SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

The PREDIMED study

Briefly, from 2003 to 2010, 7447 Spanish participants without cardiovascular disease at baseline but at high cardiovascular (CV) risk were recruited. Participants were women or men aged 55 to 80 years, with type 2 diabetes (T2D) or with at least 3 of the following CV risk factors: body mass index (BMI) ≥ 25, current smoking, hypertension, high levels of low-density lipoprotein (LDL) cholesterol, low levels of high-density lipoprotein (HDL) cholesterol, or a family history of early coronary artery disease. Participants were randomized to 1 of 3 diets: a Mediterranean diet (MedDiet) supplemented with extra virgin olive oil (EVOO), a MedDiet supplemented with mixed nuts, or a control diet (advice to reduce dietary fat). More information is available elsewhere.[1]

Metabolomics analysis

Amino acids, acylcarnitines (ACs), and other polar plasma metabolites were profiled with the use of liquid chromatography-tandem mass spectrometry on a system comprised of a Shimadzu Nexera 32 U-HPLC (Shimadzu Corp) coupled toa Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific). Carnitine and ACs were profiled with other polar metabolites in the positive ion mode using high resolution accurate mass detection. Internal standards were added during extraction and monitored to ensure performance, and pooled quality controls were inserted in the queue every 20 samples. Targeted data were processed via MultiQuant software (SCIEX) or TraceFinder (Thermo Scientific) to integrate chromatographic peaks of known identity and data were inspected to

ensure quality signal integration. Identification was conducted by matching measured retention times and masses to a database of > 1200 characterized compounds from the in-house Broad library or exact masses to a database of > 40 000 metabolites found in the Human Metabolome Database v3.14.[2] The reliability of the metabolomic platforms in an epidemiologic research setting has previously been demonstrated.[3] A total of 23 known ACs could be finally identified.

Diagnosis criteria for heart failure and atrial fibrillation

The diagnosis criteria for heart failure (HF) was defined according to the 2005 guidelines of the European Society of Cardiology available at the beginning of the endpoint adjudication in the study.[4] Patients were diagnosed as having symptoms and/or signs of HF (more frequently breathlessness or fatigue at rest or during exertion, or ankle swelling) attributable to an objective evidence of cardiac dysfunction at rest (preferably by echocardiography [ECG]). Atrial fibrillation (AF) was initially identified from an annual review of all outpatient and inpatient medical records of each participant or yearly electrocardiograms (ECGs) performed during follow-up examinations in the health care centers. If AF was mentioned anywhere in the medical record or AF was present in the ECG, all relevant documentation was submitted to the Clinical Endpoint Committee. Even though AF was not a primary endpoint in the trial, the Clinical Endpoint Committee reviewed all medical charts and ECGs from potential AF cases and made a final decision about the presence or absence of AF. A diagnosis of AF was made only if both AF was present in an ECG tracing and an explicit medical diagnosis of AF was made

by a physician. AF events associated with myocardial infarction (MI) or cardiac surgery (usually transient) were not included.

Statistical analysis

Since branched-chain amino acids are related to short-chained ACs, we included them as covariates in additional models using individual ACs as continuous variables (per standard deviation [SD]). For HF, we additionally adjusted for n-terminal pro-brain natriuretic peptide (type B) (NT-proBNP) in a subsample with available values for this biomarker. We used < 900 pg/mL as cutoff value for the 80th percentile of NT-proBNP, considered a threshold risk for cardiovascular (CVD) mortality.[5]

We created weighted metabolite scores combining all ACs, short-chain ACs (C2-C7), medium-chain ACs (C8-C14) and long-chain ACs (C16-C26) with the respective individual coefficients from the fitted multivariable logistic regression models. We applied the leave-one-out cross-validation approach to obtain unbiased estimates of these models and to avoid overfitting when creating the score. Briefly, in each run, logistic regression models were applied to the all-but-one sample (ie, the training data set), and the regression coefficient obtained was the weight applied to the remaining 1 sample (ie, the testing data set) to calculate the score. For each AC score (short-chain, medium-chain, long-chain and total), we adjusted for the same variables as for analysis of individual metabolites. In another model we mutually adjusted for short, medium, and long AC scores.

We assessed the potential effect modification of the intervention (MedDiet groups vs control group), obesity (BMI ≥ 25), and type 2 diabetes (T2D). We calculated the product term

between these variables and the AC scores (continuous and quartiles). We used the likelihood ratio test to compare the multivariable models with and without the product term and we obtained the *P* value for multiplicative interaction. We also calculated the relative excess risk due to interaction to estimate additive interaction. We ran joint analyses to observe changes in HF or AF risk according to the combination of AC levels and intervention group, obesity, and T2D status. In addition, we explored the potential effect modification by sex and age group (≤ 70 vs > 70) in the association between AC scores (short-chain, medium-chain, long-chain) and HF or AF risk.

We repeated the above analysis with a composite outcome of participants with HF and AF. We ran multinomial logistic regression models for an outcome with 4 possible categories (no disease, only HF, only AF, both HF, and AF). We adjusted for the same previously described covariates and variables used for matching (age, sex, and recruitment center).

Figures were created with the command coefplot for Stata and with Python v. 3.7.8.

REFERENCES

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5. Fitó M, Estruch R, Salas-Salvadó J, et al. Effect of the Mediterranean diet on heart failure biomarkers: a randomized sample from the PREDIMED trial. *Eur J Heart Fail.* 2014;16:543-550.

