ORIGINAL RESEARCH

Serum Acylcarnitines and Risk of Cardiovascular Death and Acute Myocardial Infarction in Patients With Stable Angina Pectoris

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***Background*—**Excess levels of serum acylcarnitines, which are intermediate products in metabolism, have been observed in metabolic diseases such as type 2 diabetes mellitus. However, it is not known whether acylcarnitines may prospectively predict risk of cardiovascular death or acute myocardial infarction in patients with stable angina pectoris.

***Methods and Results*—**This study included 4164 patients (median age, 62 years; 72% men). Baseline serum acetyl-, octanoyl-, palmitoyl-, propionyl-, and (iso)valerylcarnitine were measured using liquid chromatography/tandem mass spectrometry. Hazard ratios (HRs) and 95% CIs for quartile 4 versus quartile 1 are reported. The multivariable model included age, sex, body mass index, fasting status, current smoking, diabetes mellitus, apolipoprotein A1, apolipoprotein B, creatinine, left ventricular ejection fraction, extent of coronary artery disease, study center, and intervention with folic acid or vitamin B6. During median 10.2 years of follow-up, 10.0% of the patients died of cardiovascular disease and 12.8% suffered a fatal or nonfatal acute myocardial infarction. Higher levels of the even-chained acetyl-, octanoyl-, and palmitoyl-carnitines were significantly associated with elevated risk of cardiovascular death, also after multivariable adjustments (HR [95% CI]: 1.52 [1.12, 2.06]; P=0.007; 1.73 [1.23, 2.44]; P=0.002; and 1.61 [1.18, 2.21]; P=0.003, respectively), whereas their associations with acute myocardial infarction were less consistent.

***Conclusions*—**Among patients with suspected stable angina pectoris, elevated serum even-chained acylcarnitines were associated with increased risk of cardiovascular death and, to a lesser degree with acute myocardial infarction, independent of traditional risk factors.

***Clinical Trial Registration*—**URL: http://www.clinicaltrials.gov. Unique identifier: NCT00354081. **(*J Am Heart Assoc*. 2017;6: e003620. DOI: 10.1161/JAHA.116.003620.)**

Key Words: acylcarnitines • angina pectoris • cardiovascular outcomes • metabolism

Carnitine is a quaternary ammonium compound involved in fatty acid (FA) metabolism, maintaining the balance between free and esterified coenzyme A (CoA), which is crucial for normal cell function.[1] In the human organism, carnitine exists as free active L-carnitine or as esterified forms with a wide variety of chain lengths, collectively referred to as acylcarnitines.

The esterification of carnitine is catalyzed by acyltransferases specific for FAs of various chain lengths. Carnitine palmitoyltransferase (CPT)-I is regarded as a key regulator of mitochondrial β-oxidation of long-chain FAs, shuttling acylcarnitines across the mitochondrial membranes using carnitine-acylcarnitine translocase (CACT). CPT-II, located in the inner mitochondrial membrane, catalyzes the transesterification to intramitochondrial CoA, whereas carnitine acetyltransferase has the ability to reconvert short- and medium-chain acyl-CoAs to acylcarnitines in the mitochondrial matrix.

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Accompanying Tables S1 through S5 and Figures S1, S2 are available at http://jaha.ahajournals.org/content/6/2/e003620/DC1/embed/inline-supplementary-material-1.pdf

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Subsequently, acylcarnitines may leave the mitochondria through CACT and be transported out of the tissue to other destinations or for urinary excretion.[2]

Impaired FA oxidation has been demonstrated in patients with metabolic diseases.[3] In several previous studies, L-carnitine has been utilized as a dietary supplement, proposed to provide beneficial effects under such conditions, but with inconsistent results.[4] Recently, acylcarnitine profiling has evolved as a promising approach to evaluate chronic metabolic diseases like obesity and type 2 diabetes mellitus.[5–7] Fasting plasma long-chain acylcarnitines may be elevated under both conditions, whereas patients with type 2 diabetes mellitus may also have increased levels of short- and medium-chain acylcarnitines. Furthermore, certain metabolic signatures, including increased levels of acylcarnitines, are shown in plasma of patients with preeclampsia[5] and have been associated with elevated risk of cardiovascular events in those already at high risk of cardiovascular disease.[8] This indicates the usefulness of acylcarnitines for prediction of future adverse outcomes, both during pregnancy and later in life.[5,9] Elevated levels of a certain long-chain acylcarnitine have also been linked to risk of cardiovascular death among dialysis patients.[10] Moreover, in recent studies, elevated levels of short- to medium-chain dicarboxylacylcarnitines have been associated with increased risk of death or acute myocardial infarction (AMI) among patients with coronary artery disease (CAD),[11,12] whereas medium- to long-chain acylcarnitines have been associated with incident cardiovascular events in elderly patients, of whom the majority had established cardiovascular disease.[13] However, the associations between specific circulating acylcarnitines, having various chain lengths and properties, and cardiovascular outcomes in patients with stable angina pectoris (SAP) have, to our knowledge, not previously been evaluated. Thus, we aimed to investigate the relation of 5 specific serum acylcarnitines to risk of cardiovascular death and fatal or nonfatal AMI during long-term follow-up of patients referred to coronary angiography because of suspected SAP. Each of the 5 studied acylcarnitines serve as markers of a certain part of the metabolism, with acetyl-, octanoyl-, and palmitoylcarnitine representing short-, medium-, and long-chain even-chained species, whereas propionyl- and (iso)valerylcarnitine represent the odd-chained species often associated with branched chained amino acids.[14]

Methods

Study Population

The current investigation is a prospective observational cohort study among 4164 patients recruited at Haukeland (n=3413) and Stavanger (n=751) University Hospitals in Norway during the period 2000–2004. A total of 61.8% (n=2573) also participated in the Western Norway B-Vitamin Intervention Trial (WENBIT), which tested the effect of B-vitamin treatment upon mortality and cardiovascular disease.[15] All participants provided written informed consent. The study was performed in accord with the principles of the Declaration of Helsinki and was approved by the Regional Committee for Medical Research Ethics, the Norwegian Medicines Agency, and the Norwegian Data Inspectorate.

Assessment of Covariates

Demographic, clinical, and routine laboratory data were obtained by study personnel at both hospitals.[15] Venous serum samples were collected and stored at −80°C until analysis as previously described.[16] Sampling was performed usually 1 to 3 days before coronary angiography in patients recruited at Haukeland University Hospital and immediately after coronary angiography in patients recruited at Stavanger University Hospital. Standard blood laboratory parameters were assessed according to routine protocols at the central laboratories of Haukeland and Stavanger University Hospitals. Reagent kits of type Tina-quant (Roche Diagnostics, Mannheim, Germany) were applied for measuring apolipoprotein A1, apolipoprotein B, and lipoprotein (a) on the Hitachi 917 system (Roche Diagnostics). Plasma cotinine, glycated hemoglobin (HbA1c),[17] serum C-reactive protein (CRP),[18] and acylcarnitines[19,20] were determined as previously described. Precisions for the acylcarnitine assay are between 0.9% and 10.3%,[19] and the −80°C long-term storage conditions ensure stable analytes.[21] The intraclass correlation coefficients for acetyl-, octanoyl-, palmitoyl-, propionyl-, and (iso)valerylcarnitine are 0.63, 0.41, 0.69, 0.62, and 0.68, respectively.

The homeostatic model assessments of β-cell activity, insulin sensitivity, and insulin resistance were calculated among fasting, nondiabetic patients based on serum insulin, which was measured together with serum C-peptide in citrate-based samples by a solid-phase, 2-site chemiluminescent immunometric assay (Immulite 2000 C-Peptide; Siemens Healthcare Diagnostics Inc., Flanders, NJ).

Fasting was defined as not having ingested any food or beverages within 6 hours before blood sampling.[22] Current smokers included self-reported smokers, those reported having quit within the past 4 weeks, and patients with plasma cotinine ≥85 nmol/L.[23] Participants were classified as having diabetes mellitus if they were previously diagnosed, had a fasting baseline serum glucose ≥7.0 or a nonfasting glucose ≥11.1 mmol/L, or had baseline HbA1c ≥6.5%.[24] Estimated glomerular filtration rate (eGFR) was calculated applying the equation by the Chronic Kidney Disease Epidemiology Collaboration.[25] Left ventricular ejection fraction (LVEF) (%)

was determined by ventriculography or echocardiography, and values <50% indicated impaired systolic function. Extent of CAD was graded as nonsignificant (angiographically normal arteries or stenosis with luminal narrowing <50%) or as having single-, double-, or triple vessel disease (0–3).

