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Arginine catabolism metabolites and atrial fibrillation or heart failure risk: two case-control studies within the PREDIMED trial

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Short title

Arginine metabolites, AF, HF

List of abbreviations

ADMA: Asymmetric dimethylarginine

AF: Atrial fibrillation

ARIC: Atherosclerosis Risk in Communities

BMI: Body mass index

CHD: Coronary heart disease

CI: Confidence interval

CVD: Cardiovascular disease

ECG: Electrocardiogram

eNOS: Endothelial nitric oxide synthase

EPIC: European Prospective Investigation into Cancer and Nutrition

EVOO: Extra virgin olive oil

FDR: False discovery rate

HF: Heart failure

HILIC: Hydrophilic interaction liquid chromatography

HR: Hazard Ratio

LC-MS: Liquid chromatography tandem mass spectrometry

MEDAS, Mediterranean Diet Adherence Screener

MedDiet: Mediterranean diet

MET: Metabolic equivalent task

NMMA: NG-monomethylarginine

NO: Nitric oxide

NOS: Nitric oxide synthase

OR: Odds ratio

PREDIMED: PREvención con DIeta MEDiterránea

ROS: Reactive oxygen species

SD: Standard deviation

SDMA: Symmetric dimethylarginine

TNAP: Tissue-nonspecific alkaline phosphatase

ABSTRACT

Background. Arginine-derived metabolites are involved in oxidative and inflammatory processes related with endothelial function and cardiovascular risk.

Objective. To prospectively examine the associations of arginine catabolism metabolites with the risk of atrial fibrillation (AF) or heart failure (HF), and to evaluate the potential modification 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 PREDIMED trial. Five hundred and nine 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 participants were selected using incidence density sampling. Fasting blood samples were collected at baseline and arginine catabolism metabolites were measured using liquid chromatography tandem mass spectrometry. 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 vs control group) were analyzed with the likelihood ratio test.

Results. Inverse associations with incident AF were observed for arginine [$OR_{\text{per 1 SD}}$ (95% CI): 0.83 (0.73, 0.94)]) and homoarginine [0.87 (0.76, 0.98)], whereas positive associations were found for the asymmetric dimethylarginine/symmetric dimethylarginine ratio (ADMA/SDMA) [1.15 (1.01, 1.31)] and citrulline [1.19 (1.01, 1.39)]. For HF, inverse associations were found for arginine [0.82 (0.69, 0.97)] and homoarginine [0.81 (0.68, 0.96)], and positive associations for the ADMA/SDMA ratio [1.19 (1.02, 1.41)], N1-acetylspermidine [1.34 (1.12, 1.60)], and diacetylspermine [1.20 (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 ($p_{for\ interaction}$ 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.

Keywords: arginine catabolism metabolites; metabolomics; atrial fibrillation; heart failure; Mediterranean diet; case-control

INTRODUCTION

The increasing prevalence of atrial fibrillation (AF) and heart failure (HF) in the general population has led to substantial morbidity and mortality and constitutes 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 pathophysiology (endothelial dysfunction, oxidative stress and inflammation) (3). However, the risk of developing AF and HF is 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, nitric oxide (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 two cross-sectional studies have suggested that arginine and its related metabolites are associated with AF or HF risk (6–8). Furthermore, an inverse association between serum homoarginine levels and cardiovascular events suggest a similar effect on AF and HF risk (9). In contrast, ADMA and SDMA have been associated with a higher risk of heart related diseases including AF and HF (6,7,10,11). Previous cross-sectional and prospective metabolomic studies found a positive association between different polyamines and the risk 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 a lower risk of AF and HF (17–19). In this context, the first aim of the present study was to prospectively examine the association of metabolites implicated in different arginine catabolism pathways with the risk of incident AF or HF, in two case-control studies nested within the PREDIMED (PREvención con DIeta MEDiterránea) intervention trial. The second aim was to evaluate the effect of the MedDiet on the association between the arginine related metabolites and the incidence of AF or HF.