Table 1 of the supplementary data

List of carnitines identified in the HILIC-positive method

Metabolite	HMDB_ID	HMDB ID certainty	HMDB name	m/z	RT
carnitine	HMDB00062	1	L-Carnitine	162.1127	8.82
C2 carnitine	HMDB00201	1	L-Acetylcarnitine	204.1233	8.67
C3 carnitine	HMDB00824	1	Propionylcarnitine	218.1390	8.32
C3-DC-CH3 carnitine	HMDB13133	1	Methylmalonylcarnitine	262.1287	8.55
C4 carnitine	HMDB02013	1	Butyrylcarnitine	232.1546	8.00
C4-OH carnitine	HMDB13127	1	Hydroxybutyrylcarnitine	248.1494	8.85
C5 carnitine	HMDB00688	1	Isovalerylcarnitine	246.1702	7.77
C5:1 carnitine	HMDB02366	1	Tiglylcarnitine	244.1546	7.86
C5-DC carnitine	HMDB13130	1	Glutarylcarnitine	276.1443	8.31
C6 carnitine	HMDB00705	1	Hexanoylcarnitine	260.1858	7.52
C7 carnitine	HMDB13238	1	Heptanoylcarnitine	274.2013	7.41
C8 carnitine	HMDB00791	1	L-Octanoylcarnitine	288.2171	7.30
C9 carnitine	HMDB13288	1	Nonanoylcarnitine	302.2325	7.16
C10 carnitine	HMDB00651	1	Decanoylcarnitine	316.2481	7.10
C12 carnitine	HMDB02250	1	Dodecanoylcarnitine	344.2794	6.92
C12:1 carnitine	HMDB13326	2	trans-2-Dodecenoylcarnitine	342.2638	6.94
C14 carnitine	HMDB05066	1	Tetradecanoylcarnitine	372.3110	6.78
C14:1 carnitine	HMDB02014	2	cis-5-Tetradecenoylcarnitine	370.2952	6.77
C14:2 carnitine	HMDB13331	2	3, 5-Tetradecadiencarnitine	368.2795	6.84
C16 carnitine	HMDB00222	1	L-Palmitoylcarnitine	400.3420	6.66
C18 carnitine	HMDB00848	1	Stearoylcarnitine	428.3733	6.55
C18:1 carnitine	HMDB05065	2	Oleoylcarnitine	426.3578	6.57
C18:2 carnitine	HMDB06469	2	Linoleylcarnitine	424.3421	6.63
C26 carnitine	HMDB06347	1	Hexacosanoylcarnitine	540.5002	6.24

HILIC, hydrophilic interaction liquid chromatography; HMDB, Human Metabolome Database.

*1 = compound identified in our analysis at the Broad Institute and matched in HMDB; 2 = representative ID.

Table 2 of the supplementary data

β coefficients* (95% confidence interval) of acylcarnitine scores (SD) and cardiovascular risk factors (SD)

	No.	Short-chain AC	Medium-chain AC	Long-chain AC
DBP	1450	0.04 (−0.01 to 0.09)	**0.05 (0.01 to 0.09)**	0.02 (−0.02 to 0.07)
SBP	1450	0.01 (−0.05 to 0.06)	−0.01 (−0.08 to 0.06)	**−0.06 (−0.10 to −0.01)**
Total cholesterol	1237	−0.03 (−0.08 to 0.02)	0.02 (−0.04 to 0.08)	−0.04 (−0.09 to 0.01)
Fasting glucose	1235	**0.10 (0.01 to 0.18)**	0.09 (−0.01 to 0.19)	0.01 (−0.12 to 0.14)
TG	1228	0.05 (−0.01 to 0.10)	**0.12 (0.08 to 0.16)**	−0.03 (−0.08 to 0.02)
HDL-cholesterol	1222	0.00 (−0.06 to 0.06)	−0.04 (−0.12 to 0.05)	−0.05 (−0.13 to 0.03)
LDL-cholesterol	1146	−0.06 (0.14 to 0.02)	−0.01 (−0.05 to 0.03)	−0.03 (−0.10 to 0.03)

DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglycerides; HDL, high-density lipoprotein; LDL, low density lipoprotein.

*Adjusted for sex, age, intervention group (MedDiet + EVOO or MedDiet + nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), prevalent chronic diseases (dyslipidemia, hypertension, and type 2 diabetes), and medication use (angiotensin converting-enzyme inhibitors, diuretics, other antihypertensive treatments, statins and other lipid-lowering agents, insulin, oral hypoglycemic agents, and antiplatelet therapy).

Table 3 of supplementary data

Association between baseline levels of individual acylcarnitines and incident HF in a nested case-control study[a] of the PREDIMED trial

	Crude OR per 1 SD increment[b] (95%CI)	**FDR-corrected *P* value[c]**	**Crude OR (95%CI)**			
			Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*
C2 carnitine	1.18 (1.01-1.37)	.109	1.00 (ref)	1.16 (0.76-1.79)	1.22 (0.78-1.90)	1.51 (.99-2.32)
C3 carnitine	0.97 (0.84-1.13)	.804	1.00 (ref)	1.31 (0.88-1.95)	1.23 0(.81-1.89)	0.99 (0.65-1.52)
C3-DC-CH3 carnitine	1.13 (0.97-1.32)	.228	1.00 (ref)	0.85 (0.56-1.30)	1.29 (0.86-1.95)	1.14 (0.75-1.74)
C4 carnitine	1.06 (0.91-1.24)	.624	1.00 (ref)	1.77 (1.16-2.71)	1.54 (1.00-2.36)	1.13 (0.72-1.77)
C4-OH carnitine	1.29 (1.11-1.51)	.022	1.00 (ref)	0.77 (0.50-1.19)	1.26 (0.82-1.94)	1.58 (1.05-2.40)
C5 carnitine	1.03 (0.88-1.20)	.804	1.00 (ref)	0.93 (0.61-1.41)	1.28 (0.86-1.91)	1.20 (0.77-1.88)
C5:1 carnitine	1.14 (0.99-1.31)	.189	1.00 (ref)	0.97 (0.63-1.48)	1.38 (0.91-2.11)	1.40 (0.92-2.11)
C5-DC carnitine	0.98 (0.85-1.12)	.804	1.00 (ref)	1.01 (0.68-1.49)	1.00 (0.67-1.49)	0.85 (0.57-1.28)
C6 carnitine	1.13 (0.97-1.32)	.228	1.00 (ref)	0.96 (0.63-1.47)	1.24 (0.82-1.88)	1.34 (0.88-2.04)
C7 carnitine	1.23 (1.05-1.44)	.051	1.00 (ref)	1.29 (0.84-2.00)	1.65 (1.06-2.56)	1.75 (1.12-2.72)
C8 carnitine	1.00 (0.85-1.16)	.954	1.00 (ref)	1.08 (0.73-1.60)	1.44 (0.95-2.18)	1.01 (0.65-1.56)
C9 carnitine	0.98 (0.85-1.13)	.804	1.00 (ref)	0.73 (0.49-1.10)	0.96 (0.64-1.45)	0.92 (0.62-1.37)
C10 carnitine	0.94 (0.81-1.10)	.624	1.00 (ref)	1.00 (0.67-1.47)	1.31 (0.87-1.96)	0.88 (0.57-1.36)
C12 carnitine	1.06 (0.91-1.23)	.624	1.00 (ref)	0.94 (0.63-1.41)	1.19 (0.80-1.77)	1.05 (0.68-1.60)
C12:1 carnitine	1.07 (0.92-1.25)	.624	1.00 (ref)	1.31 (0.87-1.97)	1.18 (0.77-1.81)	1.40 (0.89-2.20)
C14 carnitine	1.24 (1.06-1.44)	.043	1.00 (ref)	0.87 (0.56-1.34)	1.32 (0.87-2.01)	1.65 (1.09-2.50)

