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Tricarboxylic acid cycle related-metabolites and risk of atrial fibrillation and heart failure

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Abstract

Background—Tricarboxylic acid (TCA) cycle deregulation may predispose to cardiovascular diseases, but the role of TCA cycle-related metabolites in the development of atrial fibrillation (AF) and heart failure (HF) remains unexplored. This study sought to investigate the association of TCA cycle-related metabolites with risk of AF and HF.

Methods—We used two nested case-control studies within the PREDIMED study. During a mean follow-up for about 10 years, 512 AF incident cases matched to 616 controls, and 334 HF incident cases matched to 433 controls were included in this study. Baseline plasma levels of citrate, aconitate, isocitrate, succinate, malate and D/L-2-hydroxyglutarate were measured with liquid chromatography-tandem mass spectrometry. Multivariable conditional logistic regression models were fitted to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for metabolites and the risk of AF or HF.

Results—Comparing extreme quartiles of metabolites, elevated levels of succinate, malate, citrate and D/L-2-hydroxyglutarate were associated with a higher risk of AF [OR_{Q4 vs. Q1} (95% CI): 1.84 (1.25–2.71), 2.16 (1.49–3.12), 1.78 (1.22–2.61) and 1.83 (1.26–2.67), respectively]. One SD increase in aconitate was directly associated with AF risk [OR (95% CI): 1.16 (1.01–1.33)]. The corresponding ORs (95% CI) for HF comparing extreme quartiles of malate, aconitate, isocitrate and D/L-2-hydroxyglutarate were 2.30 (1.41–3.76), 2.03 (1.22–3.39), 2.66 (1.61–4.40)

and 1.91 (1.17–3.10), respectively. These associations were confirmed in an internal validation, except for aconitate and AF.

Conclusion—These findings underscore the potential role of the TCA cycle in the pathogenesis of cardiac outcomes.

Keywords

tricarboxylic acid cycle metabolites; hydroxyglutarate; atrial fibrillation; heart failure; PREDIMED

1. Introduction

Atrial fibrillation (AF) and heart failure (HF) have emerged as important cardiac dysfunctions causing premature deaths and chronic disability [1]. The association between AF and HF was described more than one century ago, and the frequent coexistence of these cardiac diseases have been later reported in several epidemiological studies [2]. Although the causative relationship between both conditions is not fully understood, both share many common risk factors such as age, obesity, type 2 diabetes (T2D) and hypertension, as well as valvular, ischemic and non-ischemic heart disease [3]. However, other metabolic impairments affecting the energy production required to maintain contractile function and cardiac output may underlie both entities [4,5]. In this regard, alteration in the contraction and hemodynamic responses, together with hypoxia and reduced energy supply, induce substantial changes in tricarboxylic acid (TCA) cycle-related metabolites. Citrate, the first product following acetyl coenzyme A (Acetyl-CoA) generation from different energy sources, has been associated with cardiovascular mortality [6]. Elevated circulating α -ketoglutarate levels were also previously found in HF patients compared with controls [7]. Under hypoxic conditions, L-2-hydroxyglutarate is oxidized to α -ketoglutarate by hydroxyglutarate dehydrogenase to compensate the reductive stress generated in the mitochondria and to replenish the TCA cycle for its catapleurotic function [8]. Cardiac dysfunction has been reported to be initiated directly by D-2-hydroxyglutarate through inhibition of alpha-ketoglutarate dehydrogenase; however, whether this metabolite could affect AF and HF is unknown [9]. In a cross-sectional study conducted among acute stroke patients, alpha-ketoglutarate, succinate and malate were associated with cardioembolic stroke and atrial dysfunction, while only succinate was associated with left atrial enlargement and stroke recurrence [10]. Therefore, the TCA cycle is considered as a signalling hub with diverse roles and important effects in physiology and disease [11]. However, whereas certain changes in TCA cycle-related metabolites have been suggested to be a consequence of cardiac dysfunction, whether metabolic perturbations of the TCA cycle can anticipate cardiac dysregulation has been little explored. Therefore, in the present prospective examination conducted in the framework of the PREDIMED study, we aimed to assess associations between plasma levels of a set of TCA cycle-related metabolites (i.e., citrate, aconitate, isocitrate, succinate, malate, and D/L-2-hydroxyglutarate) and the risk of incident AF and HF.

