Nonesterified Fatty Acids and Risk of Sudden Cardiac Death in Older Adults

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Background—Although nonesterified fatty acids (NEFA) have been positively associated with coronary heart disease risk factors, limited and inconsistent data are available on the relation between NEFA and sudden cardiac death.

Methods and Results—Using a prospective design, we studied 4657 older men and women (mean age, 75 years) from the Cardiovascular Health Study (1992–2006) to evaluate the association between plasma NEFA and the risk of sudden cardiac death in older adults. Plasma concentrations of NEFA were measured using established enzymatic methods, and sudden death was adjudicated using medical records, death certificates, proxy interview, and autopsy reports. We used Cox proportional hazard models to estimate multivariable-adjusted relative risks. During a median follow-up of 10.0 years, 221 new cases of sudden cardiac death occurred. In a multivariable model adjusting for age, sex, race, clinic site, alcohol intake, smoking, prevalent coronary heart disease and heart failure, and self-reported health status, relative risks (95% confidence interval) for sudden cardiac death were 1.0 (ref), 1.15 (0.81–1.64), 1.06 (0.72–1.55), and 0.91 (0.60–1.38) across consecutive quartiles of NEFA concentration. In secondary analyses restricted to the first 5 years of follow-up, we also did not observe a statistically significant association between plasma NEFA and sudden cardiac death.

Conclusions—Our data do not provide evidence for an association between plasma NEFA measured late in life and the risk of sudden cardiac death in older adults. (Circ Arrhythm Electrophysiol. 2012;5:273-278.)

Key Words: epidemiology sudden death fatty acids risk factors

Each year, about 450 000 Americans die of sudden cardiac death (SCD). Coronary heart disease (CHD) accounts for a large proportion of SCD cases, and, consequently, major risk factors for CHD are also associated with SCD. Indeed, SCD is the first manifestation of CHD in about 50% of cases, setting a daunting task to prevent its occurrence among patients without overt CHD. This underscores the importance of identifying novel biomarkers for SCD that might help in risk stratification and perhaps offer new opportunities for pharmacological or other targeted interventions.

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Nonesterified fatty acids (NEFA) are metabolic byproducts of lipolysis and have been associated with abnormal glucose metabolism and diabetes, a major CHD risk factor. In the fasting state, NEFA are largely derived from adipose tissue lipolysis. Whereas insulin resistance promotes greater lipolysis, higher levels of circulating NEFA in turn may further impair insulin signaling and secretion from pancreatic β-cells and promote hepatic gluconeogenesis. In experimental models, higher plasma NEFA concentrations induce peripheral insulin resistance in vivo. Epidemiological studies have reported positive associations between NEFA and the risk of diabetes and other CHD risk factors. Potential effects of NEFA on the heart are less established. Under physiological conditions, the heart preferentially utilizes NEFA for energy, providing 60–70% of the adenosine triphosphate required by myocardial metabolism. Given a
higher oxygen demand for β-oxidation of NEFA than glucose utilization, greater availability and utilization of NEFA might predispose to severe arrhythmia18–20 and perhaps SCD. However, limited data exist on the association between NEFA and SCD in humans.21 We sought to test the hypothesis that plasma levels of NEFA are positively associated with incident SCD in older adults.

Methods

Study Population

The Cardiovascular Health Study (CHS) is a prospective, population-based cohort study of cardiovascular disease in older adults. In 1989–1990, 5201 people ages 65 years and older were recruited from a random sample of Medicare-eligible residents in 4 US communities: Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Allegheny County, PA. An additional cohort of 687 predominantly African-Americans was recruited during 1992 and 1993 from 3 of the same communities (except for Washington County), using the same sampling and recruitment methods. Each center’s institutional review board approved the study, and all participants gave informed written consent. Description of the design, sampling, and recruitment in the CHS has been published.22

The 1992–1993 examination served as the baseline for this analysis. Of the 5265 participants who were alive and participated in the 1992–1993 examination, we excluded 493 subjects without a blood sample; 57 with missing NEFA measurements; and 58 individuals with missing covariates. The final analysis sample included 4657 participants.