C14:1 carnitine	1.06 (0.91-1.24)	.624	1.00 (ref)	1.16 (0.79-1.72)	0.99 (0.65-1.51)	1.18 (0.77-1.81)
C14:2 carnitine	1.17 (1.00-1.37)	.135	1.00 (ref)	1.26 (0.83-1.92)	1.37 (0.90-2.08)	1.71 (1.10-2.64)
C16 carnitine	1.27 (1.09-1.48)	.023	1.00 (ref)	1.44 (0.94-2.20)	1.57 (1.02-2.41)	1.87 (1.21-2.89)
C18 carnitine	1.20 (1.04-1.39)	.059	1.00 (ref)	1.52 (0.99-2.32)	1.43 (0.92-2.21)	1.71 (1.11-2.62)
C18:1 carnitine	1.10 (0.95-1.29)	.109	1.00 (ref)	1.22 (0.81-1.84)	0.98 (0.64-1.49)	1.22 (0.79-1.89)
C18:2 carnitine	1.19 (1.02-1.39)	.804	1.00 (ref)	1.41 (0.92-2.15)	1.20 (0.77-1.89)	1.85 (1.20-2.85)
C26 carnitine	1.14 (0.99-1.32)	.228	1.00 (ref)	1.11 (0.73-1.68)	1.35 (0.89-2.03)	1.36 (0.91-2.05)

	Adjusted OR per 1 SD increment[b] (95%CI)	**FDR-corrected *P* value[c]**	**Multivariable model OR (95%CI)**			
			Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*
C2 carnitine	1.20 (1.02-1.42)	.085	1.00 (ref)	1.20 (0.75-1.90)	1.15 (0.72-1.84)	1.67 (1.05-2.65)
C3 carnitine	0.91 (0.77-1.08)	.397	1.00 (ref)	1.20 (0.78-1.85)	1.20 (0.75-1.90)	0.87 (0.55-1.38)
C3-DC-CH3 carnitine	1.08 (0.91-1.27)	.485	1.00 (ref)	0.88 (0.56-1.39)	1.26 (0.82-1.95)	1.04 (0.66-1.64)
C4 carnitine	1.03 (0.87-1.23)	.790	1.00 (ref)	1.85 (1.17-2.93)	1.39 (0.88-2.20)	1.23 (0.75-2.01)
C4-OH carnitine	1.21 (1.02-1.45)	.085	1.00 (ref)	0.63 (0.39-1.02)	1.06 (0.66-1.70)	1.31 (0.83-2.07)
C5 carnitine	0.88 (0.74-1.06)	.336	1.00 (ref)	0.81 (0.51-1.28)	1.13 (0.73-1.74)	0.87 (0.54-1.42)
C5:1 carnitine	1.11 (0.94-1.30)	.351	1.00 (ref)	1.01 (0.64-1.61)	1.50 (0.95-2.36)	1.46 (0.93-2.28)
C5-DC carnitine	0.98 (0.84-1.14)	.820	1.00 (ref)	1.09 (0.72-1.66)	1.05 (0.68-1.61)	0.86 (0.56-1.33)
C6 carnitine	1.14 (0.96-1.35)	.276	1.00 (ref)	1.07 (0.68-1.70)	1.27 (0.81-2.00)	1.50 (0.95-2.37)
C7 carnitine	1.22 (1.03-1.45)	.075	1.00 (ref)	1.57 (0.97-2.52)	1.97 (1.22-3.19)	1.77 (1.10-2.85)
C8 carnitine	1.03 (0.87-1.22)	.790	1.00 (ref)	1.24 (0.81-1.91)	1.67 (1.05-2.64)	1.20 (0.75-1.94)
C9 carnitine	0.95 (0.81-1.11)	.592	1.00 (ref)	0.75 (0.49-1.15)	1.06 (0.68-1.64)	0.87 (0.57-1.34)

C10 carnitine	0.99 (0.83-1.17)	.887	1.00 (ref)	1.19 (0.77-1.83)	1.48 (0.95-2.31)	1.07 (0.66-1.72)
C12 carnitine	1.10 (0.93-1.30)	.351	1.00 (ref)	0.97 (0.63-1.50)	1.26 (0.82-1.93)	1.24 (0.78-1.96)
C12:1 carnitine	1.11 (0.93-1.31)	.351	1.00 (ref)	1.45 (0.93-2.25)	1.21 (0.76-1.93)	1.64 (1.00-2.69)
C14 carnitine	1.22 (1.04-1.44)	.057	1.00 (ref)	0.89 (0.56-1.42)	1.37 (0.87-2.14)	1.66 (1.07-2.59)
C14:1 carnitine	1.12 (0.95-1.33)	.336	1.00 (ref)	1.25 (0.82-1.92)	0.95 (0.60-1.50)	1.41 (0.89-2.25)
C14:2 carnitine	1.25 (1.05-1.48)	.053	1.00 (ref)	1.51 (0.95-2.40)	1.68 (1.06-2.66)	1.96 (1.23-3.15)
C16 carnitine	1.31 (1.11-1.55)	.017	1.00 (ref)	1.39 (0.88-2.20)	1.49 (0.93-2.39)	1.95 (1.23-3.11)
C18 carnitine	1.36 (1.15-1.61)	.006	1.00 (ref)	1.48 (0.93-2.35)	1.51 (0.94-2.41)	2.03 (1.28-3.23)
C18:1 carnitine	1.20 (1.01-1.42)	.088	1.00 (ref)	1.17 (0.75-1.82)	0.97 (0.61-1.52)	1.43 (0.89-2.29)
C18:2 carnitine	1.28 (1.08-1.53)	.038	1.00 (ref)	1.46 (0.92-2.30)	1.24 (0.76-2.03)	2.14 (1.34-3.42)
C26 carnitine	1.23 (1.05-1.45)	.053	1.00 (ref)	1.12 (0.72-1.76)	1.47 (0.93-2.31)	1.55 (1.00-2.40)

[a] Cases and controls matched by sex, age, and recruitment center.

[b] Values were normalized and scaled to multiples of 1 SD using the rank-based inverse normal transformation.

[c] False discovery rate-corrected *P* value (Simes's method).