2. Methods

2.1 Study design and participants

This analysis used two case-control studies, each one including either AF or HF incident cases nested within the PREDIMED (Prevención con Dieta Mediterránea) study (ISRCTN35739639), a randomized trial designed to examine the effect of the Mediterranean diet (MedDiet) on the primary prevention of cardiovascular disease (CVD). The design and methods of PREDIMED were described elsewhere [12]. Briefly, from June 25, 2003 to June 30, 2009, 7447 elderly participants without CVD at enrollment but at high cardiovascular risk were recruited and randomly allocated to: 1) a MedDiet supplemented with extra-virgin olive oil, 2) a MedDiet supplemented with mixed nuts, or 3) advice to reduce all dietary fat (control group). Information on CVD-related outcomes, as adjudicated by the Clinical Events Committee, blinded to diet allocation, was collected and analyzed until December 2017. The primary endpoint of the PREDIMED study was a major CVD event (myocardial infarction, stroke or death from cardiovascular causes). In the present study, we analyzed AF and HF events as *a priori* defined secondary endpoints [13,14] in the PREDIMED trial protocol (www.predimed.es) identified during the period 2003–2017. Supplementary Figure S1 depicts the flow chart of the participants' selection process in both studies. Five hundred twelve incident AF events and 334 incident HF events were ascertained after excluding prevalent cases and incident cases without available blood samples. Incidence density sampling with replacement was used as the control sampling method [15]. The controls were randomly selected among participants free of AF or HF at baseline and matched by age (± 5 years), sex and recruitment 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 period. Following the strategy described above, we selected 1 to 3 matched controls per case. There were 108 overlapping cases of AF and HF. The protocol of the PREDIMED trial was in accordance with the Helsinki Declaration and was approved by the Research Ethics Committees of all participating centres. All participants provided written informed consent.

2.2 Ascertainment of AF and HF Cases

During the first study period (2003–2010), information on AF and HF was collected from contacts with participants and primary health care physicians, annual follow-up visits and yearly ad-hoc reviews of outpatient and inpatient medical charts. During the extended follow-up period (2011–2017), the study physicians collected information on AF and HF by reviewing the outpatient and inpatient medical charts of the participants. If a clinical diagnosis of heart disease 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 anonymized to the Clinical End-Point Adjudication Committee, whose members 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. The diagnostic criteria and procedures have been reported in detail elsewhere [13,14].

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.

HF was defined according to the 2005 (time of study design) guidelines of the European Society of Cardiology on the diagnosis and treatment of acute and chronic HF [16]. Based on these guidelines, an event was classified as HF if patients had symptoms and/or signs of HF (frequent breathlessness or fatigue at rest or during exertion, or ankle swelling) attributable to objective evidence of cardiac dysfunction at rest (preferably by echocardiography). The clinical picture might have had a sudden onset or develop progressively.

2.3 Metabolomic profiling

Fasting plasma samples were collected at baseline before the implementation of dietary interventions and stored at -80°C . Samples were shipped on dry ice to the Broad Institute (Boston, Massachusetts, USA) for metabolomics analyses. Liquid chromatography-tandem mass spectrometry was used to semi-quantitatively profile succinate, malate, citrate, aconitate, isocitrate and D/L-2-hydroxyglutarate (the method does not distinguish the enantiomers, that is, the D- and L-isomers co-elute) [17] on a system composed of a Shimadzu Nexera X2 U-HPLC (Shimadzu Corp.; Marlborough, MA) coupled to a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific; Waltham, MA). Metabolite identities were confirmed using authentic reference standards. Raw data were processed via TraceFinder software (Thermo Fisher Scientific; Waltham, MA). Internal standard peak areas were monitored for quality control and to ensure system performance throughout analyses. Pooled plasma reference samples were also inserted every twenty samples as an additional quality control. Information about the mass to charge ratio and retention time is shown in Supplementary Table S1.

2.4 Covariates

Trained dietitians and nurses used questionnaires at baseline to collect information in face-to-face interviews about sociodemographic and lifestyle variables, smoking status, medical conditions, family history of disease and medication use. Physical activity was measured with the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [18]. Blood pressure was measured in each arm with a validated semiautomatic oscillometer (Omron HEM-705CP, Hoofddorp, the Netherlands). The mean from two measurements of the systolic (SBP) and diastolic (DBP) blood pressure separated by 2 minutes was also calculated. Fasting plasma glucose and lipids levels were measured by locally standard measurement methods. Trained personnel performed the anthropometric and blood pressure measurements. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). 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.