METHODS

Study design and population

For the present study we designed two nested case-control studies within the PREDIMED trial, a multicenter, parallel-group, randomized, controlled trial aimed to evaluate the effect of the MedDiet on the primary prevention of 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) MedDiet enriched with extra virgin olive oil (EVOO); 2) MedDiet enriched with mixed nuts; and 3) a low-fat diet (control group). The study had two 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).

For the present study we designed two matched case-control studies nested within the PREDIMED trial. 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). Matching factors were recruitment center, year of birth (± 5 years), and sex. Per case, one to three controls (depending on availability of samples) were randomly selected from matched, disease-free (at the time of endpoint diagnosis in the matched case) study participants. Inclusion criteria of the case-control studies were availability of pre-intervention EDTA plasma samples; participants with prevalent AF or HF at baseline were excluded (Figure 1 and Figure 2).

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 Clinical Hospital, as the coordinating center.

Ascertainment of AF and HF cases

The PREDIMED protocol included AF and HF as prespecified secondary end-points (17,18). During the first period 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 reviewing 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 to

the Clinical End-Point Committee. This documentation was independently reviewed by two cardiologists and if they did not agree on the diagnosis of the event, consensus was reached 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 (SD) time from baseline to AF or HF diagnosis was 6.7 (3.3) years and 7.3 (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 healthcare 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 -80ºC 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 & Harvard using liquid chromatography tandem mass spectrometry (LC-MS). Hydrophilic interaction liquid chromatography (HILIC) coupled with high resolution positive ion mode MS detection was used to analyze arginine, ornithine, citrulline, NMMA, 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 coefficients of variation (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 history of diseases, and medication use was collected using different questionnaires at baseline visit. Trained personnel measured height and body weight, and body mass index (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

Mean and standard deviation (SD) was used to describe quantitative variables and percentage 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 association between metabolites and incident AF or HF .Matched odds ratios (OR) and their 95% confidence intervals (CIs) for AF or HF were estimated considering 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 pathways metabolites and AF or HF, we assigned the median value of each category and modeled them as continuous. On the other hand, we calculated the matched odds ratios for the SD of each metabolite as continuous variables. In addition, we conducted additional conditional logistic regression models with each metabolite as quadratic term to explore potential non-linear associations between the metabolites and AF or HF risk. P values were adjusted with the use of Simes false discovery rate (FDR) procedure to account for multiple testing (28). A multivariable model adjusted for potential confounders was fitted: model 1: age, intervention group (MedDiet+EVOO, MedDiet+nuts or control), smoking status (never, current, 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, three sensitivity analyses were performed: 1) adjusted conditional logistic regression models 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 2010/12/01 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 control group) and arginine and N1-acetylspermidine as continuous (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, former), BMI (kg/m^2), leisure-time physical activity (metabolic equivalent task -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.

RESULTS

Table 1 shows the baseline characteristics of participants in the two case-control studies nested within the PREDIMED trial separated by AF or HF incidence. The number of incident cases of AF and HF was 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 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 ($OR_{Q4\ vs.\ Q1}$ 0.55; 95% CI 0.38, 0.80; FDR-corrected P-trend = 0.018) but not ornithine, citrulline, or

homoarginine. When we considered the metabolites as continuous variables, a 1-SD increase in baseline arginine was associated with a 17% ($OR_{\text{for 1 SD increase}}$ 0.83; 95% CI 0.73, 0.94; FDR-corrected P = 0.044) lower risk of AF. Regarding polyamines, only N1-acetylspermidine by quartiles was longitudinally associated with AF risk ($OR_{\text{Q4 vs. Q1}}$ 1.58; 95% CI 1.13, 2.25; FDR-corrected P-trend = 0.049), but not as continuous variable. Similar results were observed when we add a quadratic term of the metabolites to assess a potential non-linear association between arginine metabolites and polyamines and AF (data not shown). Results from sensitivity analyses did not substantially change except when we restricted the analyses to the intervention period where the number of AF cases was 150 (Supplementary Table 1).