[d] Adjusted for intervention group (MedDiet + EVOO or MedDiet + nuts), body mass index (kg/m^2), smoking (never current former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), prevalent chronic diseases (dyslipidemia, hypertension, and diabetes), and medication use (angiotensin-converting enzyme inhibitors, diuretics, other antihypertensive treatments, statins and other lipid-lowering agents, insulin, oral hypoglycemic agents, and antiplatelet therapy).

Table 4 of the supplementary data

Association between naseline combined scores of plasma acylcarnitine levels and incident HF or AF in nested case-control studies[a] of the PREDIMED trial

	Multivariable[b] adjusted OR (95%CI)						
	Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*	*P for trend (quartiles)*	*per 1 SD increment[c]*	*P value (per SD)*
Heart failure[1]							
Short-chain-AC	1.00 (ref)	1.10 (0.67-1.80)	1.54 (0.96-2.47)	1.60 (1.00-2.57)	.024	1.15 (0.98-1.37)	.087
Medium-chain-AC	1.00 (ref)	1.34 (0.82-2.21)	0.99 (0.60-1.63)	1.87 (1.19-2.96)	.011	1.28 (1.09-1.51)	.003
Long-chain-AC	1.00 (ref)	1.45 (0.90-2.34)	1.72 (1.09-2.470	1.66 (1.07-2.60)	022	1.21 (1.04-1.42)	.014
Total AC	1.00 (ref)	1.32 (0.78-2.21)	1.42 (0.87-2.30)	2.08 (1.28-3.40)	.002	1.34 (1.14-1.58)	< .001
Atrial fibrillation[1]							
Short-chain-AC	1.00 (ref)	1.05 (0.75-1.47)	1.03 (0.73-1.46)	1.15 (0.82-1.62)	.441	1.06 (0.94-1.20)	.362
Medium-chain-AC	1.00 (ref)	1.21 (0.84-1.75)	1.08 (0.76-1.55)	1.42 (1.01-1.99)	.063	1.10 (0.97-1.24)	.133
Long-chain-AC	1.00 (ref)	0.97 (0.66-1.42)	1.41 (0.99-2.00)	1.70 (1.20-2.42)	.001	1.20 (1.06-1.36)	.005
Total AC	1.00 (ref)	1.29 (0.91-1.84)	1.17 (0.82-1.68)	1.46 (1.04-2.05)	.051	1.14 (1.01-1.29)	.035
HF or/and AF							
Short-chain-AC	1.00 (ref)	0.91 (0.65-1.27)	1.18 (0.88-1.59)	1.37 (1.02-1.84)	.461	1.11 (1.00-1.22)	.046

Medium-chain-AC	1.00 (ref)	1.00 (0.75-1.35)	1.03 (0.69-1.55)	1.37 (1.15-1.63)	.210	1.17 (1.07-1.28)	< .001
Long-chain-AC	1.00 (ref)	0.96 (0.63-1.48)	1.34 (1.16-1.54)	1.74 (1.32-2.29)	.016	1.24 (1.10-1.40)	< .001
Total AC	1.00 (ref)	1.16 (0.94-1.43)	1.20 (0.93-1.57)	1.63 (1.26-2.11)	.050	1.25 (1.13-1.40)	< .001

AC, acylcarnitine; AF, atrial fibrillation; HF, heart failure.

[a] Cases and controls matched by sex, age, and recruitment center.

[b] Adjusted for intervention group (MedDiet+EVOO. MedDiet+nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), prevalent chronic diseases (dyslipidemia, hypertension, and diabetes), and medication use (angiotensin converting-enzyme inhibitors, diuretics, other antihypertensive treatments, statins and other lipid-lowering agents, insulin, oral hypoglycemic agents, and antiplatelet therapy), and additionally for age, sex, and recruitment center for the total outcome HF + AF.

[c] Values were normalized and scaled to multiples of 1 SD using the rank-based inverse normal transformation and a weighted sum of ACs was then calculated.

Table 5 of the supplementary data

Association between baseline combined scores of plasma acylcarnitine levels and incident heart failure or AF stratified by sex and age group in nested case-control studies[a] of the PREDIMED trial

	Multivariable[b] adjusted OR (95%CI)					
	Sex			**Age group**		
	Men	*Women*	*P for interaction*	*≤70 y*	*> 70 y*	*P for interaction*
Heart failure[a]						
Short-chain-AC	1.01 (0.78-1.31)	1.27 (1.03-1.59)	.083	1.09 (0.86-1.38)	1.22 (0.97-1.54)	.538
Medium-chain-AC	1.39 (1.07-1.81)	1.40 (1.11-1.76)	.995	1.47 (1.14-1.91)	1.39 (1.01-1.75)	.798
Long-chain-AC	1.13 (0.88-1.45)	1.29 (1.04-1.61)	.586	1.13-(0.88-1.44)	1.32 (1.06-1.66)	.340
Atrial fibrillation[*]						
Short-chain-AC	1.06 (0.89-1.26)	1.08 (0.90-1.31)	.967	0.97 (0.83-1.14)	1.24 (0.99-1.55)	.102
Medium-chain-AC	1.20 (0.99-1.43)	1.15 (0.95-1.38)	.569	1.11 (0.94-1.30)	1.28 (1.03-1.60)	.295
Long-chain-AC	1.15 (0.95-1.39)	1.32 (1.09-1.59)	.377	1.19 (1.00-1.41)	1.27 (1.02-1.58)	.582

95%CI, 95% confidence interval; AC, acylcarnitine.

[*] Cases and controls matched by sex, age, and recruitment center.

