Circulating Nonesterified Fatty Acid Level as a Predictive Risk Factor for Sudden Death in the Population

Xavier Jouven, MD, PhD; Marie-Aline Charles, MD; Michel Desnos, MD; Pierre Ducimetière, PhD

Background—In ischemic conditions, concentration of circulating nonesterified fatty acids (NEFA) is increased and has a proarrhythmic effect that is responsible for ventricular tachyarrhythmias. In nonischemic patients, high NEFA plasma concentration has been shown to be associated with frequent premature ventricular complexes and increased familial risk of cardiovascular disease, but its relation to sudden death has not been studied. We assessed the role of circulating NEFA in sudden death in asymptomatic men in a long-term cohort study.

Methods and Results—A total of 5250 men employed by the city of Paris, aged 42 to 53 in 1967 to 1972, free of known ischemic cardiac disease, and included in the Paris Prospective Study I, completed a second annual examination and had fasting plasma circulating NEFA measured. Each subject underwent a physical examination and ECG, provided blood for laboratory tests, and answered questionnaires administered by trained interviewers. Vital status was obtained for each subject from specific inquiries until he retired; after retirement, it was obtained from death certificates. Body mass index, systolic and diastolic blood pressures, tobacco consumption, parental history of sudden death, fasting cholesterol level, and circulating NEFA concentration were independent factors associated with sudden death during follow up (average, 22 years). When adjusted for confounding factors, circulating NEFA concentration remained an independent risk factor for sudden death (relative risk, 1.70; 95% confidence interval, 1.21 to 2.13) but not for fatal myocardial infarction.

Conclusions—Circulating NEFA concentration is an independent risk factor for sudden death in middle-aged men. Some form of primary prevention could be envisaged in subjects at high risk of sudden death. (Circulation. 2001;104:756-761.)

Key Words: death, sudden □ fatty acids, nonesterified □ epidemiology □ risk factors

Sudden death remains a critical problem in industrially developed countries, and treatment of sudden death victims frequently is unsuccessful.¹² Therefore, early identification of subjects at high risk of sudden death is essential to preventive treatment strategies. Circulating nonesterified fatty acids (NEFA), also called free fatty acids, have been described as responsible for ventricular arrhythmias and sudden death after myocardial infarction.³⁻⁶ However, a possible arrhythmogenic role of NEFA has not been investigated in nonischemic patients. The main source of circulating NEFA is release from adipose tissue. A higher frequency of premature ventricular complexes was found to be associated with increased circulating NEFA concentration in patients with non-insulin-dependent diabetes mellitus.⁷ A recent study observed an association between high NEFA concentration in offspring and increased risk of cardiovascular disease in their parents.⁸ Additionally, direct arrhythmogenic action of an experimental high concentration of NEFA was described in nonischemic isolated perfused rat hearts.⁹

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If elevated circulating NEFA concentration has a direct arrhythmogenic effect in asymptomatic subjects, these subjects may be predisposed to sudden death. We used the long follow-up period of the Paris Prospective Study I (>20 years) to assess the role of circulating NEFA concentration as a risk factor for sudden death in middle-aged men without known cardiovascular disease.

Methods

Details of the Paris Prospective Study I concerning recruitment, design, and procedures have been described elsewhere.¹⁰ Briefly, after oral informed consent was obtained, examination of 7746 native Frenchmen employed by the Paris Civil Service and aged 42 to 53 years was performed from 1967 through 1972. Subjects underwent an ECG and a physical examination conducted by a physician, provided blood samples for laboratory tests, and answered questionnaires administered by trained interviewers regarding sociodemographic factors, family and personal medical history, and smoking habits. Subjects were asked for history of parental myocardial infarction, parental age at death, and whether these deaths were
sudden. Diabetic status was defined as known, reported diabetes, whether treated or not. A second examination was proposed 1 year after inclusion, during which subjects underwent a physical examination and provided blood samples under standardized conditions (after an overnight fast, seated subjects provided samples between 8:30 and 9 AM; subjects were prohibited from smoking and exercise (after an overnight fast, seated subjects provided samples between 8:30 and 9 AM; subjects were prohibited from smoking and exercise before blood was taken). 11–14 Laboratory tests included measurement of circulating insulin (radioimmunological determination15) and NEFA concentration (colorimetric determination after immediate centrifugation at 4°C).16 All variables used in the present analysis were issued from the second examination except tobacco consumption, which was taken to be average consumption in the 5 years preceding screening.

