0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(7):2542–2547

doi: 10.1210/jc.2006-0195

Free Fatty Acids Are Independently Associated with All-Cause and Cardiovascular Mortality in Subjects with Coronary Artery Disease

Stefan Pilz, Hubert Scharnagl, Beate Tiran, Ursula Seelhorst, Britta Wellnitz, Bernhard O. Boehm, Jürgen R. Schaefer, and Winfried März

Clinical Institute of Medical and Chemical Laboratory Diagnostics (S.P., H.S., B.T., W.M.), Medical University of Graz, A-8036 Graz, Austria; Ludwigshafen Risk and Cardiovascular Health Study Nonprofit LLC (U.S., B.W.), 79098 Freiburg, Germany; Division of Endocrinology and Diabetes (B.O.B.), Graduate School Molecular Diabetology and Endocrinology, Ulm University, 89081 Ulm, Germany; and Division of Cardiology (J.R.S.), Center of Internal Medicine, Dr. Pohl Stiftungsprofessur Preventive Cardiology, University Hospital Marburg, 35033 Marburg, Germany

Context: Free fatty acids (FFAs) are associated with several cardiovascular risk factors and exert harmful effects on the myocardium.

Objective: The aim of our study was to elucidate the relationship between FFAs and mortality in subjects who underwent coronary angiography.

Design, Setting, and Participants: Ludwigshafen Risk and Cardiovascular Health is a prospective cohort study of Caucasians who had undergone coronary angiography at baseline (1997–2000). During a median time of follow-up of 5.38 yr, 513 deaths had occurred among 3315 study participants with measured FFAs.

Main Outcome Measure: Hazard ratios for mortality according to FFA levels were measured.

Results: At the fourth quartile of FFAs, fully adjusted hazard ratios for death from any cause and cardiovascular causes were 1.58 ($P = 0.002$) and 1.83 ($P = 0.001$), respectively. In persons with angiographic coronary artery disease (CAD), stable CAD, and unstable CAD, the predictive value of FFAs was similar to that in the entire cohort, but the association did not attain statistical significance in persons without CAD analyzed separately. FFA levels were not related to the presence of angiographic CAD but were elevated in subjects with unstable CAD, compared with probands with stable CAD. Furthermore, FFAs increased with the severity of heart failure and were positively correlated with N-terminal pro-B-type natriuretic peptide ($P < 0.001$).

Conclusions: FFA levels independently predict all-cause and cardiovascular mortality in subjects with angiographic CAD. A possible diagnostic use of FFAs warrants further studies, but our results may underline the importance of therapeutic approaches to influence FFA metabolism. **(*J Clin Endocrinol Metab* 91: 2542–2547, 2006)**

CIRCULATING FREE FATTY acids (FFAs) mainly originate from lipolysis in the adipose tissue; they contribute to insulin resistance and are elevated in obesity and type 2 diabetes (1–4). Recent studies (5, 6) suggested that FFAs also exert negative effects on the vessel wall by triggering endothelial apoptosis and impairing endothelium-dependent vasodilation (7, 8). The involvement of FFAs in atherosclerosis is supported by observations of an increased risk for cardiovascular disease associated with high levels of FFAs (9, 10). Oxidation of FFAs, which requires relatively more oxygen than glucose use, is the main energy source for the myocardium under physiological conditions (11). However, elevation of FFAs as observed in myocardial ischemia has been shown to increase the ischemic damage of the myocardium and to be proarrhythmic (11–14).

The negative clinical outcome associated with elevated FFAs in myocardial infarction might be attributed to metabolic alterations like accumulation of toxic intermediates, suppression of glucose use, or mitochondrial dysfunctions (11–13, 15, 16). In addition, there are also studies suggesting that altered cardiac FFA metabolism, especially increased myocardial FFA uptake, might be related to the development of heart failure (17, 18). Despite this compelling evidence for a pivotal role of FFA in cardiovascular disease, clinical studies addressing the relationship between FFA and mortality are sparse (11, 16). Among the rare data on this topic is the Paris Prospective Study I of 5250 men free of ischemic cardiac disease, which demonstrated that FFAs are an independent risk factor for sudden death but not for myocardial infarction or overall cardiovascular mortality (19, 20). The same study also found an unexpected association between FFAs and mortality from cancer (20). Furthermore, high FFAs were predictive for increased mortality in type 2 diabetics (21) and subjects with acute myocardial infarction (11, 16), but to our knowledge there are currently no data available on this issue in a large well-defined cohort of subjects presenting with different stages of coronary artery disease (CAD). Thus, the present study aimed to elucidate the relationship between FFAs and mortality in subjects with and without angio-

First Published Online April 4, 2006

Abbreviations: BMI, Body mass index; CAD, coronary artery disease; CI, confidence interval; CRP, C-reactive protein; FFA, free fatty acid; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol; LURIC, Ludwigshafen Risk and Cardiovascular Health study; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

graphic proven CAD that were derived from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study (22). The results of our work support a role of FFAs as a (cardiovascular) risk factor and may promote therapeutic approaches to influence FFA metabolism.

