Metabolism Clinical and Experimental 125 (2021) 154915

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

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

Mònica Bulló [a,b,c,d,1], Christopher Papandreou [a,b,c,d,1], Jesus García-Gavilán [a,b,c,d], Miguel Ruiz-Canela [c,e,f], Jun Li [g], Marta Guasch-Ferré [g,h], Estefanía Toledo [c,e,f], Clary Clish [i], Dolores Corella [c,j], Ramon Estruch [c,k], Emilio Ros [c,l], Montserrat Fitó [c,m], Chih-Hao Lee [g,n], Kerry Pierce [i], Cristina Razquin [c,e,f], Fernando Arós [c,o], Lluís Serra-Majem [c,p], Liming Liang [q,r], Miguel A. Martínez-González [c,e,f,g], Frank B. Hu [g,h,q,r], Jordi Salas-Salvadó [a,b,c,d,*]

[a] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, Reus, Spain
[b] Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
[c] Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[d] University Hospital of Sant Joan de Reus, Nutrition Unit, Reus, Spain
[e] University of Navarra, Department of Preventive Medicine and Public Health, Pamplona, Spain
[f] Navarra Institute for Health Research (IdiSNA), Pamplona, Navarra, Spain
[g] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[h] Channing Division for Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, MA, USA
[i] Broad Institute of MIT and Harvard University, Cambridge, MA, USA
[j] Department of Preventive Medicine, University of Valencia, Valencia, Spain
[k] Department of Internal Medicine, Department of Endocrinology and Nutrition Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain
[l] Lipid Clinic, Department of Endocrinology and Nutrition Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain
[m] Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona, Spain
[n] Department of Molecular Metabolism (C.-H.L.), Harvard T.H. Chan School of Public Health, Boston, MA, USA
[o] Department of Cardiology, University Hospital of Alava, Vitoria, Spain
[p] Institute of Health Sciences IUNICS, University of Balearic Islands and Hospital Son Espases, Palma de Mallorca, Spain
[q] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[r] Department of Statistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA

ARTICLE INFO

Article history:
Received 5 July 2021
Accepted 14 October 2021

Keywords:
Tricarboxylic acid cycle metabolites
Hydroxyglutarate
Atrial fibrillation
Heart failure
PREDIMED

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 and 334 HF incident cases matched by age (±5 years), sex and recruitment center to 616 controls and 433 controls, respectively, 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. Potential confounders included smoking, family history of premature coronary heart disease, physical activity, alcohol intake, body mass index, intervention groups, dyslipidemia, hypertension, type 2 diabetes and medication use.
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): 1.80 (1.21–2.67), 2.13 (1.45–3.13), 1.87 (1.25–2.81) and 1.95 (1.31–2.90), respectively]. One SD increase in aconitate was directly associated with AF risk [OR (95% CI): 1.16 (1.01–1.34)]. The corresponding ORs (95% CI) for HF comparing extreme quartiles of malate,

Abbreviations: Acetyl-CoA, acetyl coenzyme A; AF, atrial fibrillation; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; ECGs, electrocardiograms; DBP, diastolic blood pressure; FDR, false discovery rate; HF, heart failure; MedDiet, Mediterranean diet; PREDIMED, Prevención con Dieta Mediterránea; SBP, systolic blood pressure; TCA, tricarboxylic acid; T2D, type 2 diabetes.

☆ Pubmed indexing: Bulló M, Papandreou C, García-Gavilán J, Ruiz-Canela M, Li J, Guasch-Ferré M, Toledo E, Clish C, Corella D, Estruch R, Ros E, Fitó M, Lee CH, Pierce K, Razquin C, Arós F, Serra-Majem L, Liang L, Martínez-González MA, Hu FB, Salas-Salvadó J.

* Corresponding author at: Human Nutrition Unit, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, C/Sant Llorenç 21, 43201 Reus, Spain.
E-mail address: jordi.salas@urv.cat (J. Salas-Salvadó).

[1] These authors contributed equally to this work and share co-first authorship.

https://doi.org/10.1016/j.metabol.2021.154915

aconitate, isocitrate and D/L-2-hydroxyglutarate were 2.15 (1.29–3.56), 2.16 (1.25–3.72), 2.63 (1.56–4.44) and 1.82 (1.10–3.04), 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.

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 dehydrogonase; 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 explorative analysis 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

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) were included in these analyses. The design and methods of PREDIMED have been detailed in [12]. Briefly, from June 25, 2003 to June 30, 2009, 7447 elderly participants free of 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 was collected and analyzed by the Clinical Events Committee members who were blinded to diet allocation and incident cases. 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. Fig. 1 depicts the flow chart of the participants' selection process in both studies.