Follow-Up
Each year, the administrative department in charge of the population provided a list of deceased subjects. All available data relevant to cause of death were collected by use of specific inquiries (ie, medical records from hospital departments or general practitioners indicated by the relatives of the deceased). Data were reviewed by an independent medical committee. Sudden death was defined as natural death that occurred within 1 hour after onset of acute symptoms. Death was coded as fatal myocardial infarction only if found to be related strictly to coronary disease. After retirement, causes of death mostly were obtained from death certificates. The eighth16 and ninth revisions of the International Classification of Diseases were used for coding (sudden death, code 798.1; coronary heart disease, codes 410 to 414).

Deadline of the follow-up period was January 1, 1994. Vital status could not be obtained for 355 subjects (4.6%). Men (n=245) born outside of France and men (n=169) with a diagnosis of ischemic heart disease (myocardial infarction or angina) at inclusion in the study (from personal medical history, clinical examination, or ECG) were excluded from analysis. The present analysis was conducted on the 5250 remaining men who completed the second examination.

Statistical Analysis
ANOVA and χ² analysis were used for global comparisons among groups. Because of the skewed distribution of triglycerides and insulin, log-transformed values were used in the analysis. Distribution of NEFA also was partially skewed. Untransformed values of NEFA were used. However, results were similar after log transformation. Spearman’s rank correlations were used for correlations. Relative risks (RR) of mortality were adjusted for confounding factors and estimated by the Cox proportional hazard model. SAS procedures (Statistical Analysis System) were used for analysis.

Results
Among the 5250 men followed for an average of 22 years, 1601 deaths occurred. Of these deaths, 463 were cardiovascularly related: 91 were sudden deaths (19.7%), 145 were fatal myocardial infarctions (31.3%), 22 were from cardiac failure (4.8%), 85 were from other cardiac causes (18.4%), 83 were from stroke (17.9%), and 37 were from other vascular causes (8.0%).

Table 1 gives characteristics of the subjects. Except for parental sudden death (P=0.08), all reported parameters differed significantly between the 3 groups of men: those who died from sudden death, those who died from myocardial infarction, and all other subjects (taken as controls), regardless of whether they were alive at the end of follow-up. Men who died from myocardial infarction had values for body mass index, tobacco consumption, heart rate, blood pressure, and triglyceride concentration that were intermediate between controls and men who died suddenly. Age and cholesterol concentrations were similar among men who died from myocardial infarction and those who died suddenly but were higher than in controls. Prevalence of diabetic men and increased plasma glucose, insulin, and NEFA concentrations were significantly higher only in subjects who died suddenly compared with controls and subjects who died from myocardial infarction. Prevalence of ECG abnormalities (premature ventricular complexes, premature supraventricular complexes, and complete or incomplete bundle branch block) was similar for the 3 groups (data not shown).

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**TABLE 1. Characteristics at Inclusion of Subjects Who Will Die of Sudden Death or Myocardial Infarction During Follow-Up and of All Other Participants Taken as Control**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sudden Death (n=91)</th>
<th>Fatal Myocardial Infarction (n=145)</th>
<th>Controls (n=5014)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48.3±1.7</td>
<td>48.4±1.8</td>
<td>48.0±1.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.4±3.7</td>
<td>26.3±3.1</td>
<td>26.0±3.3</td>
<td>0.0003</td>
</tr>
<tr>
<td>Tobacco consumption, g/d</td>
<td>13.1±9.6</td>
<td>12.1±10.5</td>
<td>10.4±10.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetic status, n (%)</td>
<td>7 (7.7)</td>
<td>5 (5.3)</td>
<td>134 (2.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Parental sudden death, n (%)</td>
<td>15 (16.5)</td>
<td>15 (10.3)</td>
<td>505 (10.1)</td>
<td>0.08</td>
</tr>
<tr>
<td>Parental myocardial infarction, n (%)</td>
<td>9 (9.9)</td>
<td>20 (13.8)</td>
<td>308 (6.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>70.5±10.3</td>
<td>68.2±10.2</td>
<td>67.1±10.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>155±29</td>
<td>152±28</td>
<td>144±22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>88±16</td>
<td>85±15</td>
<td>81±13</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>231±36</td>
<td>229±42</td>
<td>214±42</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>150±121</td>
<td>141±117</td>
<td>125±103</td>
<td>0.0008</td>
</tr>
<tr>
<td>Glycemia, mg/dL</td>
<td>108±27</td>
<td>103±16</td>
<td>102±15</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>110±92</td>
<td>84±47</td>
<td>88±68</td>
<td>0.02</td>
</tr>
<tr>
<td>Nonesterified fatty acids, μmol/L</td>
<td>384±334</td>
<td>310±123</td>
<td>313±144</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD unless otherwise indicated. Tobacco consumption is average consumption during 5 y preceding screening. P is by ANOVA and χ² for global comparisons among groups.
Fasting plasma NEFA concentration was significantly correlated with all variables except body mass index. Strength of this association was marked only by heart rate ($r = 0.28$) and blood pressure ($r = 0.25$); correlation coefficients were $<0.15$ for all other variables. Risk of sudden death increased gradually as NEFA concentration increased (divided into quintiles), whereas no such relationship existed for fatal myocardial infarction (Figure).