Table 6 of the supplementary dat

Association between baseline levels of individual acylcarnitines and incident AF in a nested case-control study[a] of the PREDIMED trial

	Crude OR per 1 SD increment[b] (95%CI)	**FDR-corrected *P* value[c]**	**Crude OR (95%CI)**			
			Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*
C2 carnitine	1.16 (1.03-1.30)	.036	1.00 (ref)	1.07 (0.77-1.51)	1.47 (1.05-2.06)	1.39 (1.00-1.94)
C3 carnitine	1.08 (0.96-1.21)	.273	1.00 (ref)	1.17 (0.84-1.62)	1.1 (.78-1.53)	1.52 (1.09-2.12)
C3-DC-CH3 carnitine	1.07 (0.95-1.21)	.374	1.00 (ref)	0.95 (0.69-1.32)	1.16 (0.83-1.61)	1.12 (0.80-1.57)
C4 carnitine	1.00 (0.89-1.12)	.992	1.00 (ref)	1.15 (0.83-1.60)	1.23 (0.89-1.69)	0.90 (0.64-1.27)
C4-OH carnitine	1.09 (0.97-1.23)	.254	1.00 (ref)	0.92 (0.66-1.28)	0.96 (0.68-1.35)	1.23 (0.89-1.70)
C5 carnitine	1.02 (0.91-1.16)	.800	1.00 (ref)	1.09 (0.78-1.52)	1.08 (0.77-1.53)	1.00 (0.700-1.42)
C5:1 carnitine	1.07 (0.95-1.21)	.313	1.00 (ref)	0.97 (0.69-1.35)	0.78 (0.56-1.10)	1.25 (0.91-1.71)
C5-DC carnitine	1.00 (0.89-1.13)	.992	1.00 (ref)	1.01 (0.72-1.40)	0.84 (0.60-1.17)	0.98 (0.70-1.36)
C6 carnitine	1.17 (1.05-1.32)	.027	1.00 (ref)	1.02 (0.73-1.43)	1.08 (0.77-1.49)	1.42 (1.03-1.95)
C7 carnitine	1.11 (0.99-1.25)	.158	1.00 (ref)	1.15 (0.82-1.60)	1.10 (0.78-1.55)	1.35 (0.97-1.88)
C8 carnitine	1.10 (0.98-1.23)	.196	1.00 (ref)	1.14 (0.82-1.59)	1.29 (0.92-1.82)	1.22 (0.87-1.70)
C9 carnitine	1.00 (0.89-1.12)	.992	1.00 (ref)	0.98 (0.70-1.39)	1.03 (0.73-1.44)	0.99 (0.71-1.38)
C10 carnitine	1.10 (0.98-1.23)	.194	1.00 (ref)	0.90 (0.65-1.24)	1.13 (0.82-1.57)	1.21 (0.88-1.66)
C12 carnitine	1.14 (1.02-1.29)	.058	1.00 (ref)	1.08 (0.76-1.53)	1.10 (0.78-1.56)	1.44 (1.04-1.99)
C12:1 carnitine	1.19 (1.06-1.34)	.016	1.00 (ref)	1.08 (0.76-1.54)	1.34 (0.95-1.89)	1.53 (1.10-2.13)
C14 carnitine	1.19 (1.06-1.34)	.015	1.00 (ref)	1.25 (0.89-1.76)	1.32 (0.92-1.91)	1.66 (1.20-2.31)
C14:1 carnitine	1.12 (1.00-1.26)	.108	1.00 (ref)	0.97 (0.70-1.34)	1.05 (0.75-1.47)	1.40 (1.02-1.92)
C14:2 carnitine	1.16 (1.03-1.30)	.044	1.00 (ref)	1.16 (0.82-1.63)	1.20 (0.85-1.70)	1.60 (1.15-2.24)

C16 carnitine	1.26 (1.12-1.43)	.005	1.00 (ref)	1.14 (0.80-1.62)	1.46 (1.04-2.06)	1.83 (1.30-2.57)
C18 carnitine	1.23 (1.09-1.38)	.010	1.00 (ref)	1.07 (0.75-1.53)	1.29 (0.91-1.82)	1.71 (1.22-2.40)
C18:1 carnitine	1.17 (1.04-1.32)	.036	1.00 (ref)	1.05 (0.75-1.47)	0.81 (0.56-1.17)	1.50 (1.08-2.09)
C18:2 carnitine	1.21 (1.07-1.37)	.015	1.00 (ref)	1.22 (0.87-1.71)	1.39 (0.98-1.97)	1.74 (1.23-2.46)
C26 carnitine	1.06 (0.94-1.19)	.405	1.00 (ref)	1.18 (0.85-1.66)	1.01 (0.72-1.43)	1.16 (0.83-1.63)

	Ajusted OR per 1 SD increment[b] (95%CI)	**FDR-adjusted P value[c]**	**Multivariable[d] adjusted OR (95%CI)**			
			Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*
C2 carnitine	1.13 (1.00-1.28)	.106	1.00 (ref)	1.05 (0.74-1.49)	1.46 (1.03-2.07)	1.33 (0.95-1.88)
C3 carnitine	1.03 (0.92-1.17)	.753	1.00 (ref)	1.08 (0.77-1.53)	0.97 (0.68-1.37)	1.29 (0.91-1.83)
C3-DC-CH3 carnitine	0.99 (0.87-1.13)	.916	1.00 (ref)	0.90 (0.64-1.27)	1.01 (0.72-1.43)	0.98 (0.68-1.39)
C4 carnitine	0.98 (0.87-1.11)	.853	1.00 (ref)	1.15 (0.82-1.61)	1.15 (0.82-1.62)	0.84 (0.59-1.21)
C4-OH carnitine	1.07 (0.94-1.21)	.456	1.00 (ref)	0.91 (0.65-1.28)	0.95 (0.67-1.36)	1.17 (0.82-1.67)
C5 carnitine	0.99 (0.87-1.12)	.913	1.00 (ref)	1.03 (0.73-1.46)	0.94 (0.66-1.35)	0.89 (0.61-1.29)
C5:1 carnitine	1.07 (0.95-1.22)	.400	1.00 (ref)	0.96 (0.68-1.37)	0.78 (0.54-1.12)	1.19 (0.85-1.66)
C5-DC carnitine	0.97 (0.86-1.10)	.818	1.00 (ref)	0.89 (0.63-1.26)	0.73 (0.51-1.04)	0.85 (0.60-1.21)
C6 carnitine	1.14 (1.01-1.29)	.093	1.00 (ref)	1.00 (0.70-1.42)	1.07 (0.76-1.51)	1.33 (0.96-1.86)
C7 carnitine	1.09 (0.96-1.23)	.281	1.00 (ref)	1.18 (0.83-1.67)	0.99 (0.69-1.43)	1.29 (0.91-1.83)
C8 carnitine	1.10 (0.97-1.24)	.232	1.00 (ref)	1.17 (0.83-1.64)	1.29 (0.90-1.84)	1.25 (0.88-1.77)
C9 carnitine	1.01 (0.89-1.14)	.916	1.00 (ref)	1.00 (0.70-1.42)	0.99 (0.69-1.41)	1.00 (0.71-1.41)
C10 carnitine	1.10 (0.98-1.24)	.216	1.00 (ref)	0.90 (0.65-1.26)	1.16 (0.82-1.63)	1.22 (0.87-1.70)
C12 carnitine	1.13 (1.00-1.27)	.126	1.00 (ref)	1.12 (0.79-1.61)	1.13 (0.79-1.62)	1.45 (1.04-2.03)
C12:1 carnitine	1.15 (1.02-1.31)	.093	1.00 (ref)	1.16 (0.81-1.68)	1.34 (0.94-1.92)	1.44 (1.02-2.03)
C14 carnitine	1.15 (1.02-1.29)	.093	1.00 (ref)	1.22 (0.85-1.74)	1.19 (0.82-1.75)	1.49 (1.06-2.09)