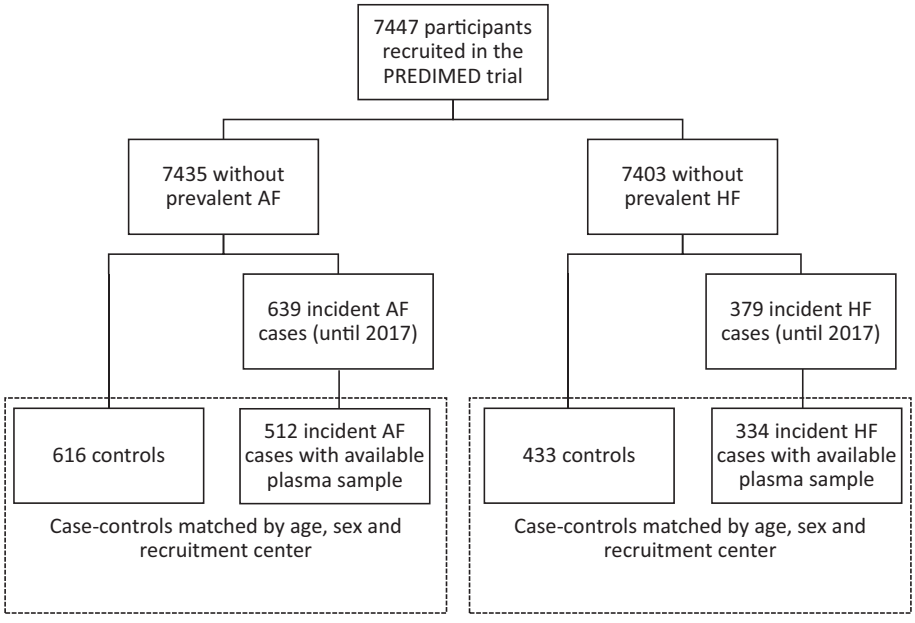

Fig. 1. Flow-chart of study participants. Abbreviations: AF, atrial fibrillation; HF, heart failure.

Five hundred twelve incident AF events and 334 incident HF events were ascertained. 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 in a ratio of 1 to 3 controls per case. Controls were matched by age (±5 years), sex and recruitment center. There were 108 overlapping cases of AF and HF. The protocol of the PREDIMED trial was in accordance with the Helsinki Declaration and it was approved by the Research Ethics Committees of all recruitment centres. All participants provided written informed consent 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. The present PREDIMED metabolomics project in relation to HF and AF was approved by the Research Ethics Committee of the University of Navarra, also a coordinating center. According to this approval it was not required an extra informed consent to be signed by the participants.

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 or by yearly ad-hoc reviews of outpatient and inpatient medical charts. During the extended follow-up period (2011–2017), or until 2014 in one center, electronic information on AF and HF by reviewing the outpatient and inpatient medical charts was recorded. When a clinical diagnosis of heart disease was made, all relevant documents including clinical records of hospital discharge, outpatient clinics and family physicians were sent anonymized to the Clinical End-Point Adjudication Committee, who adjudicated the events according to pre-specified criteria. Two independent cardiologists evaluated the documents, and a third cardiologist was contacted in case of disagreement. The diagnostic criteria and procedures have been reported in detail elsewhere [13,14].

For AF, annual reviews of all medical records and yearly electrocardiograms (ECGs) performed during follow-up examinations were used for ascertainment. 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.

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

Plasma samples were collected in fasting conditions prior to any dietary intervention and stored at −80 °C. Metabolomic analyses were conducted at the Broad Institute (Boston, Massachusetts, USA), using liquid chromatography-tandem mass spectrometry 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. Internal standard peak areas were monitored for quality control and to ensure system performance throughout analyses and pooled plasma reference samples were systematically analyzed as an additional quality control. Information about the mass to charge ratio and retention time is shown in Supplementary Table S1.

2.4. Covariates

Information about sociodemographic and lifestyle variables, smoking status, medical conditions, family history of disease and medication use were collected at baseline. Physical activity was estimated with the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [18]. Blood pressure was measured twice in each arm with a validated semiautomatic oscillometer (Omron HEM-705CP, Hoofddorp, the Netherlands). Fasting plasma glucose and lipids levels were measured by locally standard measurement methods. 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

We computed that we will have 80% power to detect a metabolite associated with AF and HF with odds ratio of 1.39 and 1.49, respectively, (corresponding to one standard deviation increase in metabolite level) using the combined controls at nominal false positive rate of 0.05. 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/m^2), 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, diuretics and antiarrythmic drugs). 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.), at a two-tailed α of 0.05.