Endpoints and Follow-up

The primary endpoint was cardiovascular death, which included death causes coded as I00 to I99 or R96 according to International Classification of Disease (ICD)-10. Fatal or nonfatal AMI was evaluated as a secondary endpoint and classified according to the diagnostic criteria of the revised definition of myocardial infarction (MI) from 2000.[26] Procedure-related nonfatal AMI occurring ≤24 hours after coronary angiography, percutaneous coronary intervention (PCI), or coronary artery bypass graft surgery (CABG) were excluded. Information on study outcomes until December 31, 2006 was collected from the Western Norway Cardiovascular Disease Registry and from the Norwegian Cause of Death Registry. Extended follow-up data were obtained from the Cardiovascular Disease in Norway project (www.cvdnor.no),[27] through 2009 (fatal or nonfatal AMI) and 2012 (cardiovascular death).

Statistical Analyses

Medians (25th, 75th percentile) and proportions were reported for baseline continuous and categorical variables, and sex differences were tested using the Mann–Whitney *U* test for continuous variables and the chi-square test for binary variables. Correlation analyses were performed using partial Spearman correlations adjusted for sex.

Participants were ranked into quartiles according to serum acetyl-, octanoyl-, palmitoyl-, propionyl-, and (iso)valerylcarnitine. Associations between acylcarnitine levels and endpoints were estimated by Cox proportional hazard regression comparing quartiles 4 to 1, and linear trends were assessed across quartiles. In addition, hazard ratios (HRs) and 95% CIs were reported per 1-SD increment. Nonlinear relationships were analyzed by generalized additive modeling (GAM), visualizing risk relationships for acylcarnitines on a continuous scale. The basic Cox model included age and sex. Additional covariates in the multivariable model were selected on the basis of clinical relevance and included the following: body mass index (BMI; continuous); fasting (yes or no); current smoker (yes or no); diabetes mellitus (yes or no); apolipoprotein A1 (Apo A-I; continuous); apolipoprotein B (Apo B; continuous); creatinine (continuous); LVEF (continuous); extent of CAD (nonsignificant; single-, double-, or triple-vessel disease); study center (dichotomous); and intervention with folic acid or vitamin B6 (dichotomous). Interaction product terms were added to the Cox model to test for effect modifications of diabetes mellitus (dichotomous), BMI (below and above median), and fasting (dichotomous) with the acylcarnitines (quartiles).

Net reclassification improvement (NRI) was evaluated by adding individual serum acylcarnitines to established cardiovascular disease risk indicators. To be able to account for all endpoints and because no established risk categories exist for subjects with cardiovascular disease, we applied the continuous or category free NRI (>0) method based on logistic regression,[28] containing the same variables as the multivariate Cox model.

Statistical analyses were performed using IBM SPSS Statistics for Windows (version 22; SPSS, Armonk, NY) and R software (version 3.2.1; R Development Core Team, Vienna, Austria). Probability values were 2-sided and in general considered statistically significant when $P<0.05$, with the exception of correlations of which the significance level was set to $P<0.01$.

Results

Baseline Characteristics

Characteristics of the 4164 participants with SAP (72% men) are presented according to sex in Table 1. At baseline, median (25th, 75th percentiles) age was 62.0 (55.0, 70.0) years and BMI was 26.3 (24.2, 29.0) kg/m^2. A total of 1137 (27.3%) patients reported to be fasting at the time of blood sampling. Furthermore, 46.7% had hypertension, 31.7% were current smokers, and 38.5% had diabetes mellitus. Coronary angiography showed that 25.1% had no or nonsignificant CAD, 23.2% had 1-vessel, 22.3% had 2-vessel, and 29.4% had triple-vessel disease. At discharge from the hospital, 80.1% and 72.5% of the participants used statins and β-blockers, respectively. As compared to men, women had lower serum levels of carnitine and its precursors, trimethyllysine and γ-butyrobetaine, and lower levels of the acylcarnitines, palmitoyl-, propionyl-, and (iso)valerylcarnitine. Serum acetyl- and octanoylcarnitine were, however, higher in women compared to men.

Associations Between Acylcarnitines and Selected Baseline Variables

Overall, the acylcarnitines were positively correlated with BMI ($\rho\geq0.05$; $P<0.01$) and plasma glucose ($\rho\geq0.15$; $P<0.001$) and negatively correlated with eGFR ($\rho\leq-0.21$; $P<0.001$; Figure 1). Additionally, age was positively correlated with acetyl-, octanoyl-, and palmitoylcarnitine ($\rho\geq0.19$; $P<0.001$). C-peptide, insulin, β-cell activity, and insulin resistance were positively correlated with both propionyl- and

Table 1. Baseline Characteristics of Participants*

	Total n=4164	Women n=1168	Men n=2996	*P* Value†
Age, y	62.0 (55.0, 70.0)‡	64.0 (56.0, 71.0)	61.0 (54.0, 69.0)	<0.001
BMI, kg/m^2	26.3 (24.2, 29.0)	26.1 (23.3, 29.4)	26.4 (24.4, 28.7)	0.007
Fasting, n (%)§	1137 (27.3)	247 (21.1)	890 (29.7)	<0.001
Coronary history, n (%)				
MI	1680 (40.3)	330 (28.3)	1350 (45.1)	<0.001
PCI	799 (19.2)	157 (13.4)	642 (21.4)	<0.001
CABG surgery	479 (11.5)	85 (7.3)	394 (13.2)	<0.001
Coronary risk factors, n (%)				
Hypertension‖	1946 (46.7)	590 (50.5)	1356 (45.3)	0.003
Current smoker¶	1320 (31.7)	306 (26.2)	1014 (33.8)	<0.001
Diabetes mellitus#	1603 (38.5)	477 (40.8)	1126 (37.6)	0.06
Serum lipids				
TG, mmol/L	1.50 (1.08, 2.14)	1.39 (1.00, 1.91)	1.55 (1.10, 2.24)	<0.001
LDL cholesterol, mmol/L	2.90 (2.40, 3.70)	3.00 (2.50, 3.80)	2.90 (2.30, 3.60)	<0.001
HDL cholesterol, mmol/L	1.20 (1.00, 1.50)	1.40 (1.20, 1.70)	1.20 (1.00, 1.40)	<0.001
Non-HDL cholesterol, mmol/L	3.60 (3.00, 4.40)	3.60 (3.00, 4.40)	3.60 (2.96, 4.40)	0.18
Apo A-I, g/L	1.30 (1.13, 1.48)	1.45 (1.26, 1.62)	1.24 (1.10, 1.41)	<0.001
Apo B, g/L	0.87 (0.73, 1.04)	0.88 (0.74, 1.06)	0.86 (0.72, 1.04)	0.005
Lp(a), g/L	0.30 (0.15, 0.59)	0.31 (0.15, 0.64)	0.29 (0.14, 0.57)	0.02
Glucose homeostasis				
Glucose, mmol/L	5.6 (5.1, 6.6)	5.5 (4.9, 6.4)	5.7 (5.1, 6.7)	<0.001
HbA1c (%)	6.1 (5.4, 6.9)	6.2 (5.5, 7.0)	6.1 (5.3, 6.8)	<0.001
C-peptide, pmol/L**	770 (552, 1027)	713 (514, 982)	783 (552, 1053)	0.03
Insulin, pmol/L**	28.0 (19.7, 69.5)	24.4 (19.7, 71.6)	28.0 (19.7, 69.5)	0.63
Homa2, insulin††				
β-cell activity (%)	54.1 (44.7, 79.7)	55.3 (46.8, 82.0)	53.7 (43.3, 78.6)	0.29
Insulin sensitivity (%)	233 (91.6, 266)	255 (94.7, 268)	218 (91.0, 265)	0.19
Insulin resistance	0.40 (0.40, 1.10)	0.40 (0.40, 1.10)	0.50 (0.40, 1.10)	0.64
Inflammation markers, renal function, and troponin T				
CRP, mg/L	1.78 (0.87, 3.67)	1.79 (0.90, 3.93)	1.77 (0.86, 3.56)	0.10
Creatinine, μmol/L	74.0 (64.7, 84.3)	64.8 (57.3, 73.6)	77.4 (68.7, 86.8)	<0.001
eGFR, mL/min	91.0 (78.0, 99.0)	87.0 (74.0, 96.0)	92.0 (81.0, 100)	<0.001
Troponin T, ng/L	4.0 (3.0, 10.0)	3.0 (3.0, 7.0)	5.0 (3.0, 11.0)	<0.001
Treatment, LVEF, and severity of CAD at baseline angiography, n (%)				
PCI	1374 (33.0)	293 (25.1)	1081 (36.1)	<0.001
CABG surgery	897 (21.5)	163 (14.0)	734 (24.5)	<0.001
LVEF <50%	412 (9.9)	66 (5.7)	346 (11.5)	<0.001
Triple-vessel disease	1224 (29.4)	207 (17.7)	1017 (33.9)	<0.001