C14:1 carnitine	1.11 (0.98-1.25)	.204	1.00 (ref)	0.93 (0.66-1.31)	1.06 (0.75-1.51)	1.34 (0.96-1.86)	
C14:2 carnitine	1.15 (1.01-1.30)	.093	1.00 (ref)	1.16 (0.81-1.67)	1.14 (0.80-1.64)	1.60 (1.12-2.26)	
C16 carnitine	1.21 (1.07-1.38)	.029	1.00 (ref)	1.08 (0.75-1.56)	1.40 (0.98-2.01)	1.68 (1.17-2.41)	
C18 carnitine	1.25 (1.11-1.42)	.009	1.00 (ref)	1.04 (0.72-1.51)	1.35 (0.94-1.93)	1.78 (1.25-2.54)	
C18:1 carnitine	1.16 (1.02-1.31)	.093	1.00 (ref)	1.04 (0.73-1.47)	0.79 (0.53-1.16)	1.44 (1.02-2.03)	
C18:2 carnitine	1.21 (1.06-1.38)	.029	1.00 (ref)	1.20 (0.84-1.71)	1.34 (0.93-1.94)	1.70 (1.18-2.44)	
C26 carnitine	1.08 (0.95-1.22)	.337	1.00 (ref)	1.21 (0.85-1.72)	1.03 (0.72-1.49)	1.21 (0.85-1.72)	

95%CI, 95% confidence interval; AC, acylcarnitine; OR, odds ratio.

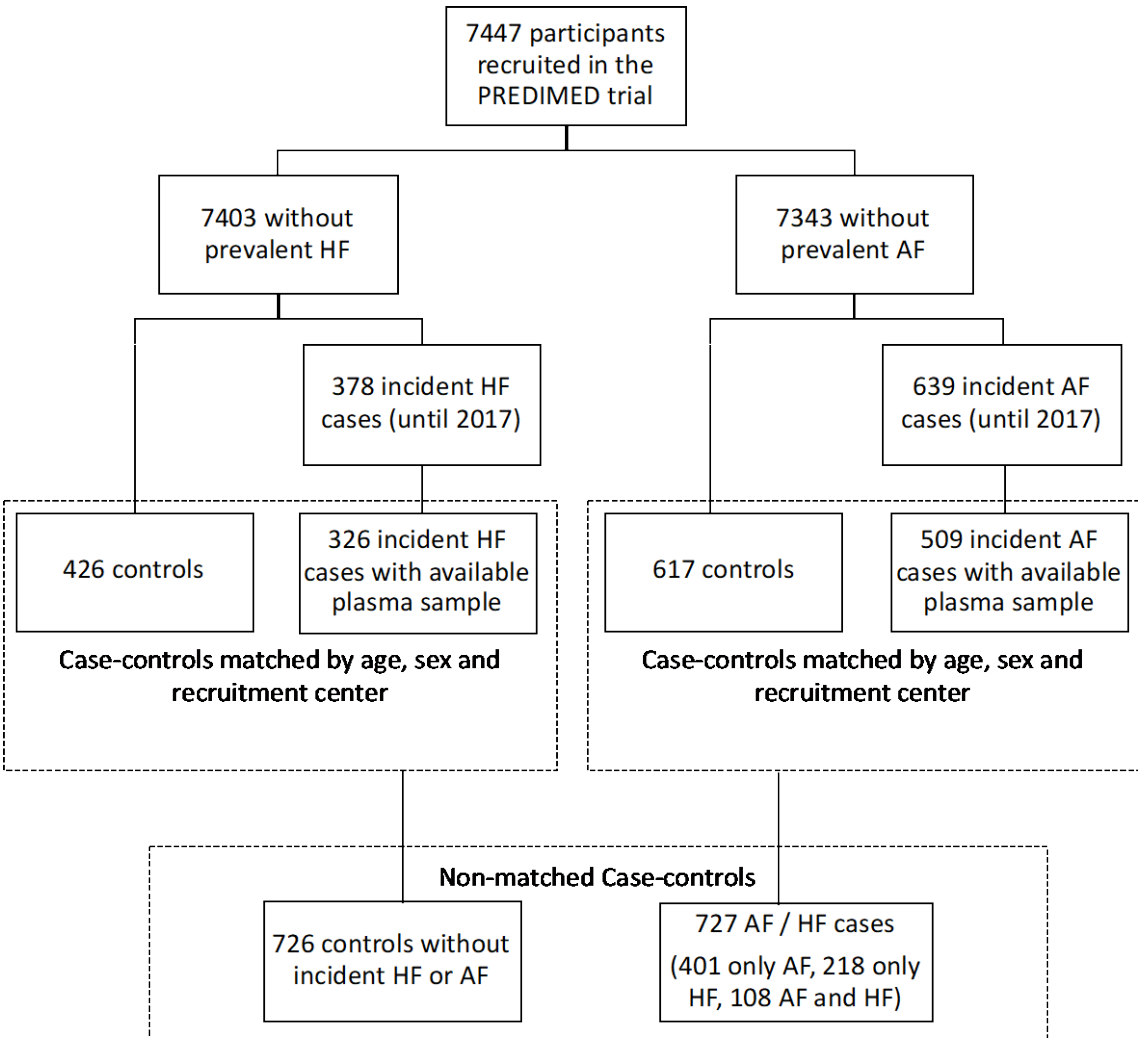
[a] Cases and controls matched by sex, age, and recruitment center.

[b] Values were normalized and scaled to multiples of 1 SD using the rank-based inverse normal transformation.

[c] False discovery rate-corrected *P* value (Simes's method).

