# Elevated plasma free fatty acids are associated with sudden death: A prospective community-based evaluation at the time of cardiac arrest

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**BACKGROUND** In cohort studies, elevated levels of plasma nonesterified free fatty acids (NEFAs) have been associated with increased risk of sudden cardiac death (SCD) in men, but blood samples were drawn several years before SCD.

**OBJECTIVE** To confirm this relationship by evaluating levels of plasma NEFAs at the time of the SCD event in a group of both men and women.

**METHODS** From the ongoing Oregon Sudden Unexpected Death Study, we compared levels of plasma NEFAs in 149 SCD cases presenting with ventricular fibrillation (mean age 64 ± 12 years; 73% men) and 149 age- and sex-matched controls with coronary artery disease. Plasma was processed from blood drawn at the time of arrest (cases) and at a routine visit (controls). The levels of plasma NEFAs were compared after categorizing into quartiles on the basis of control values. Conditional logistic regression was used to predict adjusted odds ratio for SCD associated with plasma NEFA levels per increased quartile.

**RESULTS** The plasma NEFA levels were significantly higher in SCD cases than in controls (median 0.39 mmol/L [interquartile range 0.28–0.60 mmol/L] vs 0.32 mmol/L [interquartile range 0.20–0.49 mmol/L]; $P = .002$). There were no significant differences in body mass index, smoking, and diabetes. The odds ratio for SCD was 1.42 (95% confidence interval 1.14–1.78) per quartile increase in the plasma NEFA level ($P = .002$). Individuals with plasma NEFA levels above the prespecified cutoff point of 0.32 mmol/L were at increased risk of SCD (odds ratio 2.00; 95% confidence interval 1.20–3.34; $P = .008$).

**CONCLUSION** These findings strengthen the role of plasma NEFA as a potential biomarker for the assessment of SCD risk.

**KEYWORDS** Fatty acids; Sudden cardiac death; Cardiac arrest; Biomarker; Risk prediction

**ABBREVIATIONS** **CAD** = Coronary artery disease; **CI** = Confidence interval; **IQR** = Interquartile range; **LVEF** = Left ventricular ejection fraction; **NEFA** = Nonesterified free fatty acid; **OR** = Odds ratio; **SCD** = Sudden cardiac death; **VF** = Ventricular fibrillation

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## Introduction

Sudden cardiac death (SCD) has been estimated to account for as much as 50% of all cardiovascular mortality in the United States.[1] Even though the majority of cases are found to have significant coronary artery disease (CAD), most will present with sudden cardiac arrest as the first manifestation of cardiac disease.[2] This observation highlights the need for, and importance of, improved SCD risk prediction models that incorporate the cumulative effects of multiple biomarkers.[3,4] In order to be useful for risk stratification in the general population, such tools need to be easy to deploy and affordable.[5,6] Therefore, the analysis of a marker must be robust, readily available on a larger scale, and easy to perform. Some, but not all, plasma biomarkers fit that description and there has been an increased interest in this field of research in recent years.

One biomarker with robust analysis methodology that has been evaluated in several cohort studies is the level of circulating nonesterified free fatty acids (NEFAs), compounds released from triglycerides stored in adipose tissue. In experimental settings, elevated levels of NEFAs have

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been shown to be proarrhythmic,[7,8] associated with risk of SCD in larger cohort studies,[9–11] and not associated in other reports.[12] However, definitions of SCD have not been consistent, the cohorts may not always be reflective of the general population, and the numbers of events have been relatively low. Most importantly, the samples have been drawn several years and even decades before the SCD event, which is the inherent weakness of such analyses.[13]

Therefore, we sought to validate the utility of elevated plasma NEFA levels as a risk predictor of SCD by analyzing samples drawn at the time of the SCD event from an ongoing study with well-characterized SCD cases and control participants.

