	36.8	36.3	36.5
Smoking, %				
Never	57.8	58.7	61.7	59.8
Former	28.2	27.1	27.1	25.8
Current	14.0	14.2	11.2	14.4
Physical activity, MET-min/d	232.3 (222.7)	227.6 (215.9)	211.2 (217.3)	215.8 (204.4)
Education, %				
Elementary or lower	79.3	76.1	81.6	85.0
Secondary or higher	20.7	23.9	18.4	15.0
Total energy intake, kcal/d	2340 (604.1)	2285 (600.0)	2282 (634.5)	2217 (632.4)
MEDAS	8.7 (1.9)	8.7 (2.0)	8.6 (2.0)	8.5 (2.0)
Alcohol consumption, g/d	10.0 (15.5)	8.9 (13.4)	8.2 (12.4)	8.1 (15.3)
Waist circumference, cm	100.3 (9.9)	103.3 (9.9)	98.6 (9.8)	103.8 (10.1)
BMI, kg/m^2	29.7 (3.8)	30.7 (3.8)	29.3 (3.6)	31.1 (3.8)
Family history of premature CHD, %	20.2	19.2	19.9	19.3
Hypertension, %	82.0	88.3	82.1	87.4
Dyslipidemia, %	69.6	65.2	68.9	64.1
Type 2 diabetes, %	51.3	47.9	51.5	59.5
Atrial fibrillation, %	—	—	0.0	3.7
Heart failure, %	0.1	0.4	—	—

[1]Data are shown as the mean (SD), unless otherwise indicated. Abbreviations: AF, atrial fibrillation; CHD, coronary heart disease; EVOO, extra virgin olive oil; HF, heart failure; MEDAS, Mediterranean Diet Adherence Screener; MedDiet, Mediterranean diet; MET, metabolic equivalent task.

change, except when we restricted the analyses to the intervention period, where the number of AF cases was 150 (**Supplemental Table 1**).

Table 3 presents the results of the associations between arginine catabolism metabolites and HF. Arginine and homoarginine were negatively associated with the risk of HF, showing ORs for a 1-SD increase of 0.82 (95% CI: 0.69–0.97; FDR-corrected $P = 0.049$) and 0.81 (95% CI: 0.68–0.96; FDR-corrected $P = 0.049$), respectively, whereas citrulline was positively associated with the HF risk (OR for 1-SD increase, 1.19; 95% CI: 1.01–1.39; FDR-corrected $P = 0.049$) in the multivariable-adjusted model and after correcting for multiple testing. For ADMA/SDMA, we observed a positive association with the risk of HF when we analyzed the ratio as a continuous variable (OR for 1-SD increase, 1.19; 95% CI: 1.02–1.41; FDR-corrected $P = 0.049$). In this case, 2 polyamines were associated with the HF risk: N1-acetylspermidine and diacetylspermine. For both N1-acetylpermidine [OR for Q4 compared with Q1, 1.77 (95% CI: 1.12–2.79; FDR-corrected P-trend $= 0.018$); OR for 1-SD increase, 1.34 (95% CI: 1.12–1.60; FDR-corrected $P = 0.013$)] and diacetylspermine (OR for 1-SD increase, 1.20; 95% CI: 1.02–1.41; FDR-corrected $P = 0.049$), higher values were associated with a higher risk of the disease. When we added a quadratic term, similar associations were found between arginine metabolites or polyamines and HF risks (data not shown). The results were robust in the sensitivity analyses, except when we restricted the analyses to the intervention period (number of HF cases = 94), in which some of the metabolites did not remain statistically significantly associated with the HF risk (**Supplemental Table 2**).

Finally, the potential interactions between arginine or N1-acetylspermidine and the intervention group (both MedDiet groups merged together compared with the control group) on the risks of AF and HF were analyzed. Regarding AF, no significant interactions were observed (data not shown). However, we observed that the MedDiet interventions modified the effect of arginine on the HF risk (P-interaction $= 0.044$; **Figure 3**). Participants in the MedDiet intervention group (EVOO + nuts) showed a lower HF incidence risk for higher values of arginine (OR for 1-SD increase, 0.72; 95% CI: 0.58–0.90); meanwhile, this association was absent in the control group (OR for 1-SD increase, 1.01; 95% CI: 0.79–1.30). The interaction between N1-acetylspermidine and MedDiet on the HF risk was not statistically significant (P-interaction $= 0.765$) (Figure 3).