Subjects and Methods

Subjects

We studied 3315 Caucasians of the LURIC study (22). LURIC is an ongoing prospective cohort study of white individuals investigating risk factors for CAD. The Institutional Review Board at the Ärztekammer Rheinland-Pfalz approved the study. Informed written consent was obtained from each of the participants. With the exception of acute coronary syndromes, the patients had to present in a stable clinical condition without major concomitant noncardiovascular disease. Clinical indications for angiography were chest pain or noninvasive tests consistent with myocardial ischemia (22).

CAD was assessed by angiography using the maximum luminal narrowing estimated by visual analysis. Clinically relevant CAD was defined as the occurrence of at least one stenosis 20% or more in at least one of 15 coronary segments. Individuals with stenosis less than 20% were considered as controls, and in 49 participants the information about this CAD status was not available.

Type 2 diabetes mellitus was diagnosed if plasma glucose was greater than 1.25 g/liter in the fasting state or greater than 2.00 g/liter 2 h after the oral glucose load, respectively, or if individuals were receiving oral antidiabetics or insulin. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mm Hg or if there was a clinical history significant of hypertension. The definition of the National Cholesterol Education Program Adult Treatment Panel III for the metabolic syndrome was used (23). Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as the product of the fasting insulin value (microunits per milliliter) and the fasting glucose value (millimoles per liter) divided by 22.5 (24).

Fasting measurements of FFAs, lipoproteins, C-reactive protein (CRP), creatinine, homocysteine, and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) were complete in 3266 individuals with coronary angiograms. Among these, 2567 persons had angiographic CAD, and 1535 were in a clinically stable condition, whereas 1032 presented within 7 d after onset of symptoms of unstable angina, non-ST-elevation myocardial infarction (troponin T $>$ 0.1 μg/liter), or ST-elevation myocardial infarction (troponin T $>$ 0.1 μg/liter).

Information on vital status was obtained from local person registries. No patients were lost to follow-up. Among the 3315 persons with measured FFAs, 513 deaths had occurred during a median time of follow-up of 5.38 yr. Death certificates were obtained for 498 of the decedents and were missing for 15 decedents who were included in the total mortality analysis but excluded from the cardiovascular mortality analysis. Cardiovascular death included the following categories: sudden death, fatal myocardial infarction, death due to congestive heart failure, death immediately after intervention to treat CAD, fatal stroke, and other causes of death due to CAD.

Laboratory analysis

The standard laboratory methods used have been described (22). Fasting blood collection was done before coronary angiography, and samples were snap frozen in liquid nitrogen at −80 C until analysis. Free (nonesterified) fatty acids were measured enzymatically (ACS-ACOD method) with a nonesterified fatty acid C kit (Wako Chemicals GmbH, Neuss, Germany) on a Wako 30R analyzer. The assay was calibrated by multicalibrators, which were double checked before use against primary standards. Inter- and intraassay coefficients of variation for the FFA assay were 3.0 and 1.6%, respectively. Lipoproteins were separated by a combined ultracentrifugation-precipitation method. Sensitive CRP was measured by immunonephelometry (N High Sensitivity CRP, Dade Behring, Marburg, Germany). NT-pro-BNP was measured by electrochemiluminescence on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Continuous parameters following a nonnormal distribution like FFAs were logarithmically transformed before being used in parametric procedures. Clinical and biochemical characteristics of CAD patients and controls are presented as percentages for categorical variables and as medians and 25th and 75th percentiles for continuous variables. Comparisons between groups were performed by Student's t test for continuous and by χ^2 testing for categorical variables. Simple and partial correlation analysis of FFAs and other variables with controlling for possible confounders was applied. We established quartiles of FFA values according to the values of all study participants. To examine the impact of FFAs on all-cause and cardiovascular mortality, we calculated hazard ratios and 95% confidence intervals (CIs) using the Cox proportional hazards model. Multivariable adjustment was carried out as indicated. All statistical tests were two sided. $P < 0.05$ was considered statistically significant. The SPSS 11.5 statistical package (SPSS Inc., Chicago, IL) was used.