## Methods

The Oregon Sudden Unexpected Death Study is an ongoing, prospective, community-wide study of out-of-hospital SCD that has been described in detail previously.[13]

In brief, the study is geographically located in the Portland, OR, metropolitan area with a population of ~1,000,000. Out-of-hospital SCD cases were identified through multiple sources: the Medical Examiner's Office, Emergency Medical Services, and all local hospitals. All available medical records (including prearrest medical records as well as Medical Examiner's Officer and Emergency Medical Services records) were obtained for each participant and reviewed in detail. A diagnosis of SCD was assigned by majority consensus after in-house adjudication by 3 physicians who closely evaluated the circumstances of arrest in combination with all available clinical information. SCD was defined as a sudden and unexpected pulseless condition of likely cardiac etiology.[3] An exhaustive review was performed of all available clinical and autopsy information in order to exclude all SCD cases with noncardiac cause and those with trauma/violent death, presence of a terminal illness, or death due to drug overdose.

During the same period, controls with CAD without a history of SCD were enrolled from the same geographic area. The rationale for using a population with CAD as a control group is based on prior studies showing that the vast majority (>80%–95%) of patients older than 50 years who died of sudden cardiac arrest, have significant postmortem findings of CAD.[1,14,15] This study design facilitates the identification of factors associated with SCD in the presence of CAD. Control participants across a broad spectrum of CAD were identified from patients undergoing coronary angiography at one of the region's major participating health systems, patients transported by the region's Emergency Medical Service system with complaints suggestive of coronary ischemia, and patients with existing CAD enrolled in a regional health maintenance organization. Medical records for each potential control were reviewed after obtaining consent; those with documented CAD (defined as below) were enrolled during a single visit.

This study was approved by the Institutional Review Board of Cedars-Sinai Medical Center, Oregon Health and Science University, as well as by all participating hospitals and health systems.

### Study population

For this analysis, cases identified from February 1, 2002, to January 31, 2011, were eligible if they were aged 35–84 years, were white, had a first monitored rhythm of ventricular fibrillation (VF), and had a sufficient blood sample drawn at the time of cardiac arrest. Cases (n = 150) were randomly selected from the entire pool of eligible cases. Controls (n = 150), also white, were age- and sex-matched to cases.

### Definitions

Details of clinical history such as hypertension, hyperlipidemia, heart failure, and diabetes mellitus were obtained from the clinical records. Any participant with a stenosis occluding >50% of the coronary artery lumen on coronary angiogram (before SCD) or autopsy, or a history of myocardial infarction or coronary vascularization, was considered as having CAD. Body mass index was calculated as mass (kg) divided by the height squared ($m^2$). A subset of patients had undergone quantitative assessment of left ventricular systolic function with echocardiogram before but not related to the SCD event or enrollment visit. Left ventricular ejection fraction (LVEF), a variable associated with SCD,[16] was assessed and treated as a categorical variable and dichotomized as >35% or ≤35% in order to make it comparable to clinically relevant cutoff values.[2]

### Plasma analysis

For cases, blood samples were drawn during resuscitation but before time of death. For controls, blood was drawn during a routine outpatient visit. The collection containers were nonheparinized, and none of the participants were on heparin therapy. Plasma was separated and stored at −80ºC until thawed for assays. The total NEFA levels were measured by using ATAC 8000 (Elan Diagnostics, Smithfield, RI) with a NEFA kit (Wako Diagnostics, Richmond, VA) for enzymatic colorimetric assays. Intra- and interassay coefficients of variation were <5%. The sensitivity of the method was 0.0014 mmol/L.

### Canine model to assess effects of VF arrest on NEFA levels

In order to evaluate the potential effect of the VF arrest event on the levels of circulating NEFAs, 17 female hound dogs (aged 9–19 months) were included in a canine ancillary study. Blood was drawn 1 week before inducing VF, just before cardiac arrest, 1 minute after VF, and 5 minutes after VF. Dogs were intubated and placed on a ventilator. Preanesthesia, anesthesia, and paralysis were performed using acepromazine subcutaneously, propofol intravenously, and isoflurane and oxygen as gas anesthesia. VF was induced by rapid ventricular pacing. Blood was collected in EDTA-coated tubes. Plasma was processed and stored at −80°C. NEFA levels were measured by using a Free Fatty Acid