Continued

Table 1. Continued

	Total n=4164	Women n=1168	Men n=2996	*P* Value†
Medication at discharge from hospital, n (%)				
Statins	3335 (80.1)	837 (71.7)	2498 (83.4)	<0.001
β-blockers	3018 (72.5)	783 (67.0)	2235 (74.6)	<0.001
ACE inhibitors/ARBs	1330 (31.9)	361 (30.9)	969 (32.3)	0.39
Serum carnitine precursors and esters, μmol/L				
Trimethyllysine	0.85 (0.69, 1.06)	0.74 (0.62, 0.93)	0.89 (0.72, 1.11)	<0.001
γ-Butyrobetaine	1.02 (0.87, 1.19)	0.89 (0.77, 1.01)	1.08 (0.93, 1.24)	<0.001
Free carnitine	39.0 (34.2, 44.0)	37.2 (31.8, 42.2)	39.6 (35.0, 44.6)	<0.001
Acetylcarnitine	5.9 (4.8, 7.5)	6.1 (4.9, 7.7)	5.9 (4.8, 7.4)	0.008
Octanoylcarnitine	0.137 (0.091, 0.201)	0.141 (0.093, 0.214)	0.135 (0.091, 0.195)	0.02
Palmitoylcarnitine	0.082 (0.068, 0.098)	0.078 (0.064, 0.094)	0.083 (0.069, 0.099)	<0.001
Propionylcarnitine	0.43 (0.34, 0.54)	0.40 (0.31, 0.49)	0.45 (0.35, 0.55)	<0.001
(Iso)valerylcarnitine	0.112 (0.085, 0.146)	0.097 (0.074, 0.125)	0.119 (0.091, 0.154)	<0.001

*ACE indicates angiotensin-converting enzyme; Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft surgery; CAD, coronary artery disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein A; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; TG, triglycerides.
†Calculated by using the Mann–Whitney *U* test for continuous variables and chi-square for independence on binary variables.
‡Values are presented as medians (25th, 75th percentiles) or counts (percentages).
§Includes 3989 participants with data on fasting status.
‖Receiving medical treatment for hypertension.
¶Current smoker (self-reported, ex-smoker <1 month, or cotinine level ≥85 nmol/L).
#Diabetes mellitus was defined as clinically diagnosed, as having a fasting glucose ≥7.0 or a nonfasting glucose ≥11.1 mmol/L, or as having HbA1c ≥6.5%.
**Includes participants who had fasting blood samples (total, n=1081; 236 women and 845 men).
††Includes participants without diabetes mellitus who had fasting blood samples (total, n=621; 127 women and 494 men).

(iso)valerylcarnitine (ρ≥0.30; *P*<0.001), whereas insulin sensitivity was negatively correlated (ρ≤−0.29; *P*<0.001).

All acylcarnitines were positively associated with non-HDL (high-density lipoprotein) cholesterol (ρ≥0.05; *P*<0.01), Apo B (ρ≥0.06; *P*<0.001), and CRP (ρ≥0.08; *P*<0.001; Figure 1). Propionyl- and (iso)valerylcarnitine were positively correlated with triglycerides (TGs; ρ≥0.22; *P*<0.001) and negatively correlated with HDL cholesterol (ρ≤−0.13; *P*<0.001). Furthermore, palmitoylcarnitine was positively associated with LDL cholesterol (ρ=0.18; *P*<0.001).

Follow-up and Events

Median (25th, 75th percentiles) length of follow-up was 10.2 (9.2, 11.5) years. Cardiovascular death occurred in 415 cases (10.0%), whereas a total of 534 participants (12.8%) experienced a fatal or nonfatal AMI.

Serum Acylcarnitines and Risk of Cardiovascular Death and AMI

In age- and sex-adjusted analyses, the upper quartiles of serum acetyl-, octanoyl-, palmitoyl-, propionyl-, and (iso)-valerylcarnitine were associated with an increased risk of cardiovascular death (Table 2). Risk estimates remained significant for acetyl-, octanoyl-, palmitoyl-, and propionylcarnitine also after multivariable adjustments (HR [95% CI]: 1.52 [1.12, 2.06]; *P*=0.007; 1.73 [1.23, 2.44]; *P*=0.002; 1.61 [1.18, 2.21]; *P*=0.003; 1.37 [1.01, 1.86]; *P*=0.04, respectively). The GAM plot in Figure 2A verifies a linear trend linked to risk of cardiovascular death for increasing levels of the 3 even-chained acylcarnitines, which showed the strongest associations. The crude event-free survival time from cardiovascular death by acylcarnitine quartiles are illustrated in Figure S1. Risk estimates of acylcarnitines for cardiovascular death events throughout 2006 (median follow-up, 4.7 years) are given in Table S1.

Age- and sex-adjusted Cox models also indicated an increased risk of AMI among those with high levels of serum acetyl-, octanoyl-, palmitoyl-, and (iso)valerylcarnitine (Table 3). However, after multivariable adjustment, risk estimates remained statistically significant for acetyl- and octanoylcarnitine only (HR [95% CI]: 1.33 [1.01, 1.75]; *P*=0.04; 1.38 [1.05, 1.81]; *P*=0.02, respectively). Risk associations were approximately linear (Figure 2B). The crude event-free survival time from AMI by acylcarnitine quartiles are illustrated by Figure S2. Risk estimates of acylcarnitines for AMI events throughout 2006 (median follow-up, 4.6 years)

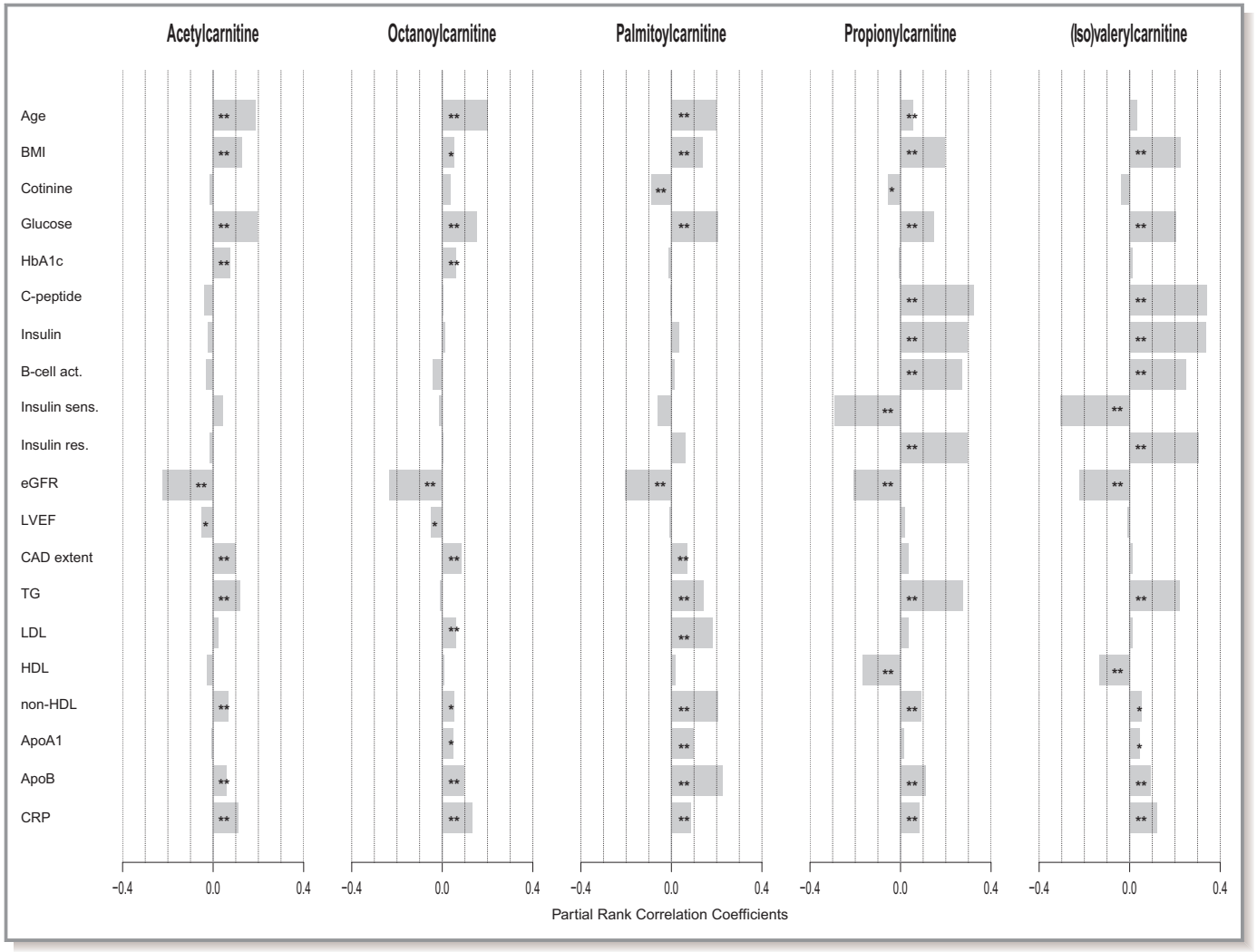

Figure 1. Association between acylcarnitines and clinical relevant covariates. Spearman's rho of ranked values of the serum acylcarnitines, acetyl-, octanoyl-, palmitoyl-, propionyl-, and (iso)valerylcarnitine, with important covariates at baseline are illustrated. The model was adjusted for sex. ApoA1 indicates apolipoprotein A1; ApoB, apolipoprotein B; B-cell act., β-cell activity; BMI, body mass index; CAD, coronary artery disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein cholesterol; Insulin res., insulin resistance; Insulin sens., insulin sensitivity; LDL, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; TG, triglycerides. *P<0.01; **P<0.001.

are given in Table S2. For both endpoints, risk estimates are additionally reported per 1-SD increment (Table S3).