Heart rate, systolic and diastolic blood pressures, tobacco consumption, and triglyceride and cholesterol concentrations were risk factors for both sudden death and fatal myocardial infarction by univariate analysis (Table 2). Parental sudden death, body mass index, diabetic status, fasting plasma glucose, and insulin and NEFA concentrations were risk factors for sudden death but not fatal myocardial infarction, whereas parental myocardial infarction and age were risk factors only for fatal myocardial infarction.

When age, body mass index, heart rate, systolic blood pressure (or diastolic blood pressure), tobacco consumption, parental history of myocardial infarction and parental history

<p>| TABLE 2. RRrs Associated With Sudden Death and Fatal Myocardial Infarction by Univariate Analysis |
|---------------------------------------------------------------|---------------------------------------------------------------|
| <strong>Sudden Death</strong> | <strong>Fatal Myocardial Infarction</strong> |</p>
<table>
<thead>
<tr>
<th>RR (95% CI)</th>
<th>$P$</th>
<th>RR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.19 (0.96–1.45)</td>
<td>0.10</td>
<td>1.20 (1.00–1.41)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.45 (1.21–1.75)</td>
<td>0.001</td>
<td>1.08 (0.92–1.28)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>1.34 (1.10–1.62)</td>
<td>0.003</td>
<td>1.22 (1.16–1.48)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>1.34 (1.22–1.63)</td>
<td>0.0001</td>
<td>1.24 (1.05–1.48)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.52 (1.31–1.78)</td>
<td>0.0001</td>
<td>1.44 (1.26–1.66)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>1.62 (1.38–1.92)</td>
<td>0.0001</td>
<td>1.43 (1.24–1.65)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.29 (1.13–1.46)</td>
<td>0.0001</td>
<td>1.29 (1.18–1.46)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.29 (1.07–1.55)</td>
<td>0.007</td>
<td>1.23 (1.06–1.42)</td>
</tr>
<tr>
<td>Glycemia</td>
<td>1.21 (1.09–1.35)</td>
<td>0.0002</td>
<td>1.05 (0.86–1.16)</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.36 (1.11–1.69)</td>
<td>0.004</td>
<td>1.07 (0.90–1.26)</td>
</tr>
<tr>
<td>Nonesterified fatty acids</td>
<td>1.94 (1.59–2.3)</td>
<td>0.0001</td>
<td>1.04 (0.90–1.19)</td>
</tr>
<tr>
<td>Parental sudden death</td>
<td>1.78 (1.03–3.10)</td>
<td>0.04</td>
<td>1.08 (0.64–1.85)</td>
</tr>
<tr>
<td>Parental myocardial death</td>
<td>1.64 (0.82–3.26)</td>
<td>NS</td>
<td>2.49 (1.55–4.00)</td>
</tr>
<tr>
<td>Diabetic status</td>
<td>3.06 (1.42–6.62)</td>
<td>0.005</td>
<td>1.31 (0.54–3.20)</td>
</tr>
</tbody>
</table>