Discussion

In 2 case-control studies in the PREDIMED trial, we identified several arginine-derived metabolites associated with AF and HF risks. Baseline arginine was inversely associated with the AF incidence, and arginine, citrulline, and homoarginine with the HF incidence. In contrast, N1-acetylspermidine was positively associated with the risk of AF, and ADMA/SDMA,

TABLE 2 ORs (95% CIs) for the association of arginine catabolism metabolites and atrial fibrillation[1]

	Metabolite in quartile categories, OR (95% CI)					FDR *P* value	Metabolite as continuos variable,per SD		
	Q1	Q2	Q3	Q4	*P*-trend		OR (95% CI)	*P* value	FDR*P* value
Arginine									
Crude	1.00 (ref)	0.88 (0.64–1.19)	0.84 (0.61–1.16)	0.50 (0.35–0.72)	<0.001	0.009	0.80 (0.71–0.91)	0.001	0.005
Model 1	1.00 (ref)	0.89 (0.65–1.23)	0.86 (0.62–1.21)	0.55 (0.38–0.80)	0.002	0.018	0.83 (0.73–0.94)	0.005	0.044
Ornithine									
Crude	1.00 (ref)	0.87 (0.63–1.21)	0.98 (0.69–1.38)	0.99 (0.70–1.39)	0.823	0.926	0.99 (0.87–1.12)	0.834	0.900
Model 1	1.00 (ref)	0.86 (0.61–1.21)	1.02 (0.72–1.46)	1.02 (0.72–1.46)	0.615	0.791	0.78 (0.58–1.06)	0.971	0.989
Citrulline									
Crude	1.00 (ref)	0.91 (0.65–1.28)	0.92 (0.65–1.29)	0.91 (0.64–1.30)	0.661	0.850	0.99 (0.88–1.12)	0.900	0.900
Model 1	1.00 (ref)	0.98 (0.69–1.38)	0.95 (0.66–1.37)	0.98 (0.68–1.42)	0.904	0.904	1.01 (0.89–1.15)	0.863	0.989
Homoarginine									
Crude	1.00 (ref)	0.61 (0.43–0.85)	0.77 (0.55–1.06)	0.65 (0.47–0.92)	0.064	0.115	0.86 (0.76–0.98)	0.019	0.043
Model 1	1.00 (ref)	0.58 (0.41–0.83)	0.78 (0.56–1.09)	0.66 (0.46–0.93)	0.092	0.207	0.87 (0.76–0.98)	0.027	0.064
NMMA									
Crude	1.00 (ref)	0.96 (0.68–1.34)	0.89 (0.64–1.24)	1.01 (0.73–1.39)	0.986	0.986	1.01 (0.90–1.35)	0.883	0.900
Model 1	1.00 (ref)	0.95 (0.67–1.34)	0.86 (0.61–1.21)	0.98 (0.70–1.36)	0.865	0.904	1.00 (0.88–1.13)	0.988	0.989
ADMA/SDMA									
Crude	1.00 (ref)	1.06 (0.74–1.52)	1.33 (0.95–1.87)	1.37 (0.97–1.94)	0.046	0.113	1.15 (1.02–1.30)	0.019	0.043
Model 1	1.00 (ref)	1.00 (0.69–1.46)	1.34 (0.95–1.90)	1.32 (0.92–1.90)	0.070	0.207	1.15 (1.01–1.31)	0.028	0.064
N-acetylputrescine									
Crude	1.00 (ref)	1.18 (0.85–1.64)	1.02 (0.72–1.45)	1.43 (1.02–2.00)	0.050	0.113	1.11 (0.98–1.24)	0.095	0.154
Model 1	1.00 (ref)	1.11 (0.79–1.55)	0.98 (0.68–1.40)	1.24 (0.87–1.77)	0.273	0.491	1.05 (0.93–1,19)	0.433	0.658
N1-acetylspermidine									
Crude	1.00 (ref)	1.35 (0.96–1.89)	1.42 (1.01–2.00)	1.74 (1.24–2.43)	0.002	0.009	1.19 (1.06–1.34)	0.003	0.012
Model 1	1.00 (ref)	1.23 (0.86–1.75)	1.33 (0.93–1.91)	1.58 (1.13–2.25)	0.011	0.049	1.16 (1.03–1.31)	0.017	0.064
Diacetylspermine									
Crude	1.00 (ref)	1.10 (0.78–1.55)	1.11 (0.79–1.55)	1.31 (0.94–1.84)	0.107	0.161	1.10 (0.98–1.25)	0.102	0.154
Model 1	1.00 (ref)	1.01 (0.71–1.44)	1.01 (0.71–1.44)	1.14 (0.80–1.63)	0.408	0.612	1.05 (0.93–1.20)	0.420	0.658