Table 3 presents the results of the association between arginine catabolism metabolites and HF. Arginine and homoarginine were negatively associated with the risk of HF, showing an $OR_{\text{for 1 SD increase}}$ of 0.82 (95% CI 0.69, 0.97; FDR-corrected P = 0.049) and $OR_{\text{for 1 SD increase}}$ of 0.81 (95% CI 0.68, 0.96; FDR-corrected P = 0.049) respectively, whereas citrulline was positively associated with HF risk ($OR_{\text{for 1 SD increase}}$ 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 ($OR_{\text{for 1 SD increase}}$ 1.19; 95% CI 1.02, 1.41, FDR-corrected P = 0.049). In this case, two polyamines were associated with HF risk, N1-acetylspermidine and diacetylspermine. For both metabolites, N1-acetylpermidine ($OR_{\text{Q4 vs. Q1}}$ 1.77; 95% CI 1.12, 2.79; FDR-corrected P-trend = 0.018; $OR_{\text{for 1 SD increase}}$ 1.34; 95% CI 1.12, 1.60; FDR-corrected P = 0.013) and diacetylspermine ($OR_{\text{for 1 SD increase}}$ 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 risk (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 HF risk (Supplementary Table 2).

Finally, the potential interaction between arginine or N1-acetylspermidine and the intervention group (both MedDiet groups merged together vs the control group) on the risk of AF and HF was analyzed. Regarding AF, no significant interactions were observed (data not shown). However, we observed that the MedDiet interventions modified the effect of arginine on 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 HF risk was not statistically significant ($P_{interaction}$ 0.765) (Figure 3).

DISCUSSION

In two case-control studies in the PREDIMED trial, we identified several arginine-derived metabolites associated with AF and HF risk. Baseline arginine was inversely associated with AF incidence; and arginine, citrulline and homoarginine were associated with HF incidence. On the contrary, N-1acetylspermidine was positively associated with the risk of AF; and ADMA/SDMA, N-1acetylspermidine and diacetylspermine with the risk of HF. In addition, we found an interaction between the MedDiet and arginine levels on 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 AF incident risk, and between arginine and

citrulline with 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 AF risk in the Framingham Offspring prospective cohort study after a 10-year median follow-up and 247 incident cases observed (29). On the other hand, the literature regarding the association of these metabolites and HF is scarce. Cheng et al. (2015) observed that HF patients in stage C had lower levels of arginine than healthy subjects (8). Moreover, they found a significantly lower citrulline/ornithine ratio among patients with HF in stage C, but not for citrulline concentrations, as we did in our study.

In our study, higher levels of ADMA/SDMA were associated with 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. (2016) (7) and Schulze et al. (2010) (6) observed that higher levels of ADMA and SDMA, respectively, were positively associated with new onset AF. However, Schnable et al. (2016) (29) reported a non-significant association between ADMA and increased risk of AF after adjusting for traditional AF risk factors. According to this, AF risk reflected by ADMA levels appears to be explained by classical cardiovascular risk factors. Regarding HF, and consistent with our results Wirth J et al. (2017) (11) found a positive association between ADMA/SDMA and HF risk in a case-cohort study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Both dimethylarginines have been postulated as biomarkers of the severity of the AF and HF, and also with the risk of mortality in patients with AF and HF (30–32). However, more prospective studies are needed to clarify the association between ADMA and SDMA with incident AF or HF.