Results

Study participants

Compared with individuals without CAD, patients with angiographic CAD were significantly older; smoking, type 2 diabetes mellitus, and β-blocker and hydroxymethylglutaryl coenzyme A reductase inhibitor use were more prevalent (Table 1). The CAD patients had higher systolic blood pressure, higher homocysteine, higher creatinine, higher triglycerides, higher HOMA-IR, lower high-density lipoprotein cholesterol (HDL-C), and lower low-density lipoprotein cholesterol (LDL-C). CRP was higher in CAD patients than controls, a finding that in part relates to the presence of patients with acute coronary syndromes in the CAD group. CAD patients had significantly higher NT-pro-BNP. Body mass index (BMI) and diastolic blood pressure were similar in patients and controls (Table 1).

Compared with patients with stable CAD, CRP, NT-pro-BNP, and the proportions of subjects using β-blockers and statins (hydroxymethylglutaryl coenzyme A reductase inhibitors) were significantly higher in the unstable CAD group. Blood pressure, HDL-C, and LDL-C were found reduced in subjects with unstable CAD. Gender, BMI, diabetes mellitus, HOMA-IR, smoking, creatinine, and homocysteine were not significantly different between the stable and unstable angina group (Table 1).

Associations of FFAs with cardiovascular risk factors and CAD status

FFA levels were not significantly different in controls and CAD patients, but compared with subjects with stable CAD, they were significantly increased in the unstable CAD group ($P = 0.003$) (Table 1). Type 2 diabetes mellitus ($P < 0.001$), metabolic syndrome ($P < 0.001$), hypertension ($P < 0.001$), and male gender ($P < 0.001$) were associated with increased and smoking with decreased FFA levels ($P < 0.001$) in the whole study population. In simple correlation analysis, FFAs were positively correlated with age (r = 0.213, $P < 0.001$), triglycerides (r = 0.168, $P < 0.001$), HOMA-IR (r = 0.146, $P < 0.001$), CRP (r = 0.140, $P < 0.001$), BMI (r = 0.101, $P < 0.001$), homocysteine (r = 0.071, $P < 0.001$), and very slightly with HDL-C (r = 0.040, $P = 0.021$), whereas there was no significant correlation with LDL-C (data not shown). Furthermore, FFAs were positively correlated with NT-pro-BNP (r = 0.177,

TABLE 1. Clinical and laboratory characteristics of controls and patients with CAD, stable CAD, and unstable CAD

Variable	Controls (n = 699)	All CAD patients (n = 2567)	Stable CAD (n = 1535)	Unstable CAD (n = 1032)	Controls *vs.* all CAD (*P*)	Stable *vs.* unstable CAD (*P*)
Males, %	52	75	76	74	<0.001	0.253
Age, yr	60 (50–66)	64 (58–71)	65 (58–71)	64 (57–71)	<0.001	0.119
BMI, kg/m^2	26.8 (24.5–29.4)	27.2 (24.8–29.8)	27.1 (24.6–29.7)	27.2 (24.9–29.8)	0.407	0.527
Diabetes mellitus, %	18	36	35	37	<0.001	0.258
Triglycerides, mmol/liter	1.52 (1.11–2.22)	1.71 (1.29–2.30)	1.68 (1.25–2.30)	1.76 (1.35–2.34)	<0.001	0.077
FFAs, mmol/liter	0.61 (0.44–0.88)	0.63 (0.43–0.89)	0.60 (0.43–0.85)	0.67 (0.44–0.96)	0.983	0.003
HDL-C in female probands, mmol/liter	1.14 (0.98–1.35)	1.06 (0.88–1.24)	1.09 (0.91–1.27)	1.01 (0.83–1.22)	<0.001	<0.001
HDL-C in male probands, mmol/liter	0.98 (0.85–1.22)	0.91 (0.78–1.06)	0.93 (0.80–1.11)	0.88 (0.73–1.01)	<0.001	<0.001
LDL-C, mmol/liter	3.08 (2.59–3.65)	2.90 (2.38–3.55)	2.98 (2.43–3.60)	2.85 (2.36–3.44)	0.001	0.025
HOMA-IR	2.03 (1.31–3.25)	2.59 (1.57–4.32)	2.64 (1.58–4.41)	2.52 (1.57–4.20)	<0.001	0.127
SBP, mm Hg	135 (121–151)	142 (124–159)	144 (128–161)	138 (120–154)	<0.001	<0.001
DBP, mm Hg	80 (72–88)	80 (72–89)	82 (74–90)	78 (71–87)	0.219	<0.001
Smoker (ex and active), %	49	69	68	70	<0.001	0.356
Creatinine, μmol/liter	80 (71–88)	80 (71–97)	80 (71–97)	80 (71–97)	<0.001	0.063
CRP, mg/liter	2.1 (1.0–5.8)	3.8 (1.5–9.2)	2.8 (1.2–6.7)	6.2 (2.4–10.0)	<0.001	<0.001
Homocysteine, μmol/liter	11.1 (9.3–14.2)	12.6 (10.1–15.7)	12.5 (10.0–15.8)	12.6 (10.3–15.6)	<0.001	0.665
NT-pro-BNP, μg/liter	154 (68–491)	333 (127–966)	299 (114–876)	397 (144–1152)	<0.001	<0.001
β-Blockers, %	44	69	62	79	<0.001	<0.001
Statins, %	17	56	49	65	<0.001	<0.001