2.5 Statistical analyses

Baseline characteristics of AF and HF cases and matched controls are described as means (SDs) for quantitative variables and percentages for categorical variables. Baseline characteristics were compared between cases and controls using Student's t-test for continuous variables and χ^2 tests for categorical variables. We applied a natural logarithmic transformation to approximate a normal distribution of metabolites levels. A correlation (Spearman) matrix of the metabolites under study was visualized through a heat map (R statistical package version 3.6.1).

To investigate the association of the 6 individual TCA-related metabolites with AF or HF, we conducted conditional logistic regressions separately using the two different case-control sets, where the outcome was either AF or HF. A crude model and 2 multivariable-adjusted conditional logistic regression models were fitted as follows: 1) multivariable model 1 was adjusted for smoking (never, current, or former), family history of premature coronary heart disease (CHD) (yes or no), physical activity (metabolic equivalent tasks in minutes per day), alcohol intake (g/day), BMI (kg/m²), intervention group assignments (MedDiet+EVOO, MedDiet+nuts, or control group), hypertension (yes or no), dyslipidaemia (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-offs 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. To account for multiple testing, we adjusted P values and P for trend of the crude and multivariable-adjusted associations with the use of the Benjamini-Hochberg false discovery rate (FDR) procedure [19]. A FDR-adjusted P-value < 0.05 was considered to be statistically significant after adjustment for 6 tests corresponding to the 6 metabolites. For internal validation of the associations, bootstrap resampling of the original sets (1000-fold) was carried out. We further examined nonparametric associations by fitting cubic splines to a conditional logistic regression model. 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.

Potential effect modification was examined by adding a multiplicative term (1 df) between categorical variables and metabolites (continuous) either into a multivariable unconditional (age and sex) or conditional (T2D and obesity status) logistic regression to test for interactions by using the likelihood ratio tests. We also evaluated possible interactions of each metabolite with the intervention groups (MedDiet+EVOO and MedDiet+nuts vs. control group) using the likelihood ratio test. Statistical analyses were performed using Stata 14.1 (Stata Corp.).

3. Results

Study participants were followed for a mean of \approx 10 years. Table 1 shows the baseline characteristics of participants. Those with incident AF and HF were more likely to have a higher BMI and higher prevalence of hypertension (Table 1). Participants who developed

AF and HF were also more likely to use antihypertensive medications, whereas those who developed HF were more likely to use oral antidiabetic agents. Heatmaps of pairwise Spearman correlation coefficients for the 6 plasma TCA cycle-related metabolites are shown in Supplementary Figure S2. Moderate to strong correlations were found between malate, citrate, aconitate and isocitrate. Succinate was weakly correlated with the other metabolites. D/L-2-Hydroxyglutarate was weakly associated with isocitrate and succinate, while not significantly associated with the other metabolites.

3.1 TCA cycle metabolites and risk of AF

The associations between TCA cycle-related metabolites with AF risk are displayed in Table 2. In the fully adjusted model, the estimated OR for incident AF reached significance only in the highest, compared with the lowest, quartile of plasma levels of succinate, malate and citrate [1.84 (95% CI, 1.25–2.71), 2.16 (95% CI, 1.49–3.12) and 1.78 (95% CI, 1.22–2.61), respectively]. Similarly, the third and fourth quartiles of D/L-2-hydroxyglutarate, compared with the lowest, were significantly associated with AF [OR (95% CI), 1.46 (1.02–2.09) and 1.83 (1.26–2.67)]. After adjustment for multiple testing, the risk of AF significantly increased per 1-SD increase in succinate, malate, citrate, aconitate and D/L-2-hydroxyglutarate levels [OR (95% CI), 1.17 (1.02–1.33), 1.29 (1.13–1.47), 1.19 (1.04–1.37), 1.16 (1.01–1.33) and 1.24 (1.09–1.41), respectively]. Cubic spline curves (Supplementary Figure S3) showed that malate, citrate and D/L-2-hydroxyglutarate were nonlinearly associated with AF risk (P value for nonlinearity with AF risk was <0.001, 0.038, and 0.003 for malate, citrate and D/L-2-hydroxyglutarate, respectively). Succinate and aconitate were linearly associated with AF. No significant associations for isocitrate and AF risk were observed. We found that per 1-SD increase in plasma aconitate levels, the risk of AF significantly increased in individuals with diabetes, with OR= 1.36 (95% CI 1.13–1.62) (P for interaction_{AdjFDR}=0.042). Internal validation by bootstrap resampling confirmed the significant findings for the majority of metabolites except for aconitate (Supplementary Table S2).