3. Results

Study participants were followed for a mean of ≈10 years [6.8 years (SD 3.3) and 7.3 years (SD 3.3) for AF and HF occurrence, respectively]. 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. On the other hand, AF incident cases were more likely to use diuretics and take antiarrhythmic drugs. Furthermore, participants in the higher quartile of the metabolites' levels had higher prevalence of T2D (data not shown). Heatmaps of pairwise Spearman correlation coefficients for the 6 plasma TCA cycle-related metabolites are shown in Supplementary Fig. S1. 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 [1.80 (95% CI, 1.21–2.67), 2.13 (95% CI, 1.45–3.13) and 1.87 (95% CI, 1.25–2.81), respectively]. Similarly, the third and fourth quartiles of D/L-2-hydroxyglutarate, compared with the lowest, were significantly associated with AF [OR (95% CI), 1.57 (1.07–2.30) and 1.95 (1.31–2.90)]. 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 (1.02–1.34), 1.28 (1.11–1.46), 1.23 (1.06–1.43), 1.16 (1.01–1.34) and 1.26 (1.10–1.45), respectively]. Cubic spline curves (Supplementary Fig. S2) 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 malate, citrate and aconitate levels, the risk of AF significantly increased in individuals with diabetes, with $OR_{malate} = 1.45$ (95% CI 1.22–1.71) (*P* for $interaction_{AdjFDR} = 0.038$), $OR_{citrate} = 1.28$ (95% CI 1.07–1.54) (*P* for $interaction_{AdjFDR} = 0.038$) and $OR_{aconitate} = 1.36$ (95% CI 1.13–1.64) (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), 2.15 (1.29–3.56) for malate, 2.16 (1.25–3.72) for aconitate, 2.63 (1.56–4.44) for isocitrate and 1.82 (1.10–3.04) 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), 1.29 (1.09–1.53), 1.34 (1.11–1.61), 1.42 (1.19–1.69) and 1.26 (1.05–1.51), 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 Fig. S3) 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-

Table 1
Baseline characteristics of the study population.

	Arial 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
Men	50.2	50.8	41.3	45.7
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
Diuretics (%)	28.0	19.0*	25.7	24.3
Antiarrhythmic drugs (%)	19.5	13.0*	20.9	15.5
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 x^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; EVOO, extra-virgin olive oil; MedDiet, Mediterranean diet; MET, metabolic equivalent.
There were 108 overlapping cases of AF and HF.
* *P* value < 0.05.

Table 2
Associations of baseline individual TCA cycle-related metabolites levels with the risk of incident atrial fibrillation in a nested case-control study of the PREDIMED study.[1]

Metabolite	Quartiles of plasma metabolite levels						
	Q1	Q2	Q3	Q4	*P* trend[2]	OR per 1 SD increment	*P* value[2]
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.)	1.40 (0.97–2.03)	1.36 (0.91–2.03)	1.80 (1.21–2.67)	**0.010**	1.17 (1.02–1.34)	**0.037**
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.)	1.23 (0.85–1.78)	1.26 (0.86–1.85)	2.13 (1.45–3.13)	**0.002**	1.28 (1.11–1.46)	**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.)	1.21 (0.82–1.79)	1.43 (0.95–2.17)	1.87 (1.25–2.81)	**0.002**	1.23 (1.06–1.43)	**0.010**
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.)	0.95 (0.65–1.38)	1.34 (0.92–1.94)	1.32 (0.89–1.95)	0.100	1.16 (1.01–1.34)	**0.048**
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.)	0.97 (0.66–1.41)	0.97 (0.67–1.41)	1.24 (0.84–1.82)	0.189	1.09 (0.95–1.25)	0.193
D/L-2-Hydroxyglutarate							
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)	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)	0.003	1.24 (1.09–1.41)	0.003
MV2	1 (Ref.)	1.33 (0.90–1.95)	1.57 (1.07–2.30)	1.95 (1.31–2.90)	**0.002**	1.26 (1.10–1.45)	**0.003**

[1] Values 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/m^2), intervention groups, dyslipidemia, hypertension and type 2 diabetes; MV2 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication, diuretics and antiarrhythmic drugs). Abbreviations: MV, multivariable; Ref, reference; TCA, tricarboxylic acid.