Data represent median (25th-75th percentile). SBP, Systolic blood pressure; DBP, diastolic blood pressure.

$P < 0.001$), even after adjustment for age, sex, BMI, HDL-C, LDL-C, CRP, triglycerides, homocysteine, creatinine, angiographic CAD status, type 2 diabetes, hypertension, smoking status, and New York Heart Association classes ($P < 0.001$). Compared with New York Heart Association class 1 (0.59, 0.41–0.83; median and 25th to 75th percentile in millimoles per liter), FFAs were significantly increased in classes 2 (0.65, 0.45–0.93; $P < 0.001$), 3 (0.68, 0.50–1.02; $P < 0.001$), and 4 (0.82, 0.48–1.20; $P < 0.001$).

FFAs and mortality from all causes

Compared with subjects in the lowest quartile of FFAs, the unadjusted hazard ratios for death at FFA concentrations in the second, third, and fourth quartiles were 1.30 ($P = 0.076$), 1.63 ($P < 0.001$), and 2.75 ($P < 0.001$), respectively (model 1, Table 2). Inclusion of age and gender as covariables decreased these estimates only moderately to 1.14 ($P = 0.393$), 1.36 ($P = 0.036$), and 2.23 ($P < 0.001$), respectively (model 2, Table 2). FFAs retained their prognostic importance after adjusting for established and emerging cardiovascular risk factors (model 3, Table 2).

Among the 2567 subjects with angiographic CAD, 447 (17.4%) died during follow-up. In this subgroup, hazard ratios for death were slightly higher than those obtained in the entire study sample (models 1–3, Table 2).

Only 56 deaths (8.0%) occurred among the 699 subjects

TABLE 2. Hazard ratios for death from all causes according to FFAs

FFAs (mmol/liter)	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 3 OR (95% CI)	*P*
All subjects (n = 3315)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	0.618
Second quartile (0.44–0.62)	1.30 (0.97–1.74)	0.076	1.14 (0.85–1.52)	0.393	1.08 (0.80–1.46)	0.736
Third quartile (0.63–0.89)	1.63 (1.23–2.16)	<0.001	1.36 (1.02–1.80)	0.036	1.05 (0.78–1.42)	0.002
Fourth quartile (>0.89)	2.75 (2.12–3.57)	<0.001	2.23 (1.71–2.91)	<0.001	1.58 (1.19–2.09)	
All CAD (n = 2567)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	1.50 (1.09–2.05)	0.013	1.30 (0.94–1.78)	0.110	1.22 (0.88–1.70)	0.230
Third quartile (0.63–0.89)	1.78 (1.31–2.43)	<0.001	1.50 (1.10–2.05)	0.011	1.11 (0.80–1.53)	0.550
Fourth quartile (>0.89)	3.06 (2.29–4.07)	<0.001	2.50 (1.87–3.35)	<0.001	1.75 (1.29–2.39)	<0.001
Stable CAD (n = 1535)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	1.25 (0.86–1.84)	0.248	1.17 (0.80–1.72)	0.424	1.08 (0.72–1.61)	0.708
Third quartile (0.63–0.89)	1.53 (1.05–2.23)	0.025	1.39 (0.95–2.03)	0.089	1.05 (0.71–1.57)	0.798
Fourth quartile (>0.89)	2.96 (2.09–4.18)	<0.001	2.72 (1.91–3.85)	<0.001	1.60 (1.10–2.34)	0.014
Unstable CAD (n = 1032)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	2.12 (1.19–3.76)	0.010	1.62 (0.91–2.88)	0.103	1.53 (0.84–2.79)	0.164
Third quartile (0.63–0.89)	2.44 (1.41–4.23)	0.002	1.77 (1.02–3.09)	0.044	1.32 (0.73–2.38)	0.363
Fourth quartile (>0.89)	3.60 (2.15–6.04)	<0.001	2.38 (1.40–4.03)	0.001	2.04 (1.16–3.58)	0.014

Model 1, unadjusted; model 2, adjusted for age and gender; model 3, in addition adjusted for BMI, triglycerides, HDL, LDL, HOMA-IR, hypertension, smoking status, CRP, homocysteine, creatinine, NT-pro-BNP, statin, and β-blocker intake. OR, Odds ratio.

with coronary stenosis less than 20%. Although there was a tendency toward an increased risk of death at high concentrations of FFAs in this group as well (unadjusted hazard ratio 1.78, $P = 0.100$, for the fourth, compared with the first quartile), the association did not reach statistical significance.