[1]Conditional logistic regression was used. Model 1 was adjusted for age, smoking status (never, current, or former), BMI (kg/m^2), physical activity (MET-min/day), and prevalent chronic conditions at baseline (hypertension, dyslipidemia, and type 2 diabetes). Abbreviations: ADMA, asymmetric dimethylarginine; FDR, false discovery rate; MET, metabolic equivalent task; NMMA, NG-monomethylarginine; Q, quartile; SDMA, symmetric dimethylarginine ratio.

TABLE 3 ORs (95% CI) for the association of arginine catabolism metabolites and heart failure[1]

	Metabolite in quartile categories, OR (95% CI)					FDR *P* value	Metabolite as continuous variable,per SD		
	Q1	Q2	Q3	Q4	*P*-trend		OR (95% CI)	*P* value	FDR*P* value
Arginine									
Crude	1.00 (ref)	0.74 (0.50–1.11)	0.71 (0.48–1.05)	0.61 (0.40–0.92)	0.017	0.045	0.78 (0.67–0.91)	0.001	0.006
Model 1	1.00 (ref)	0.79 (0.51–1.21)	0.79 (0.51–1.21)	0.72 (0.45–1.13)	0.157	0.202	0.82 (0.69–0.97)	0.018	0.049
Ornithine									
Crude	1.00 (ref)	0.73 (0.49–1.09)	0.69 (0.46–1.05)	0.83 (0.55–1.25)	0.537	0.604	0.94 (0.80–1.09)	0.411	0.411
Model 1	1.00 (ref)	0.66 (0.43–1.02)	0.60 (0.38–0.95)	0.87 (0.55–1.38)	0.802	0.802	0.95 (0.81–1.13)	0.572	0.572
Citrulline									
Crude	1.00 (ref)	1.03 (0.69–1.55)	1.00 (0.69–1.55)	1.28 (0.85–1.92)	0.259	0.370	1.08 (0.93–1.25)	0.301	0.375
Model 1	1.00 (ref)	1.26 (0.81–1.96)	1.19 (0.76–1.87)	1.68 (1.07–2.63)	0.030	0.090	1.19 (1.01–1.39)	0.033	0.049
Homoarginine									
Crude	1.00 (ref)	0.79 (0.54–1.17)	0.95 (0.64–1.39)	0.54 (0.34–0.84)	0.020	0.045	0.80 (0.68–0.94)	0.005	0.014
Model 1	1.00 (ref)	0.78 (0.51–1.20)	0.99 (0.65–1.51)	0.55 (0.34–0.90)	0.046	0.103	0.81 (0.68–0.96)	0.015	0.049
NMMA									
Crude	1.00 (ref)	1.26 (0.85–1.89)	1.14 (0.75–1.72)	1.31 (0.86–2.00)	0.288	0.370	1.10 (0.95–1.28)	0.199	0.298
Model 1	1.00 (ref)	1.20 (0.78–1.85)	1.26 (0.80–1.97)	1.45 (0.92–2.29)	0.113	0.173	1.16 (0.99–1.36)	0.074	0.096
ADMA/SDMA									
Crude	1.00 (ref)	1.27 (0.84–1.91)	0.90 (0.59–1.36)	1.15 (0.77–1.71)	0.749	0.749	1.07 (0.93–1.24)	0.334	0.375
Model 1	1.00 (ref)	1.23 (0.78–1.94)	1.02 (0.65–1.59)	1.49 (0.95–2.33)	0.115	0.173	1.19 (1.02–1.41)	0.032	0.049
N-acetylputrescine									
Crude	1.00 (ref)	1.49 (1.00–2.24)	1.36 (0.89–2.09)	1.43 (0.93–2.21)	0.212	0.370	1.14 (0.98–1.32)	0.099	0.178
Model 1	1.00 (ref)	1.46 (0.94–2.26)	1.18 (0.74–1.88)	1.31 (0.82–2.09)	0.455	0.512	1.11 (0.94–1.31)	0.208	0.234
N1-acetylspermidine									
Crude	1.00 (ref)	0.92 (0.59–1.41)	0.91 (0.59–1.41)	1.87 (1.24–2.83)	0.001	0.009	1.34 (1.14–1.57)	<0.001	0.003
Model 1	1.00 (ref)	0.77 (0.48–1.23)	0.93 (0.58–1.49)	1.77 (1.12–2.79)	0.002	0.018	1.34 (1.12–1.60)	0.001	0.013
Diacetylspermine									
Crude	1.00 (ref)	1.06 (0.70–1.62)	1.41 (0.93–2.16)	1.76 (1.16–2.67)	0.003	0.014	1.23 (1.06–1.43)	0.006	0.014
Model 1	1.00 (ref)	0.92 (0.58–1.46)	1.13 (0.71–1.78)	1.56 (1.00–2.44)	0.014	0.063	1.20 (1.02–1.41)	0.026	0.049