3.2 TCA cycle metabolites and risk of HF

Associations between plasma metabolite levels and HF risk are depicted in Table 3. Compared to those in the lowest quartile, participants in the highest quartile of malate, aconitate, isocitrate and D/L-2-hydroxyglutarate levels showed higher odds for HF [OR (95% CI), 2.30 (1.41–3.76) for malate, 2.03 (1.22–3.39) for aconitate, 2.66 (1.61–4.40) for isocitrate and 1.91 (1.17–3.10) for D/L-2-hydroxyglutarate]. One SD increment in levels of malate, aconitate, isocitrate and D/L-2-hydroxyglutarate was associated with higher risk of HF incidence [OR (95% CI), 1.33 (1.13–1.56), 1.31(1.10–1.56), 1.38 (1.16–1.62) and 1.27 (1.06–1.51), respectively]. After adjustment for multiple testing, these associations remained significant. All the previous associations observed between metabolites and HF remained significant in the internal validation analysis (Supplementary Table S3). Restricted cubic spline analysis (Supplementary Figure S4) suggested nonlinear associations of malate, aconitate, isocitrate and D/L-2-hydroxyglutarate with HF risk (P value for nonlinearity with HF risk of malate, aconitate, isocitrate and D/L-2-hydroxyglutarate was 0.025, 0.010, <0.001 and 0.028, respectively). No significant associations of succinate and citrate with HF risk were observed.

Using the individual metabolites as the primary exposures, we observed no significant effect modification by age, sex or obesity status. Similarly, we did not find significant interactions with the dietary interventions.

4. Discussion

In this secondary analysis conducted in the PREDIMED study, we found that baseline plasma levels of TCA cycle-related metabolites were associated with higher risk of both AF and HF after 10 years of follow-up. Most of these associations were nonlinear and more pronounced among individuals with higher levels of the metabolites. Our results support a dysregulation of aerobic energy metabolism likely to precede the development of both cardiac outcomes.

From a simplistic perspective, a likely interpretation could be that when the cellular energy demands are high and oxygen is present, Acetyl-CoA, the common intermediate that links oxidative metabolism from all energy sources, enters the TCA cycle. Subsequently, a sequence of spatially optimized reversible reactions occur under different conditions, aimed to generate reducing equivalents for the synthesis of ATP, but also to provide precursors for biosynthesis of several compounds [20]. Dysregulations of the TCA cycle, characterized by changes in the anaplerotic/cataplerotic flux, have been associated with several diseases related to oxidative stress such as CVD [21], cancer [22] or neurodegenerative disorders [23]; although with some controversial results, possibly due to the complexity of the anaplerotic reactions coupled to the TCA cycle to compensate for the intermediate metabolites to maintain the cataplerotic activity of the cycle.

Although plasma citric acid [24], α -ketoglutarate [25] and oxaloacetate [26] have been inversely associated with cardiovascular outcomes, the potential benefit of augmenting TCA cycle intermediates is generally accepted and supported by several cross-sectional analyses [20]. However, these studies were not designed to investigate associations between TCA cycle-related metabolites and cardiovascular disorders prospectively, whereas our data show, for the first time, a prospective association of these metabolites with AF and HF. In our study, we found a significant association between increased plasma concentrations of citrate, aconitate, succinate and malate with a higher incidence of AF, and except for aconitate, the rest metabolites were internally replicated.

Consistently, increased incidence of HF was found for increased aconitate, isocitrate and malate. In this sense, higher citrate levels have been associated with an increased risk of all-cause mortality, as well as death from cancer and other nonvascular diseases [6]. In another cohort of 223 patients, 172 of them with significant coronary atherosclerosis and the rest disclosing clinical manifestations such as chest pain, angina and HF, malic acid and α -ketoglutaric acid significantly discriminated patients with and without coronary lesions [27]. Similarly, plasma succinate and malate were found at high concentrations in subjects with myocardial infarction [28].