Among the subjects with angiographic CAD, 1535 subjects had stable CAD, and 1032 patients presented with unstable CAD (unstable angina, non-ST-elevation myocardial infarction, or ST-elevation myocardial infarction). In these subgroups of patients, 282 (18.4%) and 165 deaths (16.0%), respectively, occurred. In both of them, we found consistent associations of FFAs with mortality from all causes (models 1–3, Table 2).

FFAs and mortality from cardiovascular causes

Because death certificates were not available for 15 deceased persons, the analysis for causes of mortality included a total of 3300 individuals. Among these, 346 (10.5%) died from cardiovascular causes, 32 (1%) from fatal infection, 57 from fatal cancer (1.7%), and 63 (1.9%) from miscellaneous causes. Hazard ratios for death from cardiovascular causes according to FFAs were higher, compared with those obtained for mortality from all causes in all models and across all subgroups examined (Table 3).

In line with our other results, multivariable adjusted (model 3, Tables 2 and 3) separate analysis of subjects with and without type 2 diabetes also showed in both groups a significantly increased all-cause and cardiovascular mortality at the fourth quartile of FFAs in subjects with CAD but not in controls without angiographic CAD (data not shown).

Discussion

This follow-up study demonstrates that high levels of FFAs predict total and cardiovascular mortality, independent of established and emerging cardiovascular risk factors. The higher hazard ratios for cardiovascular mortality, compared with all-cause mortality (Tables 2 and 3), underlines the involvement of FFAs in cardiovascular diseases. There exist sufficient data on the adverse effects of elevated FFA that may explain our results. Especially the myocardium seems to be negatively affected by high levels of FFAs (11–19, 25–27). FFAs are considered to exert proarrhythmic effects, increase the ischemic damage of the myocardium, and contribute to the development of heart failure. In this context it has been suggested that high levels of FFAs and therefore increased use of FFAs as a metabolic substrate may reduce myocardial energy efficiency and ventricular function (25–27). This could be mediated by an FFA-induced increase of mitochondrial uncoupling proteins, which leads to less efficient ATP synthesis and by a FFA-induced decrease of glucose transporter 4 that reduces the efficient myocardial glucose metabolism (28). These data support the suggestions that the beneficial effects of β-blockers on heart failure could partially be explained by their ability to reduce FFA levels (27, 29). All these processes fit well to the concept of a metabolic vicious cycle in heart failure of FFA elevation induced by the compensatory hyperadrenergic state that contributes to the worsening of ventricular function (30). NT-pro-BNP may also be involved in this vicious cycle by considering the recently discovered lipolytic effects of natriuretic peptides (31) and the strong correlations of NT-pro-BNP with FFAs and heart failure in our study. Toward this, our data confirm observations of an increase of FFAs with the severity of heart failure (32).

Apart from this, FFAs may also be involved in several crucial steps in atherogenesis, *i.e.* through their ability to induce endothelial apoptosis and endothelial dysfunction (5–8, 33). Toward this, FFAs have been shown to increase the activity of protein phosphatase type 2C, which causes apoptosis of endothelial cells (6), a process that might occur preferentially in the coronary arteries by considering that FFAs serve as the main energy source for the myocardium.