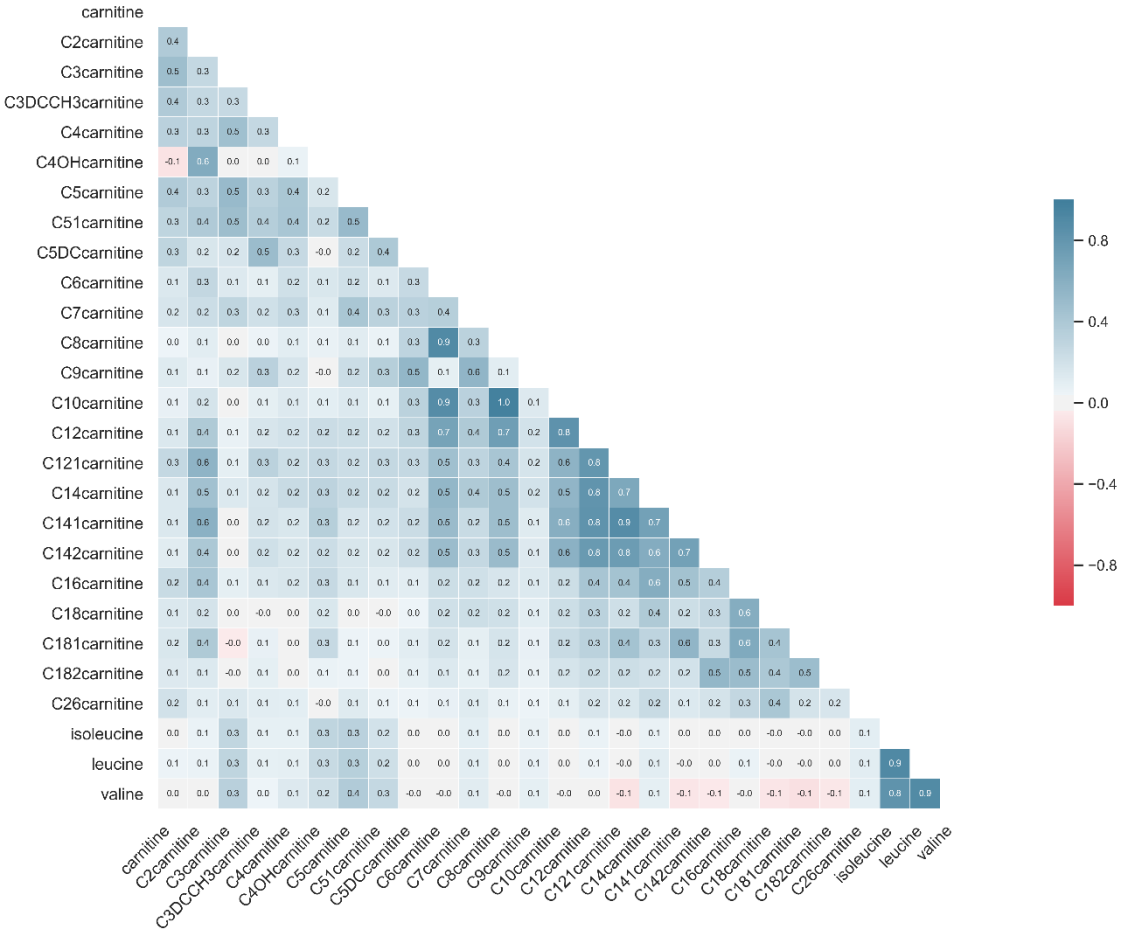
[d]Adjusted for intervention group (MedDiet + EVOO or MedDiet + nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), prevalent chronic diseases (dyslipidemia, hypertension, and diabetes), and medication use (angiotensin-converting enzyme inhibitors, diuretics, other antihypertensive treatments, statins and other lipid-lowering agents, insulin, oral hypoglycemic agents, and antiplatelet therapy.

Figure 1 of the supplementary data. Flow chart



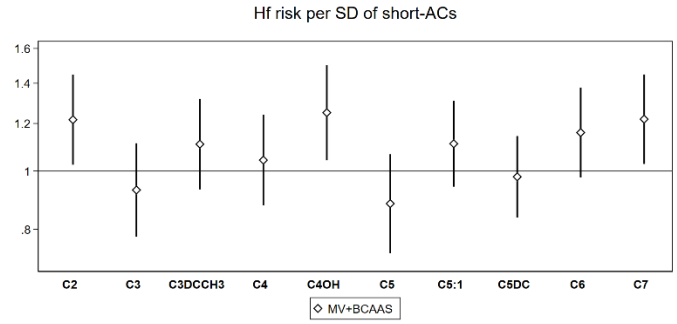
AF, atrial fibrillation; HF, heart failure.

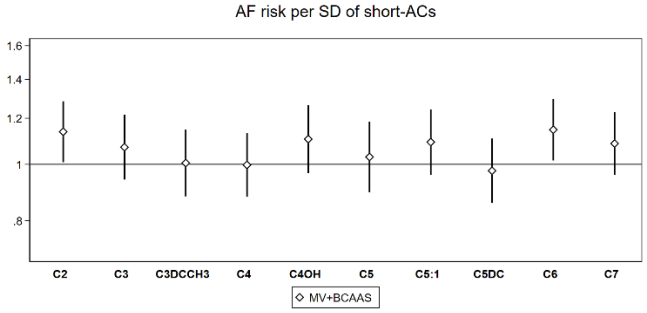
Figure 2 of the supplementary data. Heatmap of the pair correlations for ACs vs BCAAs (N=1453)*

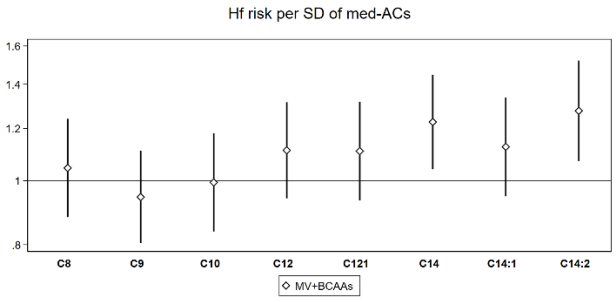


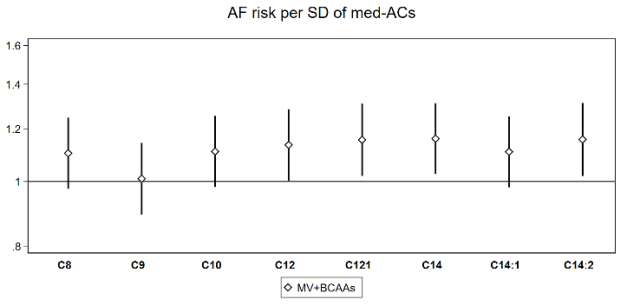
ACs, acylcarnitine; BCAAs, cranched-chain amino acids.