Similar to arginine, we found an inverse association between baseline homoarginine levels and HF incidence. A cross-sectional study within the Gutenberg health study did not observe an association between homoarginine levels and AF risk (33). However, among AF patients, homoarginine levels were lower in patients with AF at the time of blood sampling compared with AF 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 (Hazard Ratio (HR) $_{\text{per 1 SD increase in metabolite}}$=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 HF incidence. No association was observed with AF risk after FDR correction. A previous study in the Atherosclerosis Risk in Communities (ARIC) cohort study found that higher levels of acisoga, a catabolic product of spermidine formed from N1-acetylspermidine, were associated with higher risk of AF (HR $_{\text{per 1-SD difference}}$=1.15 (95% CI 1.06; 1.24)) (14). Regarding HF, higher levels of spermidine, N8-acetylspermidine, N-acetylspermidine and diacetylspermine, have been associated with greater risk of HF in 1 cross-sectional and 2 prospective cohort studies (8,12,13). Surprisingly, in a prospective and in a cross-sectional study a higher intake of spermidine has been associated with a lower incidence of cardiovascular diseases and HF (35,36). These controversial results could be explained because there is insufficient data on the contribution 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 its role 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 superoxide production, a reactive oxygen species (ROS) which interacts 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 NOS (eNOS) (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 substrate for NO synthesis (38,42). On the contrary, homoarginine can serve as NO substrate (9,34), although it is generally considered a weaker substrate than arginine (43). Moreover, homoarginine could be an specific uncompetitive inhibitor of tissue-nonspecific alkaline phosphatase (TNAP) (9), which has been directly associated with CVD and overall mortality in patients at high CVD risk and in the general population (44,45). Finally, there is paucity of data on the use of polyamines, including spermidine, spermine and their derivates, as biomarkers of AF or HF incidence. Meanwhile several studies have suggested that spermidine intake may reduce the risk of different pathophysiological conditions including CVD (35,36), metabolomic studies have reported a positive association between polyamine levels and AF or HF risk (8,12,13). A previous study suggests 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 reflects an increase in the risk 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 an abundant intake 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, see food 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 MedDiet could enhance the beneficial effects associated with high levels in 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 have not repeated measurements of metabolites, we could not analyze the effects of MedDiet on changes in metabolites. Moreover, at baseline we did not find a significant correlation between MedDiet adherence, foods rich in arginine or foods rich in potential precursors of NO, and arginine catabolism metabolites. This lack of association is consistent with other studies that did not observe correlation between the intake 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 which is especially suited to study the potential interactions between the MedDiet and the arginine pathways metabolites on AF and HF risk. Second, the number of cases was limited, particularly for HF. Although we used 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-tandem MS platform), covering central metabolites involved in the three 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 interested to analyze arginine catabolism metabolites during follow-up in order to determine if the dynamic changes of metabolites could be more important than the baseline levels in new-onset AF or HF.

Results from these two case-control studies within the PREDIMED trial indicated that several plasma metabolites implicated in arginine catabolism pathways were associated with the risk of AF or HF events. In addition, the MedDiet intervention might modulate the inverse association between arginine and HF risk since the potential health beneficial effect 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 effect of the MedDiet on plasma arginine concentrations.

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376

377 **Statement of authors' contributions to manuscript.**

378 The authors' responsibilities were as follows—LG, FBH, MAM-G, AND MR-C:
379 designed the research; LG, CR, CBC, CD, and MR-C: conducted the research; LG, CR,
380 MR-C, and ET: performed the statistical analyses; LG and MR-C: drafted the paper;
381 CR, ET, and MAM-G: contributed to drafting the manuscript; MG-F, CW, and AA:
382 critically reviewed the paper; and NB, J Li, LL, AA-G, M Fito, DC, EG-G, RE, M Fiol,
383 J Lapetra, LS-M, ER, FA, and JS-S: reviewed the final manuscript. All the authors
384 approved the final manuscript. The authors report no conflicts of interest.

385

386 **DATA SHARING PLAN**

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

REFERENCES

1. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH, Zheng ZJ, et al. Worldwide epidemiology of atrial fibrillation: A global burden of disease 2010 study. Circulation. 2014;129:837–47.
2. Cook C, Cole G, Asaria P, Jabbour R, Francis DP. The annual global economic burden of heart failure. Int J Cardiol. Elsevier Ireland Ltd; 2014;171:368–76.
3. Karnik AA, Gopal DM, Ko D, Benjamin EJ, Helm RH. Epidemiology of atrial fibrillation and heart failure: a growing and important problem. Cardiol Clin. Elsevier Inc; 2019;37:119–29.
4. Ussher JR, Elmariah S, Gerszten RE, Dyck JRB. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. J Am Coll Cardiol. 2016;68:2850–70.
5. Morris SM. Arginine metabolism revisited. J Nutr. 2016;146:2579S-2586S.
6. Schulze F, Carter AM, Schwedhelm E, Ajjan R, Maas R, von Holten RA, Atzler D, Grant PJ, Böger RH. Symmetric dimethylarginine predicts all-cause mortality following ischemic stroke. Atherosclerosis. 2010;208:518–23.
7. Ramuschkat M, Appelbaum S, Atzler D, Zeller T, Bauer C, Ojeda FM, Sinning CR, Hoffmann B, Lackner KJ, Böger RH, et al. ADMA, subclinical changes and atrial fibrillation in the general population. Int J Cardiol. 2016;203:640–6.
8. Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY, Mao CT, Lin JF, Ho HY, Yang NI. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: Diagnostic and prognostic value of metabolomics. J Am Coll Cardiol. 2015;65:1509–20.
9. Karetnikova ES, Jarzebska N, Markov AG, Weiss N, Lentz SR, Rodionov RN. Is