Tobacco consumption is average consumption (g/d) in the 5 years preceding screening. Increased risk of an event is for 1-SD increase in variables, including age (SD, 1.8 years), body mass index (SD, 3.3 kg/m²), tobacco consumption (SD, 10.2 g/d), heart rate (SD, 10.2 bpm), systolic blood pressure (SD, 22 mm Hg), diastolic blood pressure (SD, 13 mm Hg), cholesterol (SD, 42 mg/dL), triglycerides (SD, 103 mg/dL), plasma glucose (SD, 15 mg/dL), insulin (SD, 68 pmol/L) and nonesterified fatty acids (SD, 144 μmol/L).
of sudden death, cholesterol, triglycerides, fasting plasma glucose (or diabetic status) and insulin concentration were simultaneously entered into the survival model (Table 3), fasting plasma NEFA concentration remained an independent risk factor for sudden death (RR, 1.19; 95% CI, 1.06–1.34) but remained unassociated with fatal myocardial infarction (RR, 0.94; 95% CI, 0.75 to 1.09). Parental myocardial infarction remained an independent risk factor only for fatal myocardial infarction (RR, 0.56; 95% CI, 0.24 to 1.33). When deaths with causes determined from specific medical inquiries were analyzed (sudden death, n=67; fatal myocardial infarction, n=60) and their parents, results were similar, and NEFA concentration remained a risk factor for sudden death (RR, 1.19; 95% CI, 1.06–1.34) but not for fatal myocardial infarction (RR, 0.94; 95% CI, 0.75 to 1.09). Parental myocardial infarction remained a risk factor for sudden death (RR, 1.77; 95% CI, 1.33 to 2.34) but not fatal myocardial infarction (RR, 0.88; 95% CI, 0.69 to 1.18). Results were similar when diabetic subjects were excluded from analysis.

**Discussion**

After >20 years of follow-up, increased circulating NEFA concentration was an independent risk factor for sudden death but not fatal myocardial infarction in middle-aged men free of known cardiovascular disease. Whereas suspected proarrhythmic mechanisms of circulating NEFA are numerous, causes of chronic elevation of circulating NEFA are questionable.

**Proarrhythmic Mechanisms of Increased Circulating NEFA**

Proarrhythmic mechanisms of toxicity of increased NEFA are numerous and complex. NEFA have been shown to modulate the ATP-sensitive K⁺ channel and to activate ATP-insensitive K⁺ channels in rat heart cells. This may contribute to extracellular K⁺ accumulation and shortening of action-potential duration observed during early ischemia and anoxia. Even in absence of ischemia, a perfusion of high molar ratios of albumin-bound NEFA in isolated rat hearts has been shown to have a direct arrhythmogenic effect that could be mediated by Ca²⁺ overload in myocardial cells. Increased intracellular Ca²⁺ concentration induces uncoupling between cells, which supports occurrence of electrical reentry.

NEFA also may inhibit the Na⁺,K⁺ ATPase pump, thereby leading to high intracellular sodium and calcium, activating protein kinase and affecting Na⁺, K⁺, and Ca²⁺ currents. Arrhythmogenic effects of lysophospholipids, derived from the breakdown of membrane lipids during ischemia, and of acylcarnitine, derived at least in part from circulating NEFA, may be added. Accumulation of acylcarnitine can activate Ca²⁺ channels directly to produce arrhythmias through Ca²⁺ overload.

**Suspected Hyperadrenergic Tone**

Level of circulating NEFA is mediated largely by adrenergic stimulation, which may enhance its arrhythmogenic effect. A rise in plasma catecholamine concentration leads to increased release of NEFA from adipose tissue stores; in the present study, NEFA were relatively strongly correlated with heart...
rate and blood pressure. After inclusion of NEFA concentration into the multiadjusted model, heart rate was no longer an independent risk factor for sudden death. However, suspected hyperadrenergic tone is likely to play a direct role in arrhythmogenicity beyond causing a possible proarrhythmic effect of NEFA.

Coronary disease is frequent in sudden death patients >40 years of age, and we hypothesize that the expression and mechanisms of coronary disease may be variable in different subjects. For instance, although in some subjects presence of basal hyperadrenergic tone could lead to sudden death under certain ischemic conditions, under these same ischemic conditions, other subjects who do not have hyperadrenergic tone might be less likely to have arrhythmia during myocardial infarction. In the former case, an increase in NEFA concentration would be both a consequence of hyperadrenergia and a facilitating cause of sudden death through direct arrhythmogenic effects.