[2] Adjusted with the Benjamini-Hochberg False Discovery Rate method. Case and control subjects were matched on age, sex and recruitment center. Bold text indicates statistically significant *P* values.

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]. Metabolic alterations in the heart may also increase myocardial oxidative stress [4] that has been found to adversely affect atrial structure, electrical remodelling and

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 PREDIMED study.[1]

Metabolite	Quartiles of plasma metabolite levels						
	Q1	Q2	Q3	Q4	*P* trend[2]	OR per 1 SD increment	*P* value[2]
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.)	1.00 (0.63–1.60)	1.22 (0.75–1.97)	1.28 (0.78–2.10)	0.313	1.14 (0.96–1.36)	0.142
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.)	1.32 (0.79–2.18)	1.52 (0.90–2.57)	2.15 (1.29–3.56)	**0.009**	1.29 (1.09–1.53)	**0.006**
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.)	0.85 (0.54–1.33)	0.93 (0.59–1.48)	1.18 (0.71–1.95)	0.513	1.09 (0.92–1.30)	0.323
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.)	1.42 (0.86–2.34)	1.50 (0.91–2.48)	2.16 (1.25–3.72)	**0.013**	1.34 (1.11–1.61)	**0.006**
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.)	1.56 (0.94–2.59)	1.48 (0.86–2.56)	2.63 (1.56–4.44)	**0.006**	1.42 (1.19–1.69)	**0.006**
D/L-2-Hydroxyglutarate							
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.)	1.06 (0.64–1.75)	1.50 (0.91–2.47)	1.82 (1.10–3.04)	**0.013**	1.26 (1.05–1.51)	**0.019**

[1] Values 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/m^2), intervention groups, dyslipidemia, hypertension and type 2 diabetes; MV2 additionally adjusted for medication use (lipid-modifying, antihypertensive, and antidiabetic medication, diuretics and antiarrhythmic drugs). Abbreviations: MV, multivariable; Ref, reference; TCA, tricarboxylic acid.

[2] Adjusted with the Benjamini-Hochberg False Discovery Rate method. Case and control subjects were matched on age, sex and recruitment center. Bold text indicates statistically significant *P* values.

cardiac muscle function that lead to AF and HF development [30,31]. Under these conditions an increased metabolites' TCA cycle flux could be developed as defense against oxidative stress [4].

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. In a previous study conducted by our group, no associations between glycolysis metabolites neither with HF nor with AF risk were found, except for phosphoglycerate that was the only metabolite associated with a higher risk of HF [36]. Therefore, our results support the hypothesis of the mitochondrial dysfunction in the development of HF and AF [37].

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. Furthermore, the magnitude and the sign of correlations between the study TCA cycle metabolites, support that disturbances of TCA cycle are associated with incident AF and HF. With regard 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. In addition, although our results were internally validated, replication of the discovery findings in an independent cohort study would strengthen our study conclusions. Furthermore, although we carefully adjusted for many potential confounders, residual confounding cannot be ruled out. Especially, during the long follow-up many substantial metabolic changes may have occurred that could affect our results and repeated measurements in the TCA-related metabolites would better contribute to identify people with a higher risk of AF and HF. 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. As diagnosis of AF was based on hospital records and yearly ECGs, sporadic incidents of symptomatic and asymptomatic AF outside these records might have been missed. 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. Furthermore, 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. Finally, we did not have available measures of established AF/HF surrogate markers such as N-terminal-pro hormone B-type natriuretic peptide that would allow us to compare our findings [38].

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 exploratory 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.

CRediT authorship contribution statement

Mònica Bulló: Conceptualization, Investigation, Data curation, Writing – original draft. **Christopher Papandreou:** Conceptualization, Software, Formal analysis, Investigation, Data curation, Writing – original draft. **Jesus García-Gavilán:** Investigation, Writing – review & editing. **Miguel Ruiz-Canela:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Jun Li:** Investigation, Writing – review & editing, Visualization. **Marta Guasch-Ferré:** Investigation, Writing – review & editing. **Estefanía Toledo:** Investigation, Writing – review & editing. **Clary Clish:** Resources, Writing – review & editing. **Dolores Corella:** Investigation, Writing – review & editing. **Ramon Estruch:** Investigation, Writing – review & editing. **Emilio Ros:** Investigation, Writing – review & editing. **Montserrat Fitó:** Investigation, Writing – review & editing. **Chih-Hao Lee:** Investigation, Writing – review & editing. **Kerry Pierce:** Resources, Writing – review & editing. **Cristina Razquin:** Investigation, Writing – review & editing. **Fernando Arós:** Investigation, Writing – review & editing. **Lluís Serra-Majem:** Investigation, Writing – review & editing. **Liming Liang:** Investigation, Writing – review & editing. **Miguel A. Martínez-González:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Project administration. **Frank B. Hu:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Project administration, Funding acquisition. **Jordi Salas-Salvadó:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Project administration.