[1]Conditional logistic regression was used. Model 1 was adjusted for age, smoking status (never, current, or former), BMI (kg/m^2), physical activity (MET-min/day), and prevalent chronic conditions at baseline (hypertension, dyslipidemia, and type 2 diabetes). Abbreviations: ADMA, asymmetric dimethylarginine; FDR, false discovery rate; MET, metabolic equivalent task; NMMA, NG-monomethylarginine; Q, quartile; SDMA, symmetric dimethylarginine ratio.

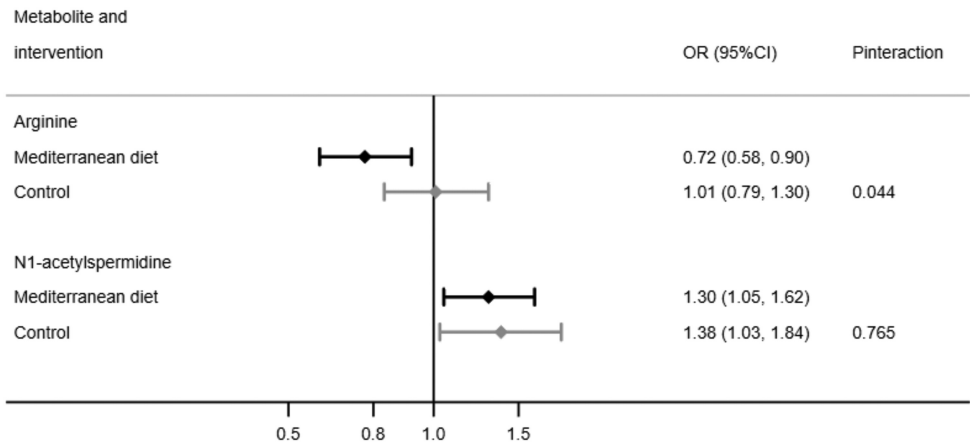

FIGURE 3 Association (per SD) between arginine or N1-acetylspermidine and incident heart failure, stratified by intervention group. Adjusted for age, sex, smoking status (never, current, or former), BMI (kg/m^2), physical activity (MET-min/day), prevalent chronic conditions at baseline (dyslipidemia, hypertension, and type 2 diabetes), and propensity scores predicting random assignment to account for small between-group imbalances at baseline. Abbreviation: MET, metabolic equivalent task.

N1-acetylspermidine, and diacetylspermine were positively associated with the risk of HF. In addition, we found an interaction between the MedDiet and arginine levels on the HF risk, observing an inverse association only within the MedDiet group.

Regarding primary urea cycle metabolites, we found a significant, inverse relationship between baseline arginine levels and the AF incident risk, and between arginine and citrulline and the HF risk. Two previous studies reported that arginine was significantly lower in patients with AF as compared to AF-free subjects (6, 7). However, no association was observed between arginine levels and the AF risk in the Framingham Offspring prospective cohort study after a 10-year median follow-up and 247 incident cases observed (29). Meanwhile, the literature regarding the associations of these metabolites with HF is scarce. Cheng et al. (8) observed that HF patients in stage C had lower levels of arginine than healthy subjects. Moreover, they found a significantly lower citrulline:ornithine ratio among patients with HF in stage C, but no association with citrulline concentrations, as was found in our study.