We also tested for possible effect modifications according to diabetes mellitus (P≥0.10) and BMI (below and above median; P≥0.15), without revealing any significant interactions. NRI demonstrated significant estimates for acetyl-, octanoyl-, palmitoyl-, and propionylcarnitine with cardiovascular death (Table 4). The estimate was particularly strong for acetylcarnitine. Furthermore, significant estimates were detected for acetyl- and palmitoylcarnitine with AMI. Estimates on all-cause death and competing risk based on death attributed to noncardiovascular causes are shown in Tables S4 and S5, respectively.

The acylcarnitine to free carnitine ratio has been proposed as a sensitive screening system for alterations in the mitochondrial metabolism in humans, and a preferred ratio is considered to be around 0.25.[29] A ratio above 0.40 has been associated with mitochondrial dysfunction. We included this ratio in the Cox analyses, as estimated based on the sum of the 5 available acylcarnitines divided by free carnitine. There were significant associations between the ratio and both endpoints, with multivariable adjusted HRs (95% CIs) being 1.56 (1.12, 2.17; P=0.008) for cardiovascular death and 1.34 (1.02, 1.76; P=0.04) for AMI, in quartile 4 versus 1, respectively.

Stratification for Fasting Status

The associations between acylcarnitines and the outcomes cardiovascular death and AMI were calculated stratified for

Table 2. Hazard Ratios (and 95% CIs) for Cardiovascular Death by Quartiles of Serum Acylcarnitines*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend
Acetylcarnitine					
No. of events	69	87	89	170	
Age/sex adjusted	1.00	1.06 (0.77, 1.46)†	1.02 (0.74, 1.39)	2.01 (1.51, 2.67)	<0.001
Multivariable‡	1.00	0.94 (0.68, 1.31)	0.88 (0.63, 1.22)	1.52 (1.12, 2.06)	0.002
Octanoylcarnitine					
No. of events	50	92	123	150	
Age/sex adjusted	1.00	1.67 (1.18, 2.35)	2.10 (1.51, 2.92)	2.32 (1.68, 3.20)	<0.001
Multivariable‡	1.00	1.53 (1.07, 2.19)	1.80 (1.28, 2.54)	1.73 (1.23, 2.44)	0.003
Palmitoylcarnitine					
No. of events	62	88	91	174	
Age/sex adjusted	1.00	1.18 (0.85, 1.63)	1.19 (0.86, 1.64)	1.98 (1.47, 2.65)	<0.001
Multivariable‡	1.00	1.08 (0.78, 1.51)	1.01 (0.72, 1.42)	1.61 (1.18, 2.21)	0.001
Propionylcarnitine					
No. of events	80	94	100	141	
Age/sex adjusted	1.00	1.10 (0.81, 1.48)	1.17 (0.87, 1.58)	1.53 (1.16, 2.01)	0.002
Multivariable‡	1.00	1.03 (0.76, 1.42)	1.08 (0.79, 1.48)	1.37 (1.01, 1.86)	0.03
(Iso)valerylcarnitine					
No. of events	89	90	103	133	
Age/sex adjusted	1.00	0.98 (0.73, 1.32)	1.14 (0.86, 1.52)	1.41 (1.08, 1.86)	0.005
Multivariable‡	1.00	0.94 (0.69, 1.27)	1.05 (0.77, 1.43)	1.21 (0.89, 1.64)	0.14

*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile.
†Hazard ratio, 95% CI in parentheses (all such values).
‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes mellitus (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of coronary artery disease (0–3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

fasting status by comparing quartiles 4 to 1 of serum acylcarnitines (Figure 3). For both outcomes, the different acylcarnitines showed generally stronger risk associations in those who were fasting as opposed to nonfasting individuals. However, effect modifications were only statistically significant for the associations between the odd-chained propionyl- and (iso)valerylcarnitines and the outcome of cardiovascular death. Of notice, acylcarnitine levels differed between nonfasting and fasting individuals, with even-chained acylcarnitine levels being higher and odd-chained acylcarnitine levels being lower in those who were fasting.

Discussion

In this large, prospective cohort study among patients with suspected SAP, elevated even-chained serum acylcarnitines, including acetyl-, octanoyl-, and palmitoylcarnitine, were associated with increased risk of cardiovascular death, whereas acetyl- and octanoylcarnitine additionally showed associations with risk of fatal or nonfatal AMI. The prediction of adverse events was independent of traditional risk factors and particularly strong for cardiovascular death.

Proposed Mechanisms

Carnitine is necessary for transferring acyl residues across most intracellular membranes, given that these are generally impermeable toward acyl-CoAs. Whereas acetylcarnitine is reversibly converted to acetyl-CoA, which is one of the most central molecules in energy metabolism, octanoyl- and palmitoylcarnitine derive from the metabolism of FAs. Propionyl- and (iso)valerylcarnitine, on the other hand, are usually derived from branched-chain amino acids[30] or, alternatively, from odd carbon-chain FA metabolites, which can be found in dairy products.[31]

Acylcarnitine levels correlated positively with both BMI and plasma glucose at baseline and also with several established risk markers for cardiovascular disease, including Apo B and CRP. Insulin homeostasis showed quite strong relations with levels of the odd-chained propionyl- and (iso)valerylcarnitine,