homoarginine a protective cardiovascular risk factor? Arterioscler Thromb Vasc Biol. 2019;39:869–75.

10. Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E, Chowdhury R. Asymmetric dimethylarginine and cardiovascular risk: Systematic review and meta-analysis of 22 prospective studies. J Am Heart Assoc. 2015;4:1–13.
11. Wirth J, Atzler D, di Giuseppe R, Cordts K, Menzel J, Böger RH, Boeing H, Weikert C, Schwedhelm E. Higher serum asymmetric dimethylarginine is related to higher risk of heart failure in the EPIC-Potsdam study. Amino Acids. Springer Vienna; 2017;49:173–82.
12. Tahir UA, Katz DH, Zhao T, Ngo D, Cruz DE, Robbins JM, Chen ZZ, Peterson B, Benson MD, Shi X, et al. Metabolomic profiles and heart failure risk in black adults: Insights from the Jackson Heart study. Circ Hear Fail. 2021;14.
13. Nayak A, Liu C, Mehta A, Ko YA, Tahhan AS, Dhindsa DS, Uppal K, Jones DP, Butler J, Morris AA, et al. N8-acetylspermidine: A polyamine biomarker in ischemic cardiomyopathy with reduced ejection fraction. J Am Heart Assoc. 2020;9.
14. Alonso A, Yu B, Sun Y V., Chen LY, Loehr LR, O'Neal WT, Soliman EZ, Boerwinkle E. Serum metabolomics and incidence of atrial fibrillation (from the Atherosclerosis Risk in Communities Study). Am J Cardiol. 2019;123:1955–61.
15. Shannon OM, Stephan BC, Minihane A-M, Mathers JC, Siervo M. Nitric oxide boosting effects of the Mediterranean Diet: a potential mechanism of action. Journals Gerontol - Ser A Biol Sci Med Sci. 2018;73:902–4.
16. Kris-Etherton PM, Hu FB, Ros E, Sabaté J. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. J Nutr.

2008;138:1746–51.

17. Martínez-González MÁ, Toledo E, Arós F, Fiol M, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fernández-Crehuet J, Lapetra J, et al. Extravirgin olive oil consumption reduces risk of atrial fibrillation: The PREDIMED (Prevención con Dieta Mediterránea) trial. Circulation. 2014;130:18–26.
18. Papadaki A, Martínez-González MÁ, Alonso-Gómez A, Rekondo J, Salas-Salvadó J, Corella D, Ros E, Fitó M, Estruch R, Lapetra J, et al. Mediterranean diet and risk of heart failure: results from the PREDIMED randomized controlled trial. Eur J Heart Fail. 2017;19:1179–85.
19. Sanches Machado D'Almeida K, Ronchi Spillere S, Zuchinali P, Corrêa Souza G. Mediterranean diet and other dietary patterns in primary prevention of heart failure and changes in cardiac function markers: a systematic review. Nutrients. 2018;10.
20. Martínez-González MÁ, Corella D, Salas-salvadó J, Ros E, Covas MI, Fiol M, Wärnberg J, Arós F, Ruíz-Gutiérrez V, Lamuela-Raventós RM, et al. Cohort profile: Design and methods of the PREDIMED study. Int J Epidemiol. 2012;41:377–85.
21. Estruch R, Ros E, Salvadó JS, Covas M, Corella D, Arós F, Gracia EG, Gutiérrez VR, Fiol M, Lapetra J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2018;34:1–14.
22. Lubin JH, Gail MH. Biased selection of controls for case-control analyses of cohort studies. Biometrics. 1984;40:63–75.
23. Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, Tavazzi L, Smiseth OA, Gavazzi A, Haverich A, et al. Guidelines for the diagnosis and treatment of chronic heart failure: Executive summary (update 2005). Eur Heart

J. 2005;26:1115–40.

24. Paynter NP, Balasubramanian R, Giulianini F, Wang DD, Tinker LF, Gopal S, Deik AA, Bullock K, Pierce KA, Scott J, et al. Metabolic predictors of incident coronary heart disease in women. Circulation. 2018;137:841–53.