The accumulation of these specific circulating metabolites from the TCA cycle could be due to perturbations in general metabolic processes that normally permit proper ATP

production, also necessary to maintain the contractile function of cardiomyocytes [29]. Under hypoxia conditions there is an increased metabolites' TCA cycle flux as defense against oxidative stress [4]. Since oxidative stress has been found to adversely affect atrial structure, electrical remodelling and cardiac muscle function that lead to AF and HF development [30,31], the increased levels of TCA metabolites in our study suggest other potential mechanisms beyond ATP production. In fact, increasing evidences indicates that beyond ATP production, TCA cycle metabolites could also control physiology through non-cell autonomous functions, acting as signaling molecules [10].

Additionally, plasma concentrations of D/L-2-hydroxyglutarate were also associated with a higher risk of AF and HF. Although D/L-2-hydroxyglutarate do not take part of the TCA cycle, it is linked to α -ketoglutarate, and both metabolites have been found to increase under hypoxia conditions [32] and relate to cardiac muscle function [8,33]. Increased cellular D/L-2-hydroxyglutarate concentrations could be considered as a mechanism inhibiting electron transport to compensate the adverse consequences of mitochondrial reductive stress induced by hypoxia [8]. It has been suggested that plasma D/L-2-hydroxyglutarate levels could be a useful marker for disease monitoring [34,35]. AF is characterized by a high-frequency excitation and contraction of cardiac muscle that would need to prioritize energy production through the TCA cycle, but AF is strongly associated with tissue ischemia and hypoxia, thus supporting the increased D/L-2-hydroxyglutarate concentrations as a counterbalance mechanism to reduce stress related to hypoxia.

Our study has several strengths such as the prospective design including well-characterized study population, the long follow-up period with few drop-outs and the blinded assessment of incident AF and HF cases by the Endpoint Adjudication Committee. With regards to limitations, we recruited older participants at high cardiovascular risk from a Mediterranean region and this might limit the generalizability of our findings to other geographical areas or younger populations with low CVD risk. Furthermore, although we carefully adjusted for many potential confounders, residual confounding cannot be ruled out. This could partially explain the lack of consistency in the associations we found for TCA cycle metabolites with both cardiac outcomes, even though this could also be explained by some differences in the physiopathology of these outcomes and by low statistical power. The metabolites in our study were measured in blood which may not reflect what occurs metabolically in cardiomyocytes, even though there is previous evidence suggesting that TCA cycle metabolites could be relevant to anaplerotic pathways. Finally, the lack of other measured TCA cycle metabolites such as oxalacetate, α -ketoglutarate and fumarate may limit a broader understanding of the metabolic processes related to TCA and cardiac outcomes.

5. Conclusions

In conclusion, our findings suggest positive associations between TCA cycle-related metabolites and incidence of AF and HF in an older population at high CVD risk. To our knowledge, this is the first prospective study assessing TCA-related metabolites for associations with both AH and HF. Although these findings need replication in other populations, they extend the current knowledge on the physiopathology of these two cardiac outcomes, which are likely to share common metabolic dysregulations. Further studies are

needed to understand the potential role of modulating TCA cycle intermediates for the prevention of AF and HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FH, JS-S, MM-G and MR-C designed research; MB, CP, JFG-G, MR-C, JL, MG-F, ET, CC, DC, RE, ER, MF, C-HL, KP, CR, FA, LS-M, LL, MM-G, FH and JS-S conducted research; DC, RE, ER, MF, FA, LS-M, MM-G and JS-S were the coordinators of subject recruitment at the outpatient clinics; CP analyzed the data; MB, CP, FBH and JS-S interpreted statistical analysis and data; CC acquired and processed metabolomics data; MB and CP drafted the paper; FH, JS-S and MM-G supervised the study and MB and JS-S took the responsibility for the integrity of the data and the accuracy of the data analysis. All authors revised the manuscript for important intellectual content, read and approved the final manuscript.