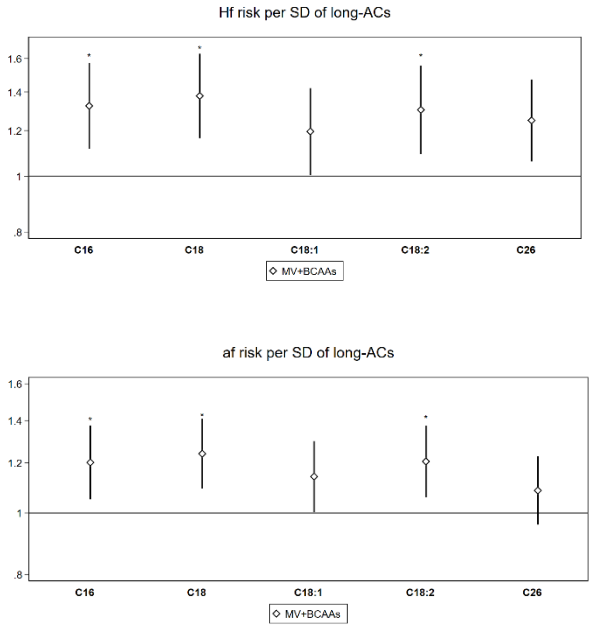
Figure 3 of the supplementary data. Multivariable adjusted odds ratios and 95% confidence intervals between baseline AC levels and incident HF or AF in nested case-control (cases and controls matched by sex, age, and recruitment center) studies additionally adjusted for branched-chain amino acids.









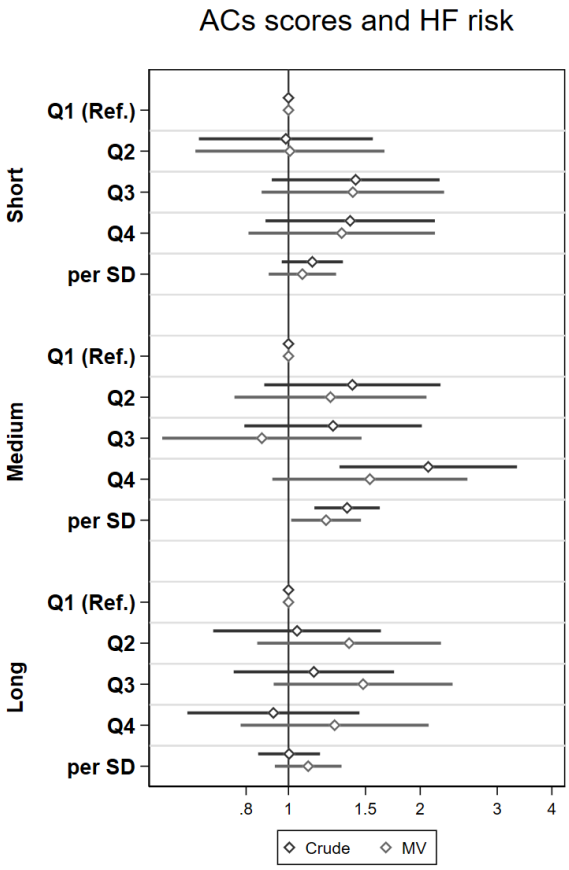


95%CI, 95% confidence interval; AC, acylcarnitine; AF, atrial fibrillation; BCAAs, branched-chain amino acids; EVOO, extra-virgin olive oil; HF, heart failure; MedDiet, Mediterranean diet; MV, multivariable model adjusted for intervention group (MedDiet + EVOO or MedDiet + nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), and prevalent chronic diseases (dyslipidemia, hypertension, and diabetes); RERI, relative excess of risk due to interaction.

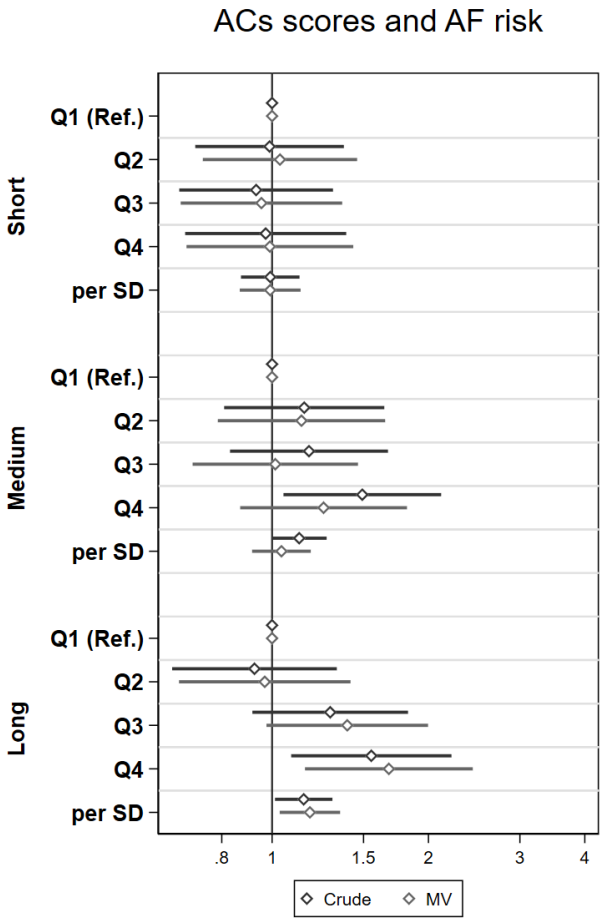
*FDR-corrected value < .05.

Figure 5 of the supplementary data. Association between baseline combined scores[a] of plasma acylcarnitine levels and Incident HF or AF in nested case-control studies[b] of the PREDIMED Trial

A)



B)



AC, acylcarnitine; AF, atrial fibrillation; HF, heart failure.

[a] Weighted sum of normalized values for each metabolite.

[b] Cases and controls matched by sex, age, and recruitment center.

Short-, medium-, and long-chain AC scores were mutually adjusted.

MV1: Multivariable model adjusted for intervention group (MedDiet + EVOO or MedDiet + nuts), body mass index (kg/m^2), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/d), prevalent chronic diseases (dyslipidemia,

hypertension, and diabetes), and medication use (angiotensin-converting enzyme inhibitor inhibitors, diuretics, other antihypertensive treatments, statins and other lipid-lowering agents, insulin, oral hypoglycemic agents, and antiplatelet therapy).