TABLE 3. Hazard ratios for death from cardiovascular causes according to FFAs

FFAs (mmol/liter)	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 3 OR (95% CI)	*P*
All subjects (n = 3300)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	1.64 (1.13–2.37)	0.009	1.44 (1.00–2.01)	0.053	1.35 (0.92–1.99)	0.122
Third quartile (0.63–0.89)	1.97 (1.38–2.82)	<0.001	1.66 (1.16–2.39)	0.006	1.25 (0.85–1.83)	0.254
Fourth quartile (>0.89)	3.29 (2.35–4.61)	<0.001	2.71 (1.92–3.81)	<0.001	1.83 (1.27–2.63)	0.001
All CAD (n = 2552)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	1.77 (1.20–2.63)	0.004	1.56 (1.05–2.32)	0.027	1.42 (0.95–2.14)	0.089
Third quartile (0.63–0.89)	1.92 (1.30–2.83)	<0.001	1.65 (1.12–2.45)	0.012	1.15 (0.76–1.74)	0.504
Fourth quartile (>0.89)	3.42 (2.39–4.91)	<0.001	2.88 (1.99–4.15)	<0.001	1.94 (1.32–2.85)	0.001
Stable CAD (n = 1527)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	1.47 (0.92–2.34)	0.108	1.39 (0.87–2.21)	0.171	1.25 (0.77–2.02)	0.373
Third quartile (0.63–0.89)	1.68 (1.06–2.66)	0.029	1.56 (0.98–2.48)	0.063	1.13 (0.69–1.84)	0.627
Fourth quartile (>0.89)	3.45 (2.25–5.28)	<0.001	3.25 (2.11–4.99)	<0.001	1.88 (1.19–2.96)	0.007
Unstable CAD (n = 1025)						
First quartile (<0.44)	1.00 Reference		1.00 Reference		1.00 Reference	
Second quartile (0.44–0.62)	2.65 (1.26–5.56)	0.010	2.04 (0.97–4.31)	0.060	1.96 (0.89–4.33)	0.096
Third quartile (0.63–0.89)	2.65 (1.28–5.49)	0.009	1.94 (0.93–4.07)	0.078	1.28 (0.57–2.86)	0.552
Fourth quartile (>0.89)	3.90 (1.96–7.77)	<0.001	2.61 (1.29–5.27)	0.007	2.22 (1.03–4.77)	0.041

Model 1, unadjusted; model 2, adjusted for age and gender; model 3, in addition adjusted for BMI, triglycerides, HDL, LDL, HOMA-IR, hypertension, smoking status, CRP, homocysteine, creatinine, NT-pro-BNP, statin, and β-blocker intake. OR, Odds ratio.

Therefore, we hypothesized that fatty acids might be a crucial factor causing myocardial infarction in man by destabilizing the endothelial layer of the coronary vessels (5). This hypothesis would perfectly fit within our finding that FFAs are elevated in subjects with unstable CAD but exhibit no significant difference between controls and subjects with angiographic-proven but stable CAD. The increase of FFAs in the unstable CAD group might be due to a surge of catecholamine activity in these patients (12). However, the missing association between FFAs and the angiographic presence of CAD may suggest that apart from (chronic) proatherosclerotic actions, other properties of FFAs may account for our results of an increased mortality associated with high levels of FFAs. Among these are also FFA-induced inflammatory processes, oxidative stress, and lipotoxic effects (1, 6, 18, 34).

To the best of our knowledge, there exists to date no other study investigating the long-term prognostic value of FFA in patients with angiographic CAD. The only large study addressing this issue so far was performed in subjects free of known ischemic cardiac disease (19). That study indicated that FFAs are independently associated with risk for sudden death, a finding that is supported by several observations of the proarrhythmic effects of FFAs, which were reported in conditions with and without cardiac ischemia (11–14, 35). Interestingly, there was no association between FFAs and death due to myocardial infarction (19). Considering in addition our results of no association between angiographic CAD and FFAs, these data might suggest that the adverse effects of FFAs on the cardiovascular system might mainly be attributed to features leading to arrhythmias and heart failure rather than to proatherosclerotic effects. In this context it should, however, be noted that sudden death and myocardial infarction are closely related, and difficulties in their classification might easily occur and influence these results (19), especially by considering the relatively small number of cases in the Paris Prospective Study (91 for sudden death and 145 for fatal myocardial infarction). Therefore, we decided to present our results without differentiating between particular causes of cardiovascular death. We are also aware of the fact that our results are mainly driven by the outcomes in patients with angiographic CAD due to the lower number of deaths occurring in individuals without CAD. Taking together the current findings and those of the Paris Prospective Study (19), we suggest that high FFAs confer an increased risk of death, regardless of the angiographic status. Nevertheless, large clinical studies with high numbers of well-defined cases are still warranted to further elucidate which causes of death are responsible for the increased cardiovascular mortality associated with high levels of FFAs in subjects with CAD.

The main message of our work is that despite the close association of FFAs with several cardiovascular risk factors (1, 7–12, 33), its predictive value for all-cause and cardiovascular mortality remains stable even after multivariable adjustments. This indicates that FFA levels provide additional information on mortality risk beyond established risk factors, supporting the idea of a direct involvement in pathophysiological processes.

Should FFAs be measured routinely as a cardiovascular risk factor? Currently this appears to be hampered by the relatively high day-to-day variability in FFA levels (36). However, considering our robust and promising results, diagnostic strategies might be devised in the future that overcome this limitation. Apart from this, the association between FFAs and mortality should further promote therapeutic strategies to influence FFA metabolism in cardiovascular disease (37–39). These therapies should aim to reduce fatty acid and increase glucose oxidation in the myocardium (37–39).