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Conflict of interest statement

ER has received grants for research through his institution from the California Walnut Commission (CWC), as well as fees for lectures and participation in the CWC's Health Research Advisory Group, and support for travel and accommodation. He is also a non-paid member of the Scientific Advisory Council of the CWC. He has also received honoraria for Advisory Board, lectures and support for travel and accommodation from Alexion; honoraria for lectures and support for travel and accommodation from Danone; and honoraria for lectures from Amarin. JS-S is a non-paid member of the Scientific Committee of the International Nut and Dried Fruit Foundation. He has received grants/research support from the American Pistachio Growers and International Nut and Dried Fruit Foundation through his Institution. He has received honoraria from Instituto Danone Spain and Eroski. He is a member of the executive committee of Instituto Danone Spain and non-paid member of International Danone Institute. None of the other co-authors has potential conflicts of interest to disclose relevant to the subject matter or materials included in this work.

Abbreviations:

TCA	tricarboxylic acid
AF	atrial fibrillation
ECGs	electrocardiograms
HF	heart failure
PREDIMED	Prevención con Dieta Mediterránea

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Table 1.

Baseline characteristics of the study population.

	Atrial Fibrillation (AF) Cases	Controls matched to AF cases	Heart Failure (HF) Cases	Controls matched to HF cases
n	512	616	334	433
Age (years)	68.3 (6.1)	68.5 (6.1)	70.3 (5.9)	70.3 (5.9)
Sex (% Women)	49.8	49.2	58.7	54.3
Body mass index, kg/m ²	30.7 (3.8)	29.8 (3.8)*	31.1 (3.8)	29.3 (3.6)*
Physical activity, MET-min/week	226 (209)	231 (217)	217 (195)	216 (218)
Intervention group, %				
MedDiet+EVOO	31.8	36.2	31.1	37.6
MedDiet+Nuts	31.2	28.4	32.3	26.3
Control group	35.4	36.9	36.5	36.0
Alcohol intake (g/day)	8.9 (13.2)	9.7 (15.0)	8.0 (15.0)	8.1 (12.6)
Family history of premature CHD, %	19.1	20.0	19.5	19.4
Type 2 diabetes, %	47.9	49.8	59.0	52.4
Hypertension, %	88.3	82.8*	87.7	82.2*
Dyslipidemia, %	64.8	68.7	64.1	69.0
Antihypertensive medication, (%)	78.9	72.5*	77.2	75.1
Oral antidiabetic agents, (%)	30.8	31.3	40.4	32.8*
Insulin medication, (%)	7.2	7.5	10.2	7.9
Lipid-lowering medication, (%)	36.1	35.7	37.4	39.0
Smoking, %				
Never	58.8	57.9	60.5	61.7
Former	26.9	28.7	25.2	27.0
Current	14.3	13.3	14.4	11.3

Data are presented as mean (SD) or percentage. The χ^2 test was used for comparison of categorical variables and Student's t-test was used for comparison of continuous variables. Abbreviations: CHD, coronary heart disease; MedDiet, Mediterranean diet; EVOO, extra-virgin olive oil; MET, metabolic equivalent.

* P value<0.05

There were 108 overlapping cases of AF and HF.

Associations of baseline individual TCA cycle-related metabolites levels with the risk of incident atrial fibrillation in a nested case-control study of the PRECIMED study¹.

Table 2.