Measurement of NEFA

Plasma samples collected at the 1992–1993 examination were stored at −70°C until analyzed at the Central Laboratory at the University of Vermont in 2010. NEFA concentration in plasma was measured in duplicate by the Wako enzymatic method and the average of the 2 measurements was used in current analyses. This technique utilizes the acylation of coenzyme A by the fatty acids in the presence of added acyl-CoA synthetase. Acyl-CoA produced is oxidized by added acyl-CoA oxidase with generation of hydrogen peroxide, which in the presence of peroxidase permits the oxidative condensation of 3-methy-N-ethyl-N(β-hydroxyethyl)-aniline with 4-aminonaphtyline to form a purple-colored adduct. The latter is then measured colorimetrically at 550 nm. Intra-assay coefficient of variation was 5%.

Ascertainment of SCD in CHS

Details of the procedures used in CHS to identify and classify cardiovascular events and deaths have been published.23,24 Information on vital status was available for 100% of participants. Cause of death is adjudicated by the CHS Events Subcommittee after review of death certificates, inpatient medical records, nursing home or hospice records, physician questionnaires, interviews with next-of-kin, and autopsy records where available. This analysis included events occurring by June 2006. SCD is defined as a sudden pulseless condition, presumed due to cardiac arrhythmia, in a previously stable individual that occurred out of the hospital or in the emergency room. For unwitnessed deaths, the participant must have been seen within 24 hours of the arrest in a stable condition and without evidence of a noncardiac cause of cardiac arrest. By definition, SCD cases could not be under hospice or nursing home care or have a life-threatening noncardiac comorbidity. SCD cases were identified and classified by a cardiologist’s record review of all CHS Events Subcommittee adjudicated cardiac deaths. A blinded second physician review of a random sample of 70 of these death records showed an 88% inter-reviewer agreement and κ = 0.74 for SCD.

Other Risk Factors

Information on age, sex, race, educational attainment, physical activity, smoking status, alcohol consumption, and dietary habits were based on self-report at baseline. Leisure-time activity (kcal/wk) was assessed using a modified Minnesota Leisure-Time Activities questionnaire.24 Usual dietary habits were assessed using a picture-sort food frequency questionnaire25 in 1989–1990 and a Willett food frequency questionnaire in 1995–1996. Weight, height, and waist circumference were measured using standardized protocols. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. Diabetes was defined as a use of insulin or oral hypoglycemic agents, a fasting glucose level of ≥7 mmol/L (126 mg/dL), or a nonfasting glucose level of ≥11.1 mmol/L (200 mg/dL). CHD end points and congestive heart failure were adjudicated by an end point committee. Detailed descriptions of cardiovascular adjudication in the Cardiovascular Health Study have been published previously.26,27 Prevalent CHD and heart failure was based on status at baseline (1992–1993 examination).

Statistical Analysis

We categorized participants into quartiles based on the distribution of NEFA concentrations in the study sample. Probability values for trends were calculated using linear regression for continuous variables and a nonparametric test across ordered groups for categorical variables. To compute probability values for difference between SCD and non-SCD groups, we used t test for normally distributed continuous variables, the Wilcoxon rank-sum test for continuous variables with skewed distributions, and the χ2 test for categorical variables. To estimate the risk of SCD associated with NEFA categories, we used Cox proportional hazards regression to estimate relative risks, with the lowest quartile serving as the reference group. An initial multivariable model adjusted for age, sex, race, and clinic site and a second model additionally adjusted for smoking status (never, former, current), alcohol consumption (0, <7, ≥7 drinks/wk), prevalent CHD, prevalent congestive heart failure, and self-reported health status (excellent, very good, good, fair, poor). Additional adjustment for body mass index, diabetes, HDL-cholesterol, hypertension, hormone replacement therapy, broiled or baked fish consumption, and physical activity did not appreciably alter the results.

We evaluated potential heterogeneity in the association of NEFA with SCD by comparing stratified hazard ratio estimates by sex, BMI (<25 versus ≥25 kg/m2), and age (<75 versus ≥75 y). In sensitivity analyses, we stratified by presence of CHD, ECG abnormality, or prevalent diabetes. Because a single measurement of NEFA may not capture changes over a long period of follow-up, we repeated the main analysis while restricting follow-up to the first 5 years. Last, we repeated main analyses restricted to the first 5 years of follow-up (n = 95 SCD cases) in a minimally adjusted model (age, sex, race, and clinic site) as well as a full adjusted model (with additional inclusion of smoking, alcohol use, CHD, heart failure, and self-reported health status). We used Schoenfeld residuals to evaluate proportional hazards assumptions and found no appreciable evidence of violations. All analyses were conducted using Stata, version 11.2 (StataCorp LP, College Station, TX).