In summary, FFA levels independently predict all-cause and cardiovascular mortality in subjects with angiographic CAD. A possible diagnostic use of FFA still warrants further studies, but our results may suggest that therapeutic approaches influencing FFA metabolism might improve the prognosis of individuals with or at high risk of cardiovascular disease.

Acknowledgments

The authors extend appreciation to the participants of the LURIC study because without their collaboration this article would not have been written. We thank the LURIC study team either temporarily or permanently involved in patient recruitment and sample and data handling and the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg and Ulm (Germany).

Received January 27, 2006. Accepted March 27, 2006.

Address all correspondence and requests for reprints to: Professor Dr. Med. Winfried März, Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria. E-mail: winfried.maerz@klinikum-graz.at.

This work was supported by Deutsche Forschungsgemeinschaft Grants SFB 518 and GRK 1041 (to B.O.B.).

References

1. **Bays H, Mandarino L, DeFronzo RA** 2004 Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J Clin Endocrinol Metab 89:463–478
2. **Poynten AM, Gan SK, Kriketos AD, Campbell LV, Chisholm DJ** 2005 Circulating fatty acids, non-high density lipoprotein cholesterol, and insulin-infused fat oxidation acutely influence whole body insulin sensitivity in non-diabetic men. J Clin Endocrinol Metab 90:1035–1040
3. **Stich V, Berlan M** 2004 Physiological regulation of NEFA availability: lipolysis pathway. Proc Nutr Soc 63:369–374
4. **Randle PJ, Garland PB, Hales CN, Newsholme EA** 1963 The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785–789
5. **Schaefer JR, Maisch B, Klumpp S, Krieglstein J** 2005 Why does atherosclerosis occur where it occurs? Atherosclerosis 180:417–418
6. **Hufnagel B, Dworak M, Soufi M, Mester Z, Zhu Y, Schaefer JR, Klumpp S, Krieglstein J** 2005 Unsaturated fatty acids isolated from human lipoproteins activate protein phosphatase type 2Cβ and induce apoptosis in endothelial cells. Atherosclerosis 180:245–254
7. **Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD** 1997 Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 100:1230–1239
8. **Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J, Baron AD** 2000 Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. Diabetes 49:1231–1238
9. **Carlsson M, Wessman Y, Almgren P, Groop L** 2000 High levels of nonesterified fatty acids are associated with increased familial risk of cardiovascular disease. Arterioscler Thromb Vasc Biol 20:1588–1594
10. **Pirro M, Mauriege P, Tchernof A, Cantin B, Dagenais GR, Despres JP, Lamarche B** 2002 Plasma free fatty acid levels and the risk of ischemic heart disease in men: prospective results from the Quebec cardiovascular study. Atherosclerosis 160:377–384
11. **Hendrickson SC, Louis JD, Lowe JE, Abdel-aleem S** 1997 Free fatty acid metabolism during myocardial ischemia and reperfusion. Mol Cell Biochem 166:85–94