Metabolite	Quartiles of plasma metabolite levels					OR per 1 SD increment	P trend ²	P value ²
	Q1	Q2	Q3	Q4	Q4			
Succinate								
Cases	101	130	116	165				
Crude model	1 (Ref.)	1.33 (0.94–1.87)	1.27 (0.87–1.84)	1.84 (1.27–2.66)	0.006	1.19 (1.04–1.35)	0.016	
MV1	1 (Ref.)	1.41 (0.99–2.01)	1.35 (0.92–1.99)	1.83 (1.25–2.68)	0.006	1.17 (1.02–1.33)	0.033	
MV2	1 (Ref.)	1.41 (0.99–2.02)	1.35 (0.92–1.99)	1.84 (1.25–2.71)	0.006	1.17 (1.02–1.33)	0.033	
Malate								
Cases	98	114	111	189				
Crude model	1 (Ref.)	1.23 (0.87–1.73)	1.24 (0.87–1.76)	2.18 (1.54–3.10)	0.006	1.28 (1.13–1.45)	0.006	
MV1	1 (Ref.)	1.23 (0.87–1.76)	1.28 (0.88–1.84)	2.14 (1.49–3.08)	0.003	1.28 (0.13–1.45)	0.003	
MV2	1 (Ref.)	1.21 (0.85–1.73)	1.28 (0.89–1.85)	2.16 (1.49–3.12)	0.003	1.29 (1.13–1.47)	0.003	
Citrate								
Cases	103	127	131	151				
Crude model	1 (Ref.)	1.24 (0.86–1.80)	1.40 (0.95–2.07)	1.78 (1.22–2.61)	0.010	1.16 (1.02–1.32)	0.026	
MV1	1 (Ref.)	1.24 (0.86–1.79)	1.39 (0.94–2.05)	1.79 (1.23–2.62)	0.004	1.20 (1.04–1.37)	0.018	
MV2	1 (Ref.)	1.24 (0.86–1.80)	1.40 (0.94–2.07)	1.78 (1.22–2.61)	0.004	1.19 (1.04–1.37)	0.022	
Aconitate								
Cases	112	111	145	144				
Crude model	1 (Ref.)	0.95 (0.67–1.34)	1.33 (0.95–1.88)	1.41 (0.98–2.00)	0.027	1.18 (1.03–1.35)	0.019	
MV1	1 (Ref.)	0.97 (0.68–1.38)	1.33 (0.94–1.90)	1.35 (0.94–1.96)	0.064	1.16 (1.01–1.33)	0.038	
MV2	1 (Ref.)	0.96 (0.67–1.37)	1.34 (0.94–1.91)	1.34 (0.93–1.94)	0.072	1.16 (1.01–1.33)	0.045	
Isocitrate								
Cases	113	122	124	153				
Crude model	1 (Ref.)	1.07 (0.75–1.52)	1.09 (0.77–1.52)	1.43 (1.01–2.02)	0.031	1.14 (1.01–1.29)	0.029	
MV1	1 (Ref.)	1.06 (0.74–1.51)	1.07 (0.75–1.52)	1.32 (0.92–1.90)	0.116	1.11 (0.98–1.26)	0.101	
MV2	1 (Ref.)	1.05 (0.73–1.50)	1.06 (0.75–1.51)	1.30 (0.90–1.88)	0.134	1.10 (0.97–1.26)	0.126	
D/L-2-Hydroxyglutarate								

Metabolite	Quartiles of plasma metabolite levels					P trend ²	OR per 1 SD increment	P value ²
	Q1	Q2	Q3	Q4	Q4			
Cases	100	110	142	160				
Crude model	1 (Ref.)	1.15 (0.81–1.63)	1.40 (0.99–1.97)	1.59 (1.13–2.25)	1.59 (1.13–2.25)	0.010	1.21 (1.07–1.37)	0.006
MV1	1 (Ref.)	1.19 (0.83–1.71)	1.46 (1.02–2.09)	1.82 (1.26–2.62)	1.82 (1.26–2.62)	0.003	1.24 (1.09–1.41)	0.003
MV2	1 (Ref.)	1.20 (0.83–1.73)	1.46 (1.02–2.09)	1.83 (1.26–2.67)	1.83 (1.26–2.67)	0.003	1.24 (1.09–1.41)	0.003

¹Values are OR (95% CI). A natural logarithmic transformation was applied to the raw values of individual metabolites. Conditional logistic regression analysis was used. MV1 adjusted for smoking, family history of premature coronary heart disease, physical activity, alcohol intake, BMI (kg/m²), intervention groups, dyslipidemia, hypertension and type 2 diabetes; MV2 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication). Abbreviations: Ref, reference; TCA, tricarboxylic acid.

²Adjusted with the Benjamini-Hochberg False Discovery Rate method. Case and control subjects were matched on age, sex and recruitment center.

Table 3.

Associations of baseline individual TCA cycle-related metabolites levels with the risk of incident heart failure in a nested case-control study of the PREMIMED study¹.