25. Chaffin MD, Cao L, Deik AA, Clish CB, Hu FB, Martínez-González MA, Razquin C, Bullo M, Corella D, Gómez-Gracia E, et al. MetProc: separating measurement artifacts from true metabolites in an untargeted metabolomics experiment. J Proteome Res. 2019;18:1446–50.

26. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota leisure time physical activity questionnaire in Spanish men. Am J Epidemiol. 1994;139:1197–209.

27. Elosua R, Garcia M, Aguilar A, Molina L, Covas M-I, Marrugat J. Validation of the Minnesota leisure time physical activity questionnaire in Spanish women. Med Sci Sport Exerc. 2000;32:1431–7.

28. Newson R. Multiple—test procedures and smile plots. Stata J. 2003;3:109–32.

29. Schnabel RB, Maas R, Wang N, Yin X, Larson MG, Levy D, Ellinor PT, Lubitz SA, McManus DD, Magnani JW, et al. Asymmetric dimethylarginine, related arginine derivatives, and incident atrial fibrillation. Am Heart J. Elsevier Inc.; 2016;176:100–6.

30. Horowitz JD, De Caterina R, Heresztyn T, Alexander JH, Andersson U, Lopes RD, Steg PG, Hylek EM, Mohan P, Hanna M, et al. Asymmetric and Symmetric Dimethylarginine Predict Outcomes in Patients With Atrial Fibrillation: An ARISTOTLE Substudy. J Am Coll Cardiol. 2018;72:721–33.

31. Büttner P, Bahls M, Böger RH, Hindricks G, Thiele H, Schwedhelm E, Kornej J. Arginine derivatives in atrial fibrillation progression phenotypes. J Mol Med.

2020;98:999–1008.

32. Pan W, Lian B, Lu H, Liao P, Guo L, Zhang M. Prognostic value of asymmetric dimethylarginine in patients with heart failure: a systematic review and meta-analysis. Biomed Res Int. 2020;2020:1–9.

33. Niekamp C, Atzler D, Ojeda FM, Sinning CR, Lackner KJ, Böger RH, Munzel T, Beutel ME, Schmidtmann I, Pfeiffer N, et al. Cross-sectional associations between homoarginine, intermediate phenotypes, and atrial fibrillation in the community—The Gutenberg health study. Biomolecules. 2018;8:1–12.

34. Atzler D, Baum C, Ojeda F, Keller T, Cordts K, Schnabel RB, Choe CU, Lackner KJ, Münzel T, Böger RH, et al. Low homoarginine levels in the prognosis of patients with acute chest pain. J Am Heart Assoc. 2016;5:1–10.

35. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, Harger A, Schipke J, Zimmermann A, Schmidt A, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med. 2016;22:1428–38.

36. Soda K, Kano Y, Chiba F. Food polyamine and cardiovascular disease-an epidemiological study. Glob J Health Sci. 2012;4:170–8.

37. Popolo A, Adesso S, Pinto A, Autore G, Marzocco S. L-Arginine and its metabolites in kidney and cardiovascular disease. Amino Acids. 2014;46:2271–86.

38. Visser M, Paulus WJ, Vermeulen MAR, Richir MC, Davids M, Wisselink W, De Mol BAJM, Van Leeuwen PAM. The role of asymmetric dimethylarginine and arginine in the failing heart and its vasculature. Eur J Heart Fail. 2010;12:1274–81.

39. Vallance P, Leiper J. Cardiovascular biology of the asymmetric

dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol. 2004;24:1023–30.

40. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation. 1998;98:1842–7.