Declaration of competing interest

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.

Acknowledgments

This work was supported by the National Institutes of Health (R01HL118264), the Spanish Ministry of Health (Instituto de Salud Carlos III) and the Ministerio de Economía y Competitividad-Fondo Europeo de Desarrollo Regional (Projects CNIC-06/2007, RTIC G03/140, RTIC 06/0045, CIBER 06/03, PI06-1326, PI07-0954, PI11/02505, SAF2016-80532, SAF2009-12304 and AGL2010-22319-C03-03) and by the Generalitat Valenciana (PROMETEO 17/2017, ACOMP2010-181, AP111/10, AP-042/11, ACOM2011/145, ACOMP/2012/190, ACOMP/2013/159 and ACOMP/213/165). Dr. CP is recipient of the Instituto de Salud Carlos III Miguel Servet fellowship (grant CP 19/00189). JFG-G has received the Contratos Predoctorales de Formaciónen Investigaciónen Salud (PFIS FI17/00255) of Acción Estratégica en Salud program (AES) from the Institute of Health Carlos III (ISCIII), Spanish Ministry of Health. Dr. MG-F was supported by American Diabetes Association grant #1-18-PMF-029. Professor JS-S, senior author of this work, is partially supported by ICREA under the ICREA Academia programme.

References

[1] Braunwald E. Cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med. 1997;337:1360–9.
[2] Anter E, Jessup M, Callans DJ. Atrial fibrillation and heart failure: treatment considerations for a dual epidemic. Circulation. 2009;119:2516–25.
[3] Heist EK, Ruskin JN. Atrial fibrillation and congestive heart failure: risk factors, mechanisms, and treatment. Prog Cardiovasc Dis. 2006;48:256–69.
[4] Doenst T, Nguyen TD, Abel ED. Cardiac metabolism in heart failure: implications beyond atp production. Circ Res. 2013;113:709–24.
[5] Harada M, Melka J, Sobue Y, Nattel S. Metabolic considerations in atrial fibrillation — mechanistic insights and therapeutic opportunities. Circ J. 2017;81:1749–57.
[6] Fischer K, Kettunen J, Würtz P, Haller T, Havulinna AS, Kangas AJ, et al. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med. 2014;11. https://doi.org/10.1371/journal.pmed.1001606.
[7] Mueller-Hennessen M, Sigl J, Fuhrmann JC, Witt H, Reszka R, Schmitz O, et al. Metabolic profiles in heart failure due to non-ischemic cardiomyopathy at rest and under exercise. ESC Hear Fail. 2017;4:178–89.
[8] Oldham WM, Clish CB, Yang Y, Loscalzo J. Hypoxia-mediated increases in L-2-hydroxyglutarate coordinate the metabolic response to reductive stress. Cell Metab. 2015;22:291–303.
[9] Karlstaedt A, Zhang X, Vitrac H, Harmancey R, Vasquez H, Wang JH, et al. Oncometabolite D-2-hydroxyglutarate impairs α-ketoglutarate dehydrogenase and contractile function in rodent heart. Proc Natl Acad Sci U S A. 2016;113:10436–41.
[10] Nelson SE, Ament Z, Wolcott Z, Gerszten RE, Kimberly WT. Succinate links atrial dysfunction and cardioembolic stroke. Neurology. 2019;92:802–10.
[11] Martínez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. Nat Commun. 2020;11. https://doi.org/10.1038/s41467-019-13668-3.
[12] Martínez-González MÁ, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fiol M, et al. PREDIMED study investigators cohort profile: design and methods of the PREDIMED study. Int J Epidemiol. 2012;41:377–85.
[13] Martínez-González MÁ, Toledo E, Arós F, Fiol M, Corella D, Salas-Salvadó J, et al. Extra virgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial. Circulation. 2014;130:18–26.
[14] Papadaki A, Martínez-González MÁ, Alonso-Gómez A, Rekondo J, Salas-Salvadó J, Corella D, et al. Mediterranean diet and risk of heart failure: results from the PREDIMED randomized controlled trial. Eur J Heart Fail. 2017;19:1179–85.
[15] Wang MH, Shugart YY, Cole SR, Platz EA. A simulation study of control sampling methods for nested case-control studies of genetic and molecular biomarkers and prostate cancer progression. Cancer Epidemiol Biomarkers Prev. 2009;18:706–11.
[16] Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005). Eur Heart J. 2005;26:1115–40.
[17] Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17:448–53.
[18] Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota leisure time physical activity questionnaire in Spanish men. The MARATHOM investigators. Am J Epidemiol. 1994;139:1197–209.
[19] Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001;125(1–2):279–84.
[20] Czibik G, Steeples V, Yavari A, Ashrafian H. Citric acid cycle intermediates in cardioprotection. Circ Cardiovasc Genet. 2014;7:711–79.
[21] Gibala MJ, Young ME, Taegtmeyer H. Anaplerosis of the citric acid cycle: role in energy metabolism of heart and skeletal muscle. Acta Physiol Scand. 2000;168(4):657–65.
[22] John AP. Dysfunctional mitochondria, not oxygen insufficiency, cause cancer cells to produce inordinate amounts of lactic acid: the impact of this on the treatment of cancer. Med Hypotheses. 2001;57:429–31.
[23] Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab. 2004;89:4963–71.
[24] Vallejo M, García A, Tuñón J, García-Martínez D, Angulo S, Martin-Ventura JL, et al. Plasma fingerprinting with GC–MS in acute coronary syndrome. Anal Bioanal Chem. 2009;394:1517–24.
[25] Bernini P, Bertini I, Luchinat C, Tenori L, Tognaccini A. The cardiovascular risk of healthy individuals studied by NMR metabonomics of plasma samples. J Proteome Res. 2011;10:4983–92.