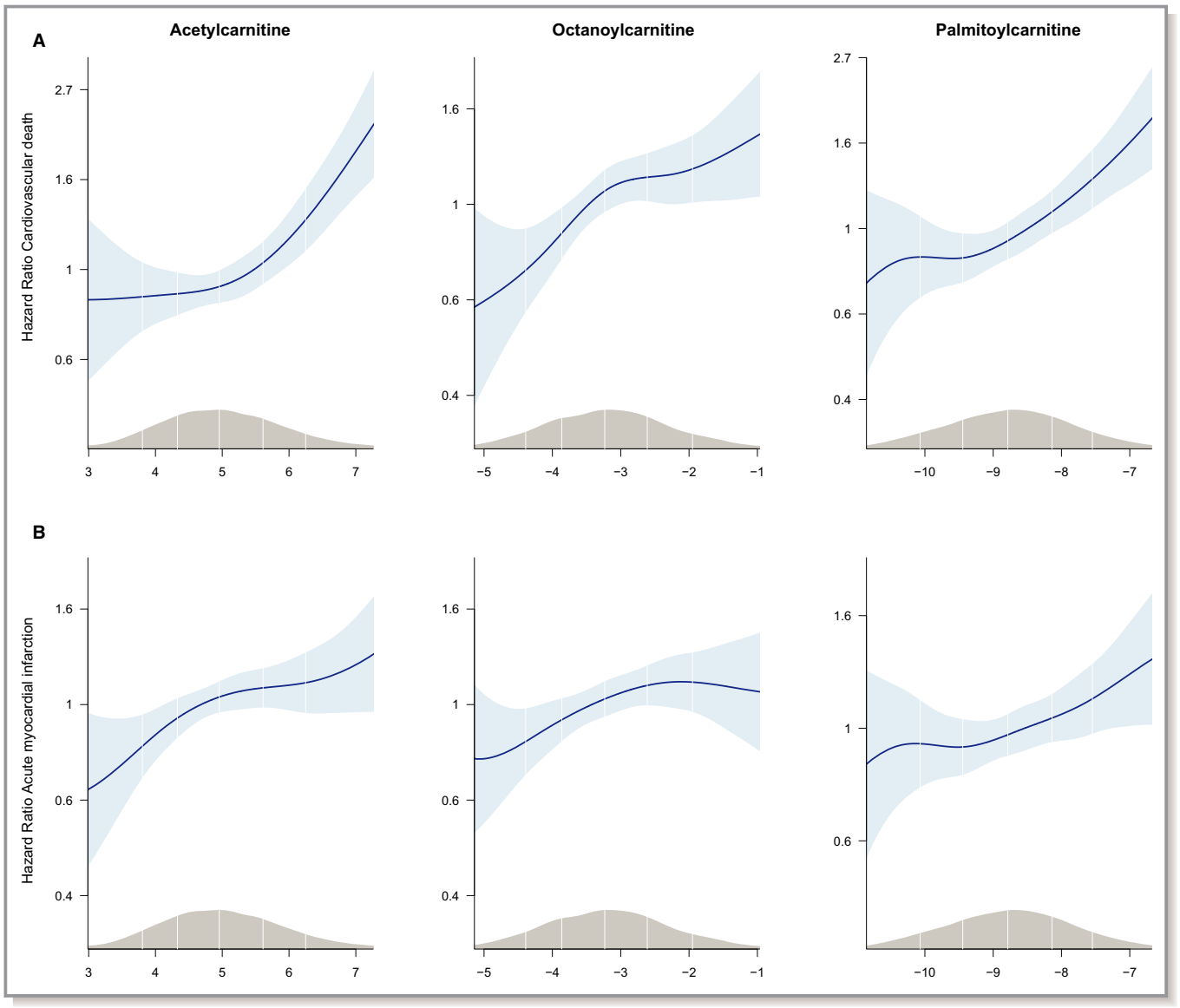

Figure 2. Association between serum even-chained acylcarnitines and risk of cardiovascular death (A) and acute myocardial infarction (B). In this generalized additive model, the hazard ratios are represented by the solid lines, whereas the 95% CIs lie within the shaded areas. Density plots show the distribution of serum acylcarnitines in the study cohort, and the vertical lines denote the 10th, 25th, 50th, 75th, and 90th percentiles. Values along the *x*-axis have been log-transformed and the plot has been cut at the 2.5th and 97.5th percentiles, respectively. The model was adjusted for age, sex, body mass index, fasting status, current smoker, diabetes mellitus, apolipoprotein A1, apolipoprotein B, creatinine, left ventricular ejection fraction, extent of coronary artery disease, study center, and intervention with folic acid or vitamin B6.

in line with previous observations in subjects with obesity or type 2 diabetes mellitus.[32] Furthermore, an accumulation of acylcarnitines in obese and type 2 diabetic individuals has been associated with impaired FA oxidation.[6,33] Also, recent studies in rodents and cell culture show that insulin resistance can be linked to increased levels of acylcarnitine intermediates in serum and muscle, attributed to incomplete β-oxidation.[34–36] Tissue accumulation of acetyl-CoA and increased generation of intermediate acylcarnitines have been hypothesized to be a result of reduced tricarboxylic acid (TCA) cycle activity attributed to impaired long-chain FA oxidation.[33] Proinflammatory pathways involved in the development of insulin resistance may subsequently be activated, as suggested based on cell studies showing an increased activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) when long-chain acylcarnitines are elevated.[33] Whereas the primary entry site of the TCA cycle is through acetyl-CoA,[37] odd carbon-chain CoAs like propionyl-CoA enter the TCA cycle through succinyl-CoA.[38] Hence, an increase in propionylcarnitine may, in addition, potentially reflect a defect in the ability to utilize succinyl-CoA in the TCA cycle.[6]

Table 3. Hazard Ratios (and 95% CIs) for AMI by Quartiles of Serum Acylcarnitines*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for Trend
Acetylcarnitine					
No. of events	92	127	146	169	
Age/sex adjusted	1.00	1.25 (0.96, 1.64)†	1.41 (1.08, 1.83)	1.69 (1.31, 2.19)	<0.001
Multivariable‡	1.00	1.15 (0.87, 1.52)	1.28 (0.98, 1.68)	1.33 (1.01, 1.75)	0.03
Octanoylcarnitine					
No. of events	92	128	133	181	
Age/sex adjusted	1.00	1.37 (1.05, 1.79)	1.36 (1.04, 1.78)	1.80 (1.40, 2.32)	<0.001
Multivariable‡	1.00	1.33 (1.01, 1.76)	1.18 (0.89, 1.56)	1.38 (1.05, 1.81)	0.07
Palmitoylcarnitine					
No. of events	100	120	134	180	
Age/sex adjusted	1.00	1.08 (0.83, 1.41)	1.21 (0.93, 1.57)	1.45 (1.13, 1.86)	0.002
Multivariable‡	1.00	1.04 (0.79, 1.37)	1.06 (0.81, 1.40)	1.24 (0.95, 1.62)	0.10
Propionylcarnitine					
No. of events	119	117	137	161	
Age/sex adjusted	1.00	0.92 (0.72, 1.19)	1.10 (0.86, 1.41)	1.22 (0.96, 1.55)	0.04
Multivariable‡	1.00	0.87 (0.67, 1.14)	0.98 (0.75, 1.28)	1.07 (0.83, 1.40)	0.37
(Iso)valerylcarnitine					
No. of events	106	140	139	149	
Age/sex adjusted	1.00	1.35 (1.05, 1.74)	1.33 (1.03, 1.72)	1.38 (1.07, 1.77)	0.03
Multivariable‡	1.00	1.29 (0.99, 1.68)	1.26 (0.96, 1.66)	1.18 (0.89, 1.58)	0.39

AMI indicates acute myocardial infarction.
*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile.
†Hazard ratio, 95% CI in parentheses (all such values).
‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes mellitus (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of coronary artery disease (0–3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

Elevated plasma levels of palmitoylcarnitine have been linked to serious adverse outcome in patients with heart failure.[39] Furthermore, increased levels of long-chain acylcarnitines, including palmitoylcarnitine, have been associated with reduced CPT activity, which are targets of the peroxisome proliferator-activated receptor (PPAR) α, a nuclear transcription factor that controls several genes involved in FA oxidation.[14] Given that CPT-I and -II is crucial for the entrance of long-chain FAs into the mitochondria before β-oxidation, an elevated level of palmitoylcarnitine suggests an overloaded or

Table 4. Net Reclassification Improvement for Cardiovascular Death and AMI*

	Cardiovascular Death	*P* Value	Acute Myocardial Infarction	*P* Value
Acetylcarnitine	0.32 (0.22, 0.42)†	0.008	0.14 (0.06, 0.23)	0.03
Octanoylcarnitine	0.15 (0.05, 0.25)	0.006	0.14 (0.05, 0.23)	0.09
Palmitoylcarnitine	0.12 (0.01, 0.22)	0.004	0.06 (−0.03, 0.16)	0.03
Propionylcarnitine	0.08 (−0.02, 0.19)	0.02	0.06 (−0.03, 0.16)	0.32
(Iso)valerylcarnitine	0.06 (−0.04, 0.17)	0.52	0.07 (−0.02, 0.17)	0.08

AMI indicates acute myocardial infarction.
*For the net reclassification improvement (95% CIs) analyses, logistic regression models were applied, containing the same variables as the Cox models. The model included age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes mellitus (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of coronary artery disease (0–3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).
†Net reclassification improvement, 95% CI in parentheses.