In our study, higher levels of ADMA/SDMA were associated with a higher risk of HF but not with AF after FDR correction. Although dimethylarginines have been widely associated with CVD (10), the results are inconclusive for the specific outcomes of AF and HF. Ramuschkat et al. (7) and Schulze et al. (6) observed that higher levels of ADMA and SDMA, respectively, were positively associated with new-onset AF. However, Schnabel et al. (29) reported a nonsignificant association between ADMA and an increased risk of AF after adjusting for traditional AF risk factors. According to this, the AF risk reflected by ADMA levels appears to be explained by classical cardiovascular risk factors. Regarding HF, and consistent with our results, Wirth et al. (11) found a positive association between ADMA/SDMA and the HF risk in a case-cohort study within the European Prospective Investigation into Cancer and Nutrition cohort. Both dimethylarginines have been postulated as biomarkers of the severity of AF and HF, and have also been associated with the risk of mortality in patients with AF and HF (30–32). However, more prospective studies are needed to clarify the associations of ADMA and SDMA with incident AF or HF.

Similar to arginine, we found an inverse association between baseline homoarginine levels and the HF incidence. A cross-sectional study within the Gutenberg health study did not observe an association between homoarginine levels and the AF risk (33). However, among AF patients, homoarginine levels were lower in patients with AF at the time of blood sampling compared with patients in sinus rhythm, and among patients with advanced AF progression phenotypes (31). Moreover, a previous study among acute chest pain patients showed an inverse association between homoarginine concentrations and prevalent AF (34). Concerning HF, and in accordance with our findings, homoarginine levels were inversely related to incident HF (HR per 1-SD increase in metabolite = 0.77; 95% CI: 0.66–0.90) in black adults in the Jackson Heart Study during a mean follow-up of 9.6 years (12).

Our results also showed a direct association between N1-acetylspermidine or diacetylspermine and the HF incidence. No association was observed with the AF risk after FDR correction. A previous study in the Atherosclerosis Risk in Communities cohort study found that higher levels of acisoga, a catabolic product of spermidine formed from N1-acetylspermidine, were associated with higher risks of AF (HR per 1-SD difference = 1.15; 95% CI: 1.06–1.24) (14). Regarding HF, higher levels of spermidine, N8-acetylspermidine, N1-acetylspermidine, and diacetylspermine were associated with greater risks of HF in 1 cross-sectional and 2 prospective cohort studies (8, 12, 13). Surprisingly, in a prospective study and in a cross-sectional study, a higher intake of spermidine was associated with lower incidences of cardiovascular diseases and HF (35, 36). These controversial results could be due to insufficient data on the contributions of dietary spermidine to plasma spermidine concentrations.

The involvement of arginine and related metabolites in the pathogenesis of AF and HF could be partially explained through their roles in NO production. Low levels of NO may contribute

to different pathological states, including atherosclerosis, a common cause of AF and HF (37). Arginine may prevent cardiovascular dysfunction by restoring NO synthesis and decreasing the production of reactive oxygen species that interact with NO, limiting its bioavailability (37). In addition, it is well known that NO synthesis can be impaired by ADMA and SDMA (38). ADMA is an endogenous inhibitor of endothelial NO synthase (NOS) (39), and changes in plasma ADMA concentrations correlate with variability in activation of NOS (40, 41). Similarly, SDMA inhibits arginine transport, reducing cellular uptake of arginine as a substrate for NO synthesis (38, 42). In contrast, homoarginine can serve as an NO substrate (9, 34), although it is generally considered a weaker substrate than arginine (43). Moreover, homoarginine could be a specific, uncompetitive inhibitor of tissue-nonspecific alkaline phosphatase (9), which has been directly associated with CVD and overall mortality, both in patients at high risk for CVD and in the general population (44, 45). Finally, there is a paucity of data on the use of polyamines, including spermidine, spermine, and their derivates, as biomarkers of AF or HF incidences. Meanwhile, several studies have suggested that spermidine intake may reduce the risks of different pathophysiological conditions, including CVD (35, 36), and metabolomic studies have reported a positive association between polyamine levels and AF or HF risks (8, 12, 13). A previous study suggested that increased levels of N8-acetylspermidine could decrease intracellular spermidine bioavailability or enhance spermidine production and degradation in response to ischemic stress (13). Whether high levels of polyamines reflect increases in the risks of AF or HF requires further exploration.