[26] Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, et al. Metabolomic identification of novel biomarkers of myocardial ischemia. Circulation. 2005;112:3868–75.
[27] Calderón-Santiago M, Priego-Capote F, Galache-Osuna JG, Luque de Castro MD. Method based on GC–MS to study the influence of tricarboxylic acid cycle metabolites on cardiovascular risk factors. J Pharm Biomed Anal. 2013;74:178–85.
[28] Yao H, Shi P, Zhang L, Fan X, Shao Q, Cheng Y. Untargeted metabolic profiling reveals potential biomarkers in myocardial infarction and its application. Mol Biosyst. 2010;6:1061–70.
[29] Neubauer S. The failing heart — an engine out of fuel. N Engl J Med. 2007;356:1140–51.
[30] Karam BS, Chavez-Moreno A, Koh W, Akar JG, Akar FG. Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes. Cardiovasc Diabetol. 2017;16. https://doi.org/10.1186/s12933-017-0604-9.
[31] Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circ Res. 2002;91:988–98.
[32] Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, et al. Hypoxia promotes isocitrate dehydrogenasedependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci U S A. 2011;108:19611–6.
[33] Ritterhoff J, Tian R. Metabolism in cardiomyopathy: every substrate matters. Cardiovasc Res. 2017;113:411–21.
[34] Lombardi G, Corona G, Bellu L, Della Puppa A, Pambuku A, Fiduccia P, et al. Diagnostic value of plasma and urinary 2-hydroxyglutarate to identify patients with isocitrate dehydrogenase-mutated glioma. Oncologist. 2015;20:562–7.
[35] Yin F, Ling Y, Keller J, Kraus D, Narayanaswamy R, Mangus H, et al. Quantitation of 2-hydroxyglutarate in human plasma via LC-MS/MS using a surrogate analyte approach. Bioanalysis. 2020;12:1149–59.
[36] Becerra-Tomás N, Ruiz-Canela M, Hernández-Alonso P, Bulló M, Li J, Guasch-Ferré M, et al. Glycolysis metabolites and risk of atrial fibrillation and heart failure in the PREDIMED trial. Metabolites. 2021;11(5):306. https://doi.org/10.3390/metabo11050306.
[37] Li X, Yang X, Li Y, Yuan M, Tian C, Yang Y, et al. Mitochondria and the pathophysiological mechanism of atrial fibrillation. Curr Pharm Des. 2018;24(26):3055–61.
[38] Natriuretic Peptides Studies Collaboration. Natriuretic peptides and integrated risk assessment for cardiovascular disease: an individual-participant-data meta-analysis. Lancet Diabetes Endocrinol. 2016;4(10):840–9.