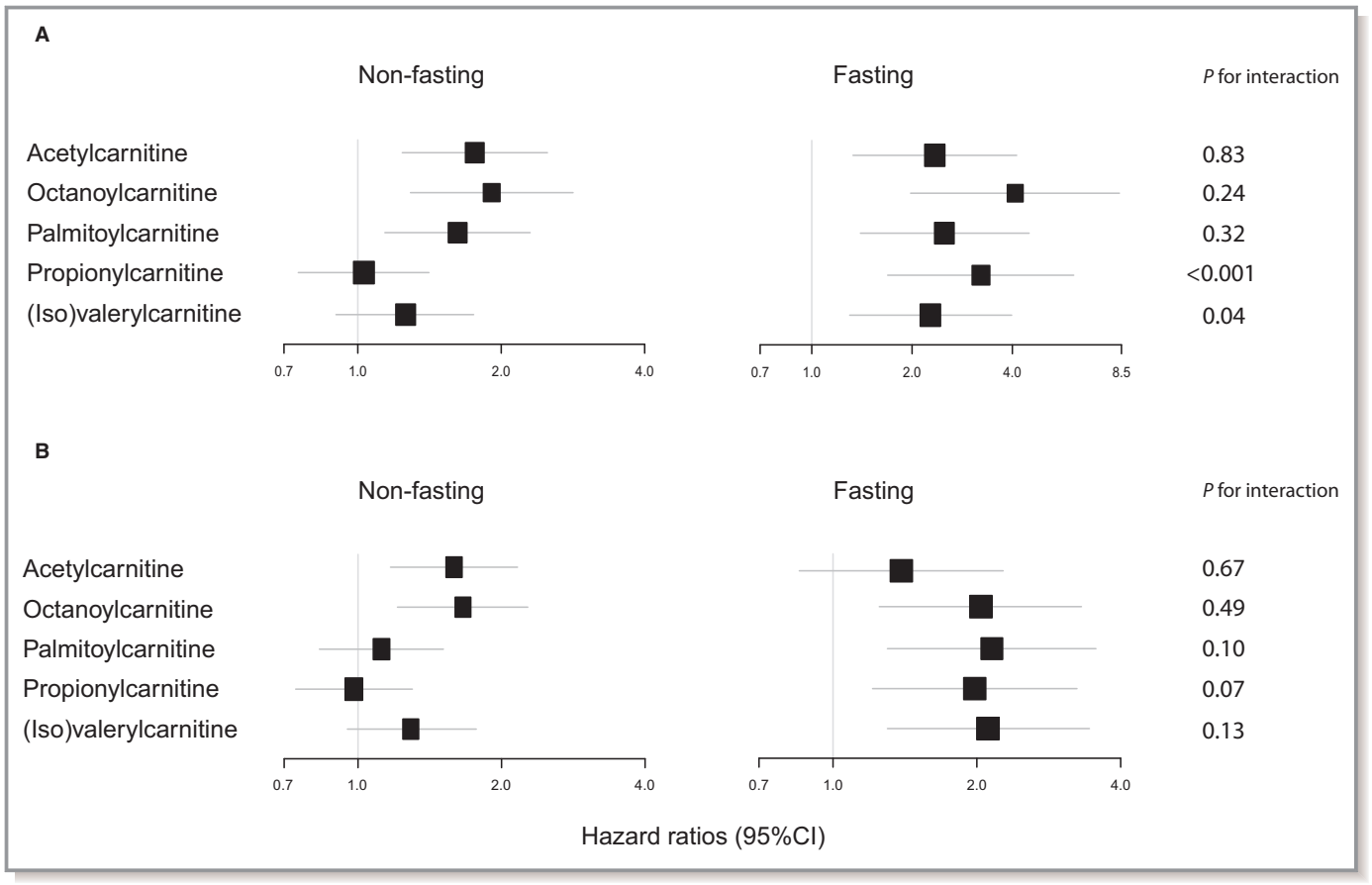

Figure 3. Forest-plot showing risk associations in the upper versus lower quartile of circulating acylcarnitines based on nonfasting and fasting measurements. Risk of cardiovascular death (A) and acute myocardial infarction (B) was calculated using Cox proportional hazards modeling. Hazard ratios (95% CIs) for quartile 4 versus 1 of each acylcarnitine are illustrated in strata of fasting status as designated. Cox proportional hazards survival analyses were adjusted for age and sex.

dysfunctional mitochondrial transport or oxidation system. Notably, in the current study, serum palmitoylcarnitine showed a moderately strong association with low-density lipoprotein (LDL) and non-HDL cholesterol, as well as with Apo B, further linking a dysfunctional mitochondrial transport of FAs with elevated proatherogenic lipoproteins.

Being one of the main products from peroxisomal β-oxidation, octanoyl-CoA needs to be exported to mitochondria for complete oxidation.[40] Based on this and the associations observed in the current study, an accumulation of octanoylcarnitine may also serve as an indicator of impaired mitochondrial oxidation of FAs, similar to elevated levels of acetyl- and palmitoylcarnitine.[41] We demonstrate an association between even-chained acylcarnitines and risk of cardiovascular events and partly also with AMI. These findings are supported by the link between the acylcarnitine to free carnitine ratio and risk. Altogether, the findings are suggestive of an association between mitochondrial dysfunction, dyslipidemia, and increased risk of cardiovascular events.[42]

Clinical Implications

Whereas high levels of all 3 even-chained acylcarnitines were associated with risk of cardiovascular death in multivariable Cox analysis, only acetyl- and octanoylcarnitine was significantly associated with risk of AMI. A weak, although significant, association was detected between the odd-chained propionylcarnitine and cardiovascular death, which also remained after multivariable adjustment.

FAs are the preferred metabolic fuel in situations where glucose levels are low.[43] Consequently, FA oxidation and mitochondrial function is of major importance during fasting conditions. Interestingly, PPARα activation, which is central for FA oxidation capacity, is particularly important in relation to FA utilization during fasting.[44] Thus, high serum concentrations of acylcarnitines in this state may represent more-sensitive indicators of mitochondrial dysfunction than the corresponding nonfasting levels, being less influenced by recent dietary intake. In those who were fasting during blood sampling, all 5 acylcarnitines, including the odd-chained

propionyl- and (iso)valerylcarnitines, were significantly associated with risk of both cardiovascular death and AMI, except from acetylcarnitine, which showed a significant association with risk of AMI only in nonfasting individuals. Hence, it may be important to apply fasting samples when evaluating these metabolites in future studies. Interestingly, the estimates were not appreciably altered after adjustments for troponin T, a strong determinant of coronary death.

Altogether, our results demonstrate that circulating levels of specific acylcarnitines may serve as potential biomarkers for cardiovascular risk in patients with SAP independent of established risk factors, including troponin T. The most promising are the even-chained acylcarnitines, acetyl-, octanoyl-, and palmitoylcarnitine, based on their association with cardiovascular death both in nonfasting and fasting individuals. Whether there are strategies with the potential to reduce risk in those having elevated acylcarnitines, such as improved lifestyle and more-healthy dietary habits, remains to be further elucidated and are interesting perspectives for future studies. One recent study has suggested a Mediterranean diet to modify the association between plasma acylcarnitines and cardiovascular disease risk.[8]

Strengths and Limitations

The strengths of this study include its large, well-characterized population with long-term follow-up and extensive clinical information. Furthermore, the associations between specific circulating acylcarnitines and outcome in such a large cohort of patients with SAP has, to our knowledge, not previously been evaluated, although some studies have investigated the associations between metabolic profiles and outcome in patients at risk of CAD.[11,12] A committee blinded to blood measurements validated all endpoints in the current study. Given that a large proportion of this cohort was part of a B-vitamin intervention trial, folic acid and vitamin B6 treatment was included in the multivariable model without affecting the risk estimates.

This study also had certain limitations. Given that acylcarnitines were measured in blood, caution should be made when extrapolating findings to intracellular levels and tissue distribution. We applied the widely used continuous NRI[28] for risk classification analyses, although we do acknowledge the limitations of this method.[45] Future validation studies should also consider alternative reclassification measures.[46] Furthermore, despite careful adjustments for important available covariates, there may still be residual confounding.

Conclusions

Elevated serum acetyl-, octanoyl-, and palmitoylcarnitine were associated with increased risk of cardiovascular death and a modest increased risk of fatal or nonfatal AMI among patients with SAP, independent of traditional risk factors. Future studies are warranted to explore underlying mechanisms of elevated acylcarnitines in patients at increased risk of adverse cardiovascular outcomes.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Hazard ratios (and 95% CIs) for cardiovascular death until 2006 by quartiles of serum acylcar

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Acetylcarnitine					
Number of events	24	24	45	75	
Age/sex adjusted	1.00	0.85 (0.48, 1.49)†	1.49 (0.91, 2.45)	2.41 (1.51, 3.83)	<0.001
Multivariable‡	1.00	0.84 (0.47, 1.49)	1.25 (0.74, 2.10)	1.72 (1.04, 2.83)	0.004
Octanoylcarnitine					
Number of events	10	30	57	71	
Age/sex adjusted	1.00	2.73 (1.33, 5.59)	4.67 (2.38, 9.16)	5.26 (2.70, 10.2)	<0.001
Multivariable‡	1.00	2.36 (1.15, 4.86)	3.65 (1.84, 7.24)	3.45 (1.74, 6.84)	<0.001
Palmitoylcarnitine					
Number of events	22	31	35	80	
Age/sex adjusted	1.00	1.17 (0.67, 2.01)	1.27 (0.74, 2.17)	2.38 (1.48, 3.84)	<0.001
Multivariable‡	1.00	1.13 (0.65, 1.97)	1.06 (0.61, 1.85)	2.01 (1.21, 3.35)	0.002
Propionylcarnitine					
Number of events	35	22	49	62	

Age/sex adjusted	1.00	0.60 (0.35, 1.02)	1.31 (0.85, 2.03)	1.49 (0.98, 2.26)	0.004
Multivariable‡	1.00	0.57 (0.33, 1.00)	1.19 (0.75, 1.89)	1.23 (0.78, 1.95)	0.06
(Iso)valerylcarnitine					
Number of events	29	32	50	57	
Age/sex adjusted	1.00	1.08 (0.65, 1.79)	1.72 (1.08, 2.73)	1.80 (1.14, 2.84)	0.002
Multivariable‡	1.00	1.03 (0.61, 1.74)	1.59 (0.97, 2.61)	1.37 (0.82, 2.29)	0.10

*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile.

†Hazard ratio, 95% CI in parentheses (all such values).

‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of CAD (0-3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

Table S2. Hazard ratios (and 95% CIs) for acute myocardial infarction until 2006 by quartiles of serum acylcar

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Acetylcarnitine					
Number of events	57	76	90	121	
Age/sex adjusted	1.00	1.21 (0.86, 1.71)†	1.39 (0.99, 1.94)	1.88 (1.37, 2.59)	<0.001
Multivariable‡	1.00	1.10 (0.78, 1.57)	1.17 (0.83, 1.65)	1.35 (0.96, 1.89)	0.07
Octanoylcarnitine					
Number of events	47	85	85	127	
Age/sex adjusted	1.00	1.74 (1.22, 2.48)	1.65 (1.15, 2.36)	2.34 (1.67, 3.29)	<0.001
Multivariable‡	1.00	1.67 (1.16, 2.41)	1.36 (0.94, 1.98)	1.72 (1.20, 2.47)	0.02
Palmitoylcarnitine					
Number of events	61	72	87	124	
Age/sex adjusted	1.00	1.04 (0.74, 1.47)	1.24 (0.90, 1.73)	1.59 (1.16, 2.17)	0.001
Multivariable‡	1.00	1.01 (0.72, 1.43)	1.05 (0.75, 1.49)	1.28 (0.91, 1.79)	0.12
Propionylcarnitine					
Number of events	77	68	91	108	

Age/sex adjusted	1.00	0.85 (0.61, 1.18)	1.11 (0.82, 1.51)	1.25 (0.93, 1.67)	0.05
Multivariable‡	1.00	0.80 (0.57, 1.12)	0.96 (0.69, 1.33)	1.05 (0.76, 1.45)	0.47
(Iso)valerylcarnitine					
Number of events	51	100	92	101	
Age/sex adjusted	1.00	1.96 (1.40, 2.75)	1.81 (1.28, 2.55)	1.89 (1.34, 2.66)	0.003
Multivariable‡	1.00	1.93 (1.35, 2.75)	1.71 (1.18, 2.48)	1.54 (1.05, 2.28)	0.17

*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile.

†Hazard ratio, 95% CI in parentheses (all such values).

‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of CAD (0-3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

Table S3. Hazard ratios (and 95% CIs) per SD for cardiovascular death and acute myocardial infarction by serum acylca

	Cardiovascular death	*P*	Acute myocardial infarction	*P*
Number of events	415		534	
Acetylcarnitine				
Age/sex adjusted	1.43 (1.29, 1.58)†	<0.001	1.24 (1.14, 1.35)	<0.001
Multivariate‡	1.29 (1.16, 1.44)	<0.001	1.14 (1.04, 1.25)	0.007
Octanoylcarnitine				
Age/sex adjusted	1.28 (1.17, 1.40)	<0.001	1.19 (1.10, 1.29)	<0.001
Multivariate‡	1.18 (1.07, 1.31)	0.001	1.08 (0.99, 1.19)	0.08
Palmitoylcarnitine				
Age/sex adjusted	1.44 (1.30, 1.60)	<0.001	1.22 (1.11, 1.33)	<0.001
Multivariate‡	1.30 (1.16, 1.46)	<0.001	1.12 (1.01, 1.23)	0.03
Propionylcarnitine				
Age/sex adjusted	1.17 (1.06, 1.29)	0.002	1.08 (0.99, 1.17)	0.11
Multivariate‡	1.12 (1.00, 1.25)	0.06	1.01 (0.92, 1.12)	0.81
(Iso)valerylcarnitine				

Age/sex adjusted	1.14 (1.04, 1.25)	0.005	1.11 (1.02, 1.20)	0.01
Multivariate[‡]	1.07 (0.96, 1.19)	0.22	1.04 (0.94, 1.14)	0.49

[*]Hazard ratios and 95% CIs were calculated by using Cox proportional hazards.

[†]Hazard ratio, 95% CI in parentheses per SD (all such values).

[‡]Multivariate model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of CAD (0-3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

Table S4. Hazard ratios (and 95% CIs) for all-cause death by quartiles of serum acylcarnitines*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Acetylcarnitine					
Number of events	163	188	222	338	
Age/sex adjusted	1.00	0.98 (0.80, 1.21)†	1.10 (0.90, 1.34)	1.76 (1.46, 2.12)	<0.001
Multivariable‡	1.00	0.93 (0.75, 1.16)	1.05 (0.85, 1.29)	1.50 (1.22, 1.84)	<0.001
Octanoylcarnitine					
Number of events	143	200	253	315	
Age/sex adjusted	1.00	1.28 (1.03, 1.58)	1.55 (1.26, 1.90)	1.77 (1.45, 2.16)	<0.001
Multivariable‡	1.00	1.18 (0.94, 1.47)	1.36 (1.10, 1.69)	1.45 (1.17, 1.80)	<0.001
Palmitoylcarnitine					
Number of events	163	194	217	337	
Age/sex adjusted	1.00	0.99 (0.81, 1.22)	1.09 (0.89, 1.33)	1.49 (1.23, 1.80)	<0.001
Multivariable‡	1.00	0.93 (0.75, 1.16)	1.02 (0.82, 1.26)	1.39 (1.13, 1.70)	<0.001
Propionylcarnitine					
Number of events	211	203	213	284	

Age/sex adjusted	1.00	0.89 (0.74, 1.08)	0.94 (0.77, 1.14)	1.16 (0.97, 1.39)	0.06
Multivariable‡	1.00	0.91 (0.74, 1.12)	0.93 (0.76, 1.14)	1.14 (0.93, 1.39)	0.14
(Iso)valerylcarnitine					
Number of events	205	211	213	282	
Age/sex adjusted	1.00	0.99 (0.82, 1.20)	1.01 (0.83, 1.22)	1.28 (1.07, 1.54)	0.006
Multivariable‡	1.00	1.00 (0.81, 1.23)	1.02 (0.82, 1.25)	1.22 (0.99, 1.50)	0.06

*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile.

†Hazard ratio, 95% CI in parentheses (all such values).

‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of CAD (0-3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

Table S5. Hazard ratios (and 95% CIs) for competing risk by quartiles of serum acylcarnitines*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Acetylcarnitine					
Number of events	94	101	133	168	
Age/sex adjusted	1.00	0.93 (0.70, 1.23)†	1.16 (0.89, 1.51)	1.56 (1.21, 2.02)	<0.001
Multivariable‡	1.00	0.92 (0.69, 1.24)	1.19 (0.90, 1.57)	1.46 (1.11, 1.92)	0.001
Octanoylcarnitine					
Number of events	93	108	130	165	
Age/sex adjusted	1.00	1.07 (0.81, 1.41)	1.25 (0.95, 1.63)	1.47 (1.14, 1.91)	0.001
Multivariable‡	1.00	0.99 (0.74, 1.33)	1.12 (0.85, 1.49)	1.31 (1.00, 1.72)	0.03
Palmitoylcarnitine					
Number of events	101	106	126	163	
Age/sex adjusted	1.00	0.88 (0.67, 1.16)	1.02 (0.79, 1.33)	1.19 (0.92, 1.53)	0.07
Multivariable‡	1.00	0.84 (0.63, 1.12)	1.03 (0.78, 1.36)	1.22 (0.93, 1.61)	0.04
Propionylcarnitine					
Number of events	131	109	113	143	

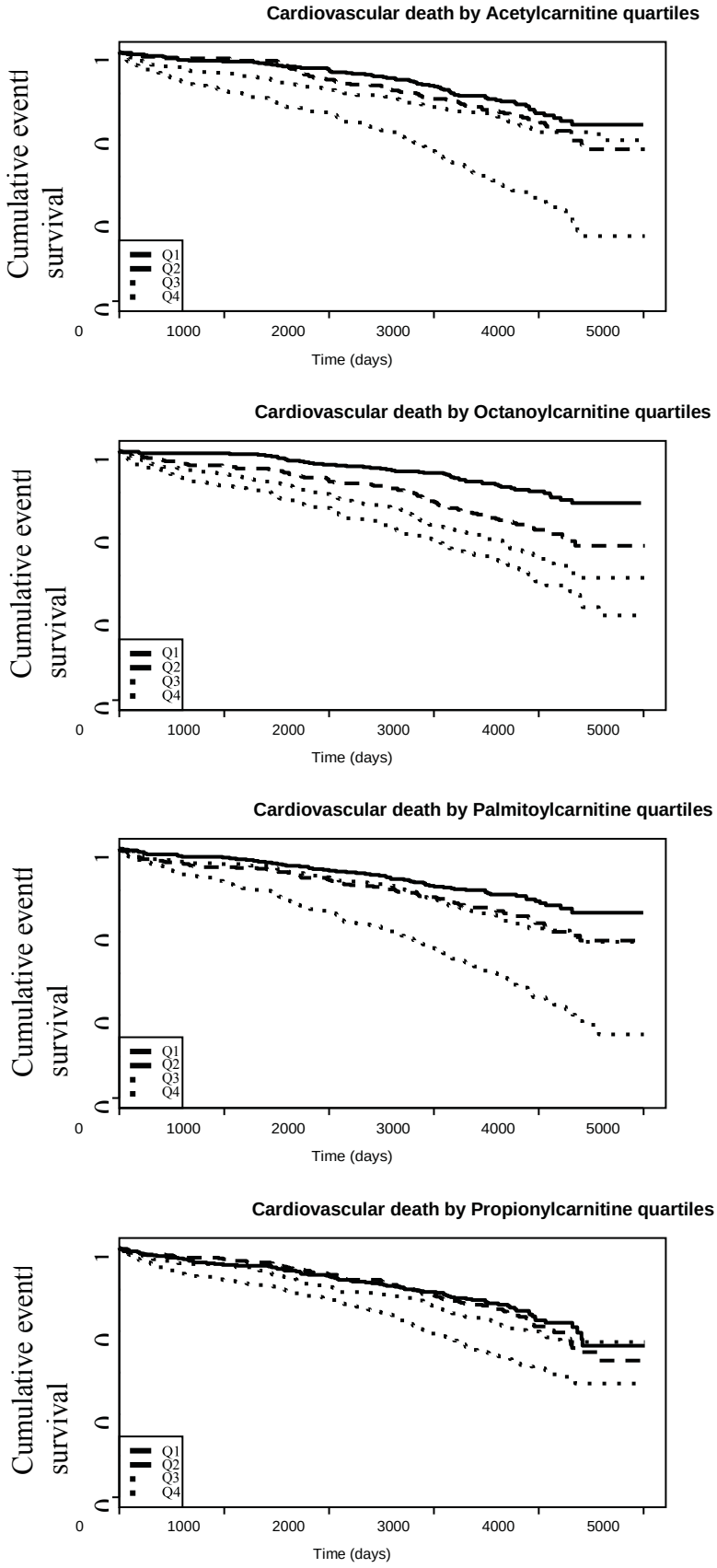
Age/sex adjusted	1.00	0.77 (0.60, 0.99)	0.79 (0.62, 1.02)	0.94 (0.74, 1.19)	0.75
Multivariable‡	1.00	0.82 (0.62, 1.08)	0.83 (0.63, 1.09)	0.96 (0.74, 1.26)	0.93
(Iso)valerylcarnitine					
Number of events	116	121	110	149	
Age/sex adjusted	1.00	1.00 (0.77, 1.29)	0.91 (0.70, 1.18)	1.19 (0.93, 1.52)	0.25
Multivariable‡	1.00	1.05 (0.80, 1.38)	0.98 (0.73, 1.30)	1.22 (0.92, 1.62)	0.23