Results

Of the 4657 participants, 58% were women and 16% were African-Americans. The mean age was 74.9 ± 5.3 years (range, 65–98 years). Table 1 shows the baseline characteristics according to plasma NEFA. Higher levels of plasma NEFA were associated with older age, higher measures of adiposity, female sex, lower levels of physical activity, poor self-reported health status, lower prevalence of smoking, higher concentration of triglycerides and HDL-cholesterol, higher prevalence of diabetes and hypertension, and lower prevalence of CHD. Table 2 presents baseline characteristics of participants who had SCD compared with those who did
People in which SCD occurred were older, more likely to be male, and to have higher waist circumference, low HDL-cholesterol, and a higher prevalence of diabetes, hypertension, coronary disease, and heart failure.

During an average follow-up of 10.0 years (maximum of 13.5 years), 221 cases of SCD occurred. Crude incidence rate of SCD were 5.15, 5.45, 4.56, and 3.78 cases per 1000 person-years from the lowest to the highest quartile of plasma NEFA. In a multivariable model adjusting for age, sex, race, and clinic site, there was no significant association between NEFA and SCD (Table 3). Additional control for smoking, alcohol consumption, self-reported health status, prevalent CHD, and heart failure did not alter these findings (Table 3). Additional adjustment for lipid-lowering drugs, β-blockers, BMI, diabetes, hypertension, exercise, hormone replacement therapy, and serum albumin did not change the results (data not shown). The association between NEFA and SCD did not differ by sex [fully adjusted relative risk (95% confidence interval [CI]): 1.0 (ref), 1.27 (0.84–1.92), 0.75 (0.44–1.31), 1.05 (0.59–1.85) from the lowest to the highest quartile of NEFA in men; corresponding values for women were 1.0 (ref), 0.99 (0.50–1.93), 1.43 (0.77–2.63), and 0.89 (0.46–1.72)]. Stratification by body mass, age, prevalent diabetes, ECG abnormality, or CHD did not show any association.
between NEFA and SCD (data not shown). The relative risk (95% CI) per unit change in plasma NEFA modeled continuously was 0.66 (0.31–1.40) for a model adjusted for age, race, sex, and clinic, and 0.72 (0.33–1.54) for a model additionally adjusted for smoking status, alcohol consumption, CHD, congestive heart failure, and health status.

In a sensitivity analysis restricted to the first 5 years of follow-up, plasma NEFA was not associated with SCD (n=95 events) in a model adjusted for age, race, sex, and clinic site [relative risks: 1.0 (ref), 1.06 (0.62–1.78), 0.75 (0.41–1.37), and 0.82 (0.45–1.51)] or fully adjusted model [relative risk (95% CI) of 1.0 (ref), 1.10 (0.65–1.86), 0.82 (0.47–1.42), and 0.66 (0.31–1.40)] across consecutive quartiles of NEFA. Exclusion of participants with prevalent CHD or heart failure did not alter the findings [multivariable adjusted relative risk for SCD 1.0 (ref), 1.09 (0.65–1.81), 1.35 (0.81–2.24), and 0.96 (0.54–1.70)] across consecutive quartiles of NEFA.

**Discussion**

In this large, prospective study of older adults with long-term follow-up and adjudicated incidence of SCD, plasma concentration of NEFA measured late in life was not associated with the risk of SCD. Considering only participants with or without prevalent diabetes or CHD at baseline or restricting follow-up to the first 5 years did not alter the results. Contrary to our results, the Paris Prospective Study \(^\text{28}\) reported a 70% increased risk of SCD per each standard deviation increase in NEFA in a multivariable model among French men [relative risk, 1.70 (1.21–2.13)]. The participants in the Paris prospective study \(^\text{28}\) were all men and were younger (age, 42–53 years at baseline) than subjects in our study (mean age, 75 years). Of note is that when we stratified our data by sex and age (<75 versus 75+ y), we did not observe any association between NEFA and SCD in any stratum. In another prospective study, Pilz et al \(^\text{17}\) observed a 76% increased risk of SCD, comparing the fourth to the first quartile of NEFA in patients referred for coronary angiography [hazard ratio, 1.76 (1.03–3.00)]. Findings from these 2 prospective studies are supported by the proarrhythmic effects of NEFA \(^\text{29–31}\). The discordance of these 2 studies with our results merits some comments.