12. **Oliver MF, Opie LH** 1994 Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. Lancet 343:155–158
13. **Oliver MF** 2002 Metabolic causes and prevention of ventricular fibrillation during acute coronary syndromes. Am J Med 112:305–311
14. **Manzella D, Barbieri M, Rizzo MR, Ragno E, Passariello N, Gambardella A, Marfella R, Giugliano D, Paolisso G** 2001 Role of free fatty acids on cardiac autonomic nervous system in noninsulin-dependent diabetic patients: effects of metabolic control. J Clin Endocrinol Metab 86:2769–2774
15. **Korge P, Honda HM, Weiss JN** 2003 Effects of fatty acids in isolated mitochondria: implications for ischemic injury and cardioprotection. Am J Physiol Heart Circ Physiol 285:259–269
16. **Azzazy HM, Pelsers MM, Christenson RH** 2006 Unbound free fatty acids and heart-type fatty acid-binding protein: diagnostic assays and clinical applications. Clin Chem 52:19–29
17. **Yagyu H, Chen G, Yokoyama M, Hirata K, Augustus A, Kako Y, Seo T, Hu Y, Lutz EP, Merkel M, Bensadoun A, Homma S, Goldberg IJ** 2003 Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. J Clin Invest 111:419–426
18. **Chiu HC, Kovacs A, Blanton RM, Han X, Courtois M, Weinheimer CJ, Yamada KA, Brunet S, Xu H, Nerbonne JM, Welch MJ, Fettig NM, Sharp TL, Sambandam N, Olson KM, Ory DS, Schaffer JE** 2005 Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. Circ Res 96:225–233
19. **Jouven X, Charles MA, Desnos M, Ducimetiere P** 2001 Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population. Circulation 104:756–761
20. **Charles MA, Fontbonne A, Thibult N, Claude JR, Warnet JM, Rosselin G, Ducimetiere P, Eschwege E** 2001 High plasma nonesterified fatty acids are predictive of cancer mortality but not of coronary heart disease mortality: results from the Paris prospective study. Am J Epidemiol 153:292–298
21. **Forsblom CM, Sane T, Groop PH, Tötterman KJ, Kallio M, Saloranta C, Laasonen L, Summanen P, Lepäntalo M, Laatikainen L, Matikainen E, Teppo AM, Koskimies S, Groop L** 1998 Risk factors for mortality in type II (non-insulin-dependent) diabetes: evidence of a role for neuropathy and a protective effect of HLA-DR 4. Diabetologia 41:1253–1262
22. **Winkelmann BR, März W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J** 2001 Rationale and design of the LURIC study: a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. Pharmacogenomics 2:1–73
23. **Boehm BO, Schiekofer S** 2006 The metabolic syndrome. Heart Metab 30:7–10
24. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419
25. **Korvald C, Elevenes OP, Myrmel T** 2000 Myocardial substrate metabolism influences left ventricular energetics *in vivo*. Am J Phys Heart Circ Physiol 278:1345–1351
26. **Chandler MP, Chavez PN, McElfresh TA, Huang H, Harmon CS, Stanley WC** 2003 Partial inhibition of fatty acid oxidation increases regional contractile power and efficiency during demand-induced ischemia. Cardiovasc Res 59: 143–151
27. **Wallhaus TR, Taylor M, DeGrado TR, Russell DC, Stanko P, Nickles RJ, Stone CK** 2001 Myocardial free fatty acid and glucose use after carvedilol treatment in patients with congestive heart failure. Circulation 103:2441–2446
28. **Murray AJ, Anderson RE, Watson GC, Radda GK, Clarke K** 2004 Uncoupling proteins in human heart. Lancet 364:1786–1788
29. **Beanlands RS, Nahmias C, Gordon E, Coates G, deKemp R, Firnau G, Fallen E** 2000 The effects of β_1-blockade on oxidative metabolism and the metabolic cost of ventricular work in patients with left ventricular dysfunction. Circulation 102:2070–2075
30. **Opie LH** 2004 The metabolic vicious cycle in heart failure. Lancet 364:1733–1734
31. **Birkenfeld AL, Boschmann M, Moro C, Adams F, Heusser K, Franke G, Berlan M, Luft FC, Lafontan M, Jordan J** 2005 Lipid mobilization with physiological atrial natriuretic peptide concentrations in humans. J Clin Endocrinol Metab 90:3622–3628
32. **Lommi J, Kupari M, Yki-Järvinen H** 1998 Free fatty acid kinetics and oxidation in congestive heart failure. Am J Cardiol 81:45–50
33. **Oram JF, Bornfeldt KE** 2004 Direct effects of long-chain non-esterified fatty acids on vascular cells and their relevance to macrovascular complications of diabetes. Front Biosci 9:1240–1253
34. **Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P** 2003 Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 52:2882–2887
35. **Paolisso G, Gualdiero P, Manzella D, Rizzo MR, Tagliamonte MR, Gambardella A, Verza M, Gentile S, Varricchio M, D'Onofrio F** 1997 Association of fasting plasma free fatty acid concentration and frequency of ventricular premature complexes in nonischemic non-insulin-dependent diabetic patients. Am J Cardiol 80:932–937
36. **Frayn KN** 2005 Plasma non-esterified fatty acids: why are we not measuring them routinely? Ann Clin Biochem 42:413–414
37. **Folmes CD, Clanachan AS, Lopaschuk GD** 2005 Fatty acid oxidation inhibitors in the management of chronic complications of atherosclerosis. Curr Atheroscler Rep 7:63–70
38. **Frayn KN, Fielding BA, Karpe F** 2005 Adipose tissue fatty acid metabolism and cardiovascular disease. Curr Opin Lipidol 16:409–415
39. **Dyck JR, Cheng JF, Stanley WC, Barr R, Chandler MP, Brown S, Wallace D, Arrhenius T, Harmon C, Yang G, Nadzan AM, Lopaschuck GD** 2004 Malonyl coenzyme A decarboxylase inhibition protects the ischemic heart by inhibiting fatty acid oxidation and stimulating glucose oxidation. Circ Res 94:78–84

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.