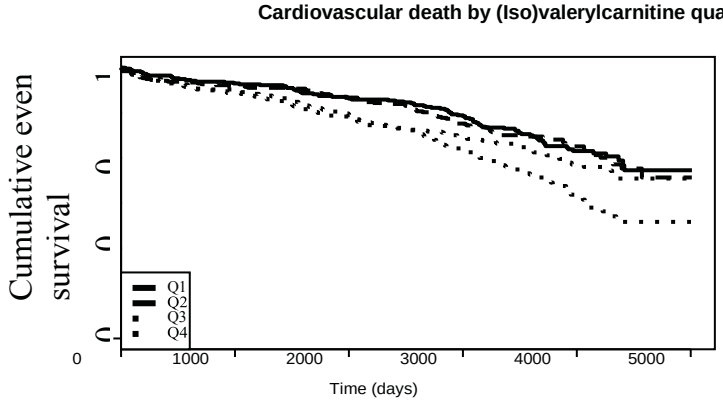
*Hazard ratios and 95% CIs were calculated by using Cox proportional hazards. There was an equal distribution into quartiles, with n=1041 patients in each quartile. Competing risk was assessed based on death of non-cardiovascular causes.

†Hazard ratio, 95% CI in parentheses (all such values).

‡Multivariable model adjusted for age (continuous), sex (dichotomous), body mass index (continuous), fasting (yes or no), current smoker (yes or no), diabetes (yes or no), apolipoprotein A1 (continuous), apolipoprotein B (continuous), creatinine (continuous), left ventricular ejection fraction (continuous), extent of CAD (0-3 affected vessels), study center (dichotomous), and intervention with folic acid or vitamin B6 (dichotomous).

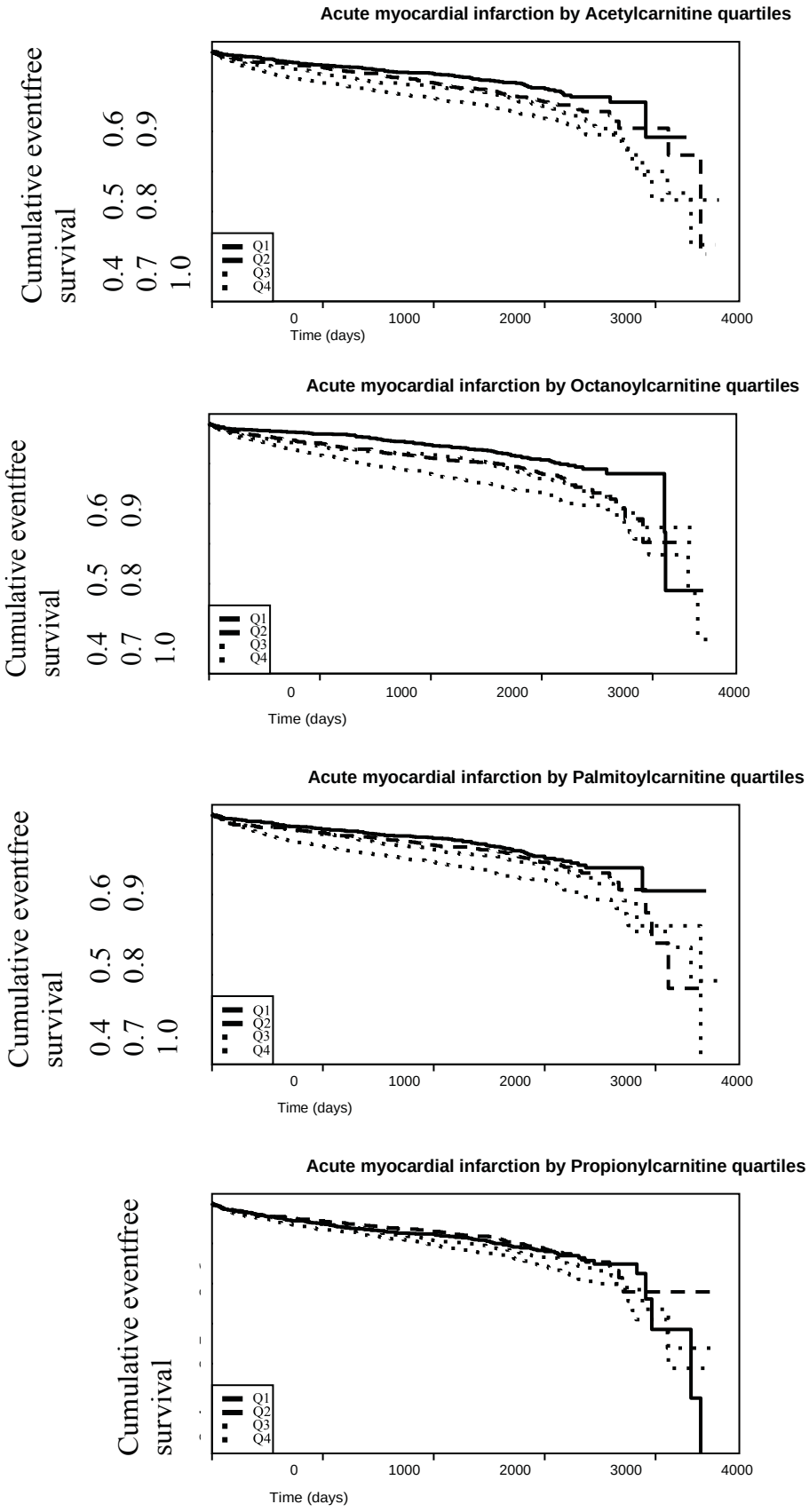
Figure S1. Kaplan-Meier plot illustrating survival of cardiovascular death.

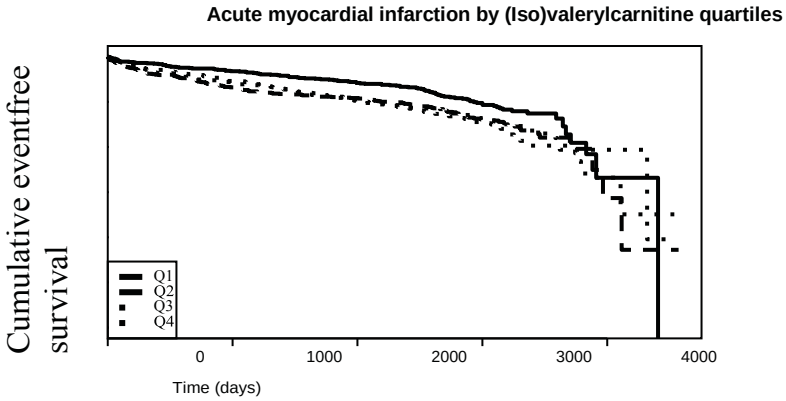




Survival plot showing time to cardiovascular death in each quartile of acylcarnitines as designated. Quartiles are illustrated as shown by Q1-Q4.

Figure S2. Kaplan-Meier plot illustrating survival of acute myocardial infarction.





Survival plot showing time to acute myocardial infarction in each quartile of acylcarnitines as designated. Quartiles are illustrated as shown by Q1-Q4.

Serum Acylcarnitines and Risk of Cardiovascular Death and Acute Myocardial Infarction in Patients With Stable Angina Pectoris

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