It is possible that a lack of an association between NEFA and SCD in our cohort may be partially explained by the relatively older age in our cohort (75 years on average) and a relatively high prevalence of subclinical cardiovascular disease, making all subjects at a higher risk of SCD than those in the Paris Prospective study (42–53 years) \(^\text{28}\) or the LURIC study (51–75 years) in Germany. \(^\text{17}\) With a higher background...
rate of SCD in the reference group, greater statistical power is required to detect a modest increase in SCD risk with higher NEFA levels. Such a conjecture is supported by the relatively higher proportion of subjects with major risk factors for SCD in our cohort including diabetes, hypertension, heart failure, and CHD. We had 80% power to detect a 70% increase risk of SCD and 59% power to detect a 50% increase in SCD risk in our study when comparing the fourth to the first quartile of NEFA. It is also possible that people at higher risk of SCD (ie, subjects with prevalent CHD) were more likely to have lower plasma NEFA concentration due to aggressive dietary and pharmacological treatment for comorbidities. Such a scenario would have led to a depletion of susceptible pool of SCD in the highest quartile of NEFA and to a paradoxical lower risk of SCD with higher concentration of plasma NEFA. This hypothesis of confounding by indication is partly supported in our data by a higher prevalence of CHD in the lowest (26%) compared with the highest quartile of NEFA (20%). Furthermore, the fact that we observed a decrease in crude incidence rates of SCD across consecutive quartiles also lend support to the above hypothesis. In an animal experiment, injection of saturated fatty acids led to ventricular arrhythmia, suggesting that the composition of NEFA may be important. In our study, we were not able to examine the role of fatty acid type as we measured total NEFA. As an observational study, we cannot exclude the possibility that unmeasured or residual confounding accounted for our results. NEFA concentrations were measured late in life in our study, and we were not able to account for changes in plasma NEFA over time in our analyses as we had only a single baseline measurement of NEFA. The limited number of SCD events in our study precluded the ability to detect small yet clinically relevant associations between NEFA and SCD. Last, our sample consisted mostly of European-American individuals with an average age of 75 years at baseline, thereby limiting the generalizability of our results to other ethnic groups or younger adults. Nonetheless, our study has several strengths including a centralized and standardized approach to adjudicated SCD, a relatively large sample size of older adults from four geographic locations across the United States, 13.5 years of follow-up, robustness of the findings in stratified analyses (sex, age, BMI, comorbidity, etc) and a large number of covariates to assess confounding. In conclusion, our data do not support a major association between plasma NEFA measured late in life and the risk of SCD in older adults. Further investigation in other cohorts and experimental models is needed to clarify the role of NEFA in the development of SCD.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Each year, about 450 000 Americans die from sudden cardiac death (SCD). SCD is the first manifestation of coronary heart disease in about 50% of cases, thereby underscoring the importance of identifying novel biomarkers for SCD that might help in risk stratification and perhaps offer new opportunities for pharmacological or other targeted interventions. Nonesterified fatty acids (NEFA) are metabolic byproducts of lipolysis and have been associated with abnormal glucose metabolism and diabetes, a major coronary heart disease risk factor. Although NEFA have been positively associated with coronary heart disease risk factors, limited and inconsistent data are available on the relation between NEFA and SCD. In a prospective design, we studied 4657 older men and women (mean age, 75 years) from the Cardiovascular Health Study (1992–2006) to evaluate the association between plasma NEFA and the risk of SCD. During a median follow-up of 10.0 years, 221 new cases of sudden cardiac death occurred. Our data did not provide evidence for an association between NEFA and SCD [multivariable adjusted relative risks (95% confidence interval) for SCD were 1.0 (ref), 1.15 (0.81–1.64), 1.06 (0.72–1.55), and 0.91 (0.60–1.38) across consecutive quartiles of NEFA concentration. Our data suggest that plasma NEFA measured late in life are not related to the risk of SCD in older adults.
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