Metabolite	Quartiles of plasma metabolite levels					P trend ²	OR per 1 SD increment	P value ²
	Q1	Q2	Q3	Q4	Q4			
Succinate								
Cases	81	70	92	91				
Crude model	1 (Ref.)	0.86 (0.56–1.31)	1.14 (0.74–1.74)	1.20 (0.77–1.86)	0.251	1.14 (0.98–1.33)	0.093	
MV1	1 (Ref.)	0.94 (0.60–1.47)	1.24 (0.79–1.95)	1.09 (0.68–1.74)	0.579	1.09 (0.93–1.28)	0.312	
MV2	1 (Ref.)	0.95 (0.60–1.50)	1.25 (0.80–1.98)	1.11 (0.69–1.78)	0.534	1.10 (0.93–1.29)	0.285	
Malate								
Cases	63	78	84	109				
Crude model	1 (Ref.)	1.35 (0.87–2.10)	1.54 (0.98–2.42)	2.18 (1.39–3.42)	0.002	1.32 (1.14–1.54)	0.002	
MV1	1 (Ref.)	1.32 (0.82–2.13)	1.56 (0.96–2.52)	2.28 (1.41–3.70)	0.003	1.34 (1.14–1.57)	0.003	
MV2	1 (Ref.)	1.35 (0.83–2.19)	1.62 (0.98–2.66)	2.30 (1.41–3.76)	0.003	1.33 (1.13–1.56)	0.003	
Citrate								
Cases	89	65	79	101				
Crude model	1 (Ref.)	0.80 (0.53–1.20)	0.92 (0.61–1.38)	1.36 (0.88–2.10)	0.198	1.12 (0.96–1.31)	0.140	
MV1	1 (Ref.)	0.83 (0.54–1.27)	0.95 (0.62–1.47)	1.24 (0.77–1.98)	0.430	1.10 (0.93–1.30)	0.310	
MV2	1 (Ref.)	0.83 (0.54–1.28)	0.96 (0.62–1.49)	1.26 (0.78–2.02)	0.390	1.10 (0.93–1.31)	0.285	
Aconitate								
Cases	56	78	91	109				
Crude model	1 (Ref.)	1.36 (0.88–2.08)	1.76 (1.13–2.74)	2.35 (1.47–3.76)	0.002	1.38 (1.17–1.62)	0.002	
MV1	1 (Ref.)	1.32 (0.83–2.10)	1.64 (1.02–2.63)	1.97 (1.19–3.26)	0.012	1.31 (1.10–1.57)	0.004	
MV2	1 (Ref.)	1.38 (0.86–2.12)	1.64 (1.02–2.64)	2.03 (1.22–3.39)	0.010	1.31 (1.10–1.56)	0.004	
Isocitrate								
Cases	58	75	75	126				
Crude model	1 (Ref.)	1.45 (0.93–2.26)	1.51 (0.95–2.39)	2.69 (1.71–4.23)	0.002	1.43 (1.23–1.68)	0.002	
MV1	1 (Ref.)	1.63 (1.01–2.63)	1.38 (0.83–2.27)	2.52 (1.54–4.11)	0.003	1.36 (1.16–1.60)	0.003	
MV2	1 (Ref.)	1.66 (1.02–2.71)	1.45 (0.87–2.41)	2.66 (1.61–4.40)	0.003	1.38 (1.16–1.62)	0.003	
D/L-2-Hydroxyglutarate								

Metabolite	Quartiles of plasma metabolite levels					P trend ²	OR per 1 SD increment	P value ²
	Q1	Q2	Q3	Q4	Cases			
Cases	62	76	86	110				
Crude model	1 (Ref.)	1.30 (0.84–2.02)	1.52 (0.98–2.35)	1.96 (1.26–3.04)		0.004	1.22 (1.05–1.43)	0.018
MV1	1 (Ref.)	1.15 (0.72–1.84)	1.54 (0.96–2.46)	1.84 (1.14–2.97)		0.012	1.24 (1.05–1.47)	0.016
MV2	1 (Ref.)	1.13 (0.70–1.81)	1.55 (0.96–2.49)	1.91 (1.17–3.10)		0.010	1.27 (1.06–1.51)	0.010

¹Values are OR (95% CI). A natural logarithmic transformation was applied to the raw values of individual metabolites. Conditional logistic regression analysis was used. MV1 adjusted for smoking, family history of premature coronary heart disease, physical activity, alcohol intake, BMI (kg/m²), intervention groups, dyslipidemia, hypertension and type 2 diabetes; MV2 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication). Abbreviations: Ref, reference ; TCA, tricarboxylic acid.

²Adjusted with the Benjamini-Hochberg False Discovery Rate method. Case and control subjects were matched on age, sex and recruitment center.