We observed a significant effect modification by the MedDiet intervention on the association between arginine and incident HF, but not between arginine and AF or N1-acetylspermidine and AF or HF. Specifically, we found that arginine was only associated with a lower risk of HF in the MedDiet intervention groups, but not in the control group. The MedDiet is characterized by abundant intakes of dietary arginine (fish, nuts, legumes, and whole-grain cereals), vitamin C and polyphenols (fruits, vegetables, whole-grain cereals, legumes, wine, nuts, olive oil), omega-3 fatty acids (fish, seafood, and walnuts), and nitrate (green, leafy vegetables), which are potential precursors of NO synthesis in the body (15). Moreover, in a substudy within the PREDIMED intervention trial, NO availability in plasma increased in participants randomized to each of the 2 the MedDiet groups (EVOO or nuts) (46). Therefore, it could be possible that the MedDiet could enhance the beneficial effects associated with high levels of arginine in participants with a high cardiovascular risk and in whom the endogenous arginine synthesis was not sufficient to meet metabolic demands. Unfortunately, in the present study, because we did not repeat measurements of metabolites, we could not analyze the effects of the MedDiet on changes in metabolites. Moreover, at baseline, we did not find a significant correlation between MedDiet adherence, foods rich in arginine or in potential precursors of NO, and arginine catabolism metabolites. This lack of an association is consistent with the results of other studies that did not observe correlations between the intakes of certain compounds and plasma or serum metabolites (47).

The present study has several strengths and limitations. First, the results may not be extrapolated to other populations, since the participants were elderly subjects from a Mediterranean country and at high cardiovascular risk. In spite of the specific characteristics of our population, we used an efficient case-control design nested in a large, long-term intervention trial that is especially suited to studying the potential interactions between the MedDiet and the arginine pathway metabolites on AF and HF risks. Second, the number of cases was limited, particularly for HF. Although we used a robust assessment method of incident AF and HF cases by a clinical adjudication committee, further, independent studies should be performed to replicate our findings. Third, metabolomic assays did not capture all the metabolites involved in arginine metabolism pathways. However, we used a validated method (LC-MS/MS platform) covering central metabolites involved in the 3 main arginine metabolism pathways, the urea cycle, NO synthesis, and the polyamine synthesis. Fourth, our analyses were extensively adjusted for potential confounders. However, due to the observational nature of our primary analyses, we cannot exclude the possibility of residual confounding. Fifth, we did not have repeated measurements of metabolites. It would be interesting to analyze arginine catabolism metabolites during follow-up in order to determine whether the dynamic changes of metabolites could be more important than the baseline levels in new-onset AF or HF.

Results from these 2 case-control studies within the PREDIMED trial indicate that several plasma metabolites implicated in arginine catabolism pathways are associated with the risks of AF or HF events. In addition, the MedDiet intervention might modulate the inverse association between arginine and the HF risk, since the potential beneficial health effects on HF of high levels of arginine was restricted to the MedDiet group. These results reinforce the cardio-protective role of the MedDiet, although prospective studies with repeated measures are needed to assess the effects of the MedDiet on plasma arginine concentrations.

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The authors' responsibilities were as follows—LG, FBH, MAM-G, and MR-C: designed the research; LG, CR, CBC, CD, and MR-C: conducted the research; LG, CR, MR-C, and ET: performed the statistical analyses; LG and MR-C: drafted the paper; CR, ET, and MAM-G: contributed to drafting the manuscript; MG-F, CW, and AA: critically reviewed the paper; NB, J Li, LL, AA-G, M Fitó, DC, EG-G, RE, M Fiol, J Lapetra, LS-M, ER, FA, and JS-S: reviewed the final manuscript; and all authors: read and approved the final manuscript.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval from the PREDIMED Steering Committee and Institutional Review Boards.

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