41. Antoniades C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, Cunnington C, Tousoulis D, Pillai R, Ratnatunga C, et al. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: Implications for endothelial function in human atherosclerosis. Eur Heart J. 2009;30:1142–50.

42. Strobel J, Mieth M, Endreß B, Auge D, König J, Fromm MF, Maas R. Interaction of the cardiovascular risk marker asymmetric dimethylarginine (ADMA) with the human cationic amino acid transporter 1 (CAT1). J Mol Cell Cardiol. Elsevier Ltd; 2012;53:392–400.

43. Atzler D, Schwedhelm E, Choe C. L-Homoarginine and cardiovascular disease. Curr Opin Clin Nutr Metab Care. 2015;18:83–8.

44. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: A meta-analysis of prospective cohort studies. Atherosclerosis. 2014;236:7–17.

45. Kunutsor SK, Apekey TA, Seddoh D, Walley J. Liver enzymes and risk of all-cause mortality in general populations: A systematic review and meta-analysis. Int J Epidemiol. 2014;43:187–201.

46. Medina-Remón A, Tresserra-Rimbau A, Pons A, Tur JA, Martorell M, Ros E,

Buil-Cosiales P, Sacanella E, Covas MI, Corella D, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. Nutr Metab Cardiovasc Dis. 2015;25:60–7.

47. De La O V, Zazpe I, Ruiz-Canela M. Effect of branched-chain amino acid supplementation, dietary intake and circulating levels in cardiometabolic diseases: an updated review. Curr Opin Clin Nutr Metab Care. 2020;23:35–50.

TABLES

Table 1. Baseline characteristics of atrial fibrillation and heart failure cases and controls

	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, METs-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)
Body mass index, 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	-	-

Mean (SD)

CHD, Coronary heart disease; EVOO, Extra virgin olive oil; MEDAS, Mediterranean Diet Adherence Screener; MedDiet, Mediterranean diet; MET, Metabolic equivalent

Table 2. Odds ratios (95% CI) for the association of arginine catabolism metabolites and atrial fibrillation

	Metabolite in quartile categories, OR (95% CI)						Metabolite as continuos variable, per SD		
	Q1	Q2	Q3	Q4	*P* trend	*FDR P* value	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

ADMA/SDMA, Asymmetric dimethylarginine/symmetric dimethylarginine ratio; NMMA, NG-monomethylarginine; 95% CI, 95% Confidence interval; FDR, False discovery rate

Conditional logistic regression

Model 1: Adjusted for age, smoking status (never, current, former), BMI (kg/m^2), physical activity (METs/day), and prevalent chronic conditions at baseline (hypertension, dyslipidemia, and type 2 diabetes).

Table 3. Odds ratios (95% CI) for the association of arginine catabolism metabolites and heart failure

	Metabolite in quartile categories, OR (95% CI)						Metabolite as continuous variable, per SD		
	Q1	Q2	Q3	Q4	*P* trend	*FDR P* value	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

ADMA/SDMA, Asymmetric dimethylarginine/symmetric dimethylarginine ratio; NMMA, NG-monomethylarginine; 95% CI, 95% Confidence interval; FDR, False discovery rate

Conditional logistic regression

Model 1: Adjusted for age, smoking status (never, current, former), BMI (kg/m^2), physical activity (METs/day), and prevalent chronic conditions at baseline (hypertension, dyslipidemia, and type 2 diabetes).

FIGURE TITLES AND LEGENDS

Figure 1. Flowchart of the case-control design for incident atrial fibrillation within the PREDIMED trial

*Incidence density sampling with replacement was used as the control sampling method.

AF, Atrial fibrillation; PREDIMED, Prevención con Dieta Mediterránea.

Figure 2. Flowchart of the case-control design for incident heart failure within the PREDIMED trial

*Incidence density sampling with replacement was used as the control sampling method.

HF, Heart failure; PREDIMED, Prevención con Dieta Mediterránea.

Figure 3. Association (per SD) between arginine or N1-acetylspermidine and incident heart failure stratifying by intervention group

Adjusted for age, sex, smoking status (never, current, former), BMI (kg/m^2), physical activity (METs/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.