NEFA, Insulin, and Glucose
A rise in plasma catecholamine concentration leads to decreased secretion of insulin by pancreatic B cells, which is necessary for glucose uptake by the myocardium. NEFA metabolism is regulated by insulin that modulates lipolysis and reesterification of triglycerides in adipose cells. In the Paris Prospective Study I, high plasma NEFA concentration was associated with deterioration in glucose tolerance, independently of plasma insulin concentration. In our present findings, by use of univariate analysis, plasma glucose and insulin concentration were risk factors for sudden death but not fatal myocardial infarction. However, after adjustment for NEFA concentration, plasma glucose and insulin were no longer independent risk factors for sudden death. For Paolisso et al., in normotensive, nonischemic patients with non-insulin-dependent diabetes mellitus, premature ventricular complexes were associated independently with both insulin and NEFA level. Increases in blood pressure and in levels of plasma glucose, insulin, and triglycerides are features of the metabolic syndrome (syndrome X). Besides an increase in NEFA, all of these variables were increased at inclusion in subjects who died suddenly during follow-up.

Lowered Circulating NEFA, Polyunsaturated Fatty Acid Intake, and Sudden Death
Numerous studies have shown that a dietary supplement of polyunsaturated fatty acids can reduce the NEFA concentration in plasma and in cell membranes and have an antiarrhythmic effect. Dietary fish consumption and n-3 fatty acid intake were associated with a reduced risk of sudden death but not myocardial infarction in the US Physician’s Health Study. Under ischemic conditions, a nicotinic-acid analogue started within 5 hours after myocardial infarction decreased the high level of circulating NEFA and proportionally decreased occurrence of ventricular tachycardia. Therefore, although its clinical benefit is not yet proven, a decrease in NEFA level in subjects at high risk for sudden death may be envisaged as a preventive target. Further studies are needed to validate this hypothesis.

Study Limitations
Men included in the Paris Prospective Study I in whom NEFA concentrations were not measured (n=1829) had the same baseline characteristics and mortality rates as men who completed both examinations (n=5250); inclusion of the former group is unlikely to have introduced spurious consequences into our results.

Assessment of the effect of the definition of sudden death on the robustness of the results remains difficult. Although sudden death may be defined in various ways, the common working definition is a natural death that occurs within 1 hour of onset of acute symptoms. Although many sudden deaths are instantaneous or unwitnessed, the elapsed time can be estimated roughly at least. Some inaccurate coding is likely to have occurred for sudden death, especially when information came from death certificates. In 5 Minnesota communities that used 6-year mortality data, sensitivities of death certificates ranged from 24% to 87%, specificities from 66% to 85%, and positive predictive values from 19% to 27%, depending on how sudden death was defined. For coronary heart disease, sensitivity and positive predictive value of death certificates compared with autopsy results ranged from 70% to 90% but were lower for finer end points, including fatal myocardial infarction.

We cannot exclude that combining causes of death from specific inquiries and from death certificates might have consequences on our results. However, when we restricted the analysis to deaths with causes issued from specific medical inquiries, the association between NEFA concentration and sudden death and fatal myocardial infarction remained unchanged. Diagnosis of sudden death is debatable and imprecise among older subjects who are susceptible to various medical disorders. Nevertheless, we found consistent results when analysis was restricted to deaths that occurred in subjects <65 years of age.

Risk of sudden death increases comitantly with increases in the level of circulating NEFA, and no threshold exists for identification of people at high risk. This prevents us from considering NEFA measurement as a simple predictor of sudden death in clinical practice for a given subject, although high levels are associated with increased risk in the general population. Further studies are needed to define how this information can be of practical interest in clinical practice.

Conclusion
Like parental sudden death and diabetic status, which were both associated with sudden death but not fatal myocardial infarction in the Paris Prospective Study I, elevated fasting circulating NEFA concentration is an independent risk factor for sudden death in middle-aged men free of known cardiovascular disease. Further studies are needed to assess the potential contribution of NEFA measurement for the prediction of sudden death in clinical practice and of decreased NEFA levels as a possible target for prevention of sudden death.

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References


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