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Erythrocyte Very Long-Chain Saturated Fatty Acids Associated with Lower Risk of Incident Sudden Cardiac Arrest

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Abstract

Prior studies suggest that circulating n-3 and *trans*-fatty acids influence the risk of sudden cardiac arrest (SCA). Yet, while other fatty acids also differ in their membrane properties and biological activities which may influence SCA, little is known about the associations of other circulating fatty acids with SCA. The aim of this study was to investigate the associations of 17 erythrocyte membrane fatty acids with SCA risk. We used data from a population-based case-control study of SCA in the greater Seattle, Washington, area. Cases, aged 25–74 years, were out-of-hospital SCA patients, attended by paramedics (n=265). Controls, matched to cases by age, sex and calendar year, were randomly identified from the community (n=415). All participants were free of prior clinically-diagnosed heart disease. Blood was obtained at the time of cardiac arrest by attending paramedics (cases) or at the time of an interview (controls). Higher levels of erythrocyte very long-chain saturated fatty acids (VLSFA) were associated with lower risk of SCA. After adjustment for risk factors and levels of n-3 and *trans*-fatty acids, higher levels of 20:0 corresponding to 1 SD were associated with 30% lower SCA risk (13%–43%, p=0.001). Higher levels of 22:0 and 24:0 were associated with similar lower SCA risk (ORs for 1 SD-difference: 0.71 [95% CI: 0.57–0.88, p=0.002] for 22:0; and 0.79 [95% CI: 0.63–0.98, p=0.04] for 24:0). These novel findings support the need for investigation of biologic effects of circulating VLSFA and their determinants.

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Keywords

cardiac arrest; fatty acids; epidemiology

1. Introduction

Sudden cardiac arrest (SCA), also known as out-of-hospital cardiac arrest due a cardiac etiology, is a major cause of mortality in the general population, accounting for 10% of total mortality and 40% of mortality due to coronary heart disease [1]. While a number of risk factors are known, such as advanced age, male gender and coronary heart disease risk factors, discovery of novel risk factors is needed to make progress on the prevention of this devastating disease [2].

Fatty acids appear to influence the risk of incident SCA, possibly by direct mechanisms influencing the ion channels' membrane environment, or by indirect mechanisms influencing risk factors for SCA [3]. In particular, in both erythrocyte membranes and whole blood, higher levels of the long-chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are associated with lower risk [4, 5], and higher levels of the *trans*-fatty acids are associated with higher risk [6, 7, 8]. In addition, we reported that fatty acid biomarkers of *de novo* fatty acid synthesis, such as palmitic acid, are associated with higher risk of SCA [9].

In addition to the fatty acids mentioned above, cell membranes include very long-chain saturated fatty acids (with 20 or more carbons), fatty acids with odd numbers of carbons, n-6 fatty acids and n-9 fatty acids. Little is known of the potential association of these fatty acids with SCA. We previously measured fatty acids in a case-control study both as a biomarker of diet and of cell membrane composition. Having reported hypothesis-based fatty acid-SCA associations earlier [5, 7, 9, 10], we now explore the associations of these other erythrocyte fatty acids with incident SCA.

2. Materials and methods

2.1. Study subjects

The study was a population-based case-control study that has been described previously, and the study subjects in this report were included in earlier hypothesis-based reports of erythrocyte membrane fatty acids (n-3 fatty acids, *trans*-fatty acids and fatty acids markers of *de novo* fatty acid synthesis) and incident SCA [5, 7, 9, 10].

Cases were SCA cases aged 25–74 years, and were attended by paramedics between October 1988 and September 2005. SCA was defined as a sudden pulseless condition in the absence of evidence of a non cardiac cause of cardiac arrest [2]. In addition to emergency service incident reports, we reviewed death certificates and medical examiner reports when available, to exclude patients with cardiac arrest due to a noncardiac cause. We excluded patients with a history of diagnosed heart disease or life-threatening co-morbidities to avoid changes in diet before SCA, and users of fish oil supplements because fish oil affects membrane fatty acid composition. Included in the study were 265 SCA patients meeting the

above criteria, who were married (in order to interview their spouse), and on whom the paramedics were able to draw a blood sample at the time of cardiac arrest. We reported previously that cases with and without blood sample for the fatty acid measurements had similar levels of traditional SCA risk factors [5]. The response rate among the spouses of cases was 73%.

Control subjects (n=415) were a random sample from the community identified by random digit dialing. They were recruited concurrently with cases and were individually matched to case on age (within 7 years), sex, and calendar year at a ratio of approximately 2 to 1. Controls met the same eligibility criteria as the cases. The response rate among the spouses of controls was 55%. The University of Washington Human Subject Review Committee approved the study protocol and study subjects or their proxy signed an informed consent.

2.2. Red blood cell membrane fatty acid measurements

Blood specimens were collected from SCA patients in the field by attending paramedics, after essential emergency medical care had been provided and either the patient was clinically stable, or resuscitation had proven ineffective, usually within 30 to 45 min of the cardiac arrest. Blood specimens from controls were obtained at the time of an interview.

Erythrocytes were separated from plasma and white blood cells, washed three times with isotonic saline and stored until extraction at -80°C . Fatty acid methyl esters were prepared by direct trans-esterification using the method of Lepage and Roy [11]. The fatty acid methyl esters were separated on a 100m X 0.25mm internal diameter capillary silica column using a gas chromatograph equipped with a flame ionization detector as described previously [7]. Laboratory analyses were conducted by technicians blinded to case and control status. Quality control included the use of pooled red blood cells and internal standards. Specimens from each case and matched controls were submitted to gas chromatography in the same batch. Fatty acid levels were expressed as percentages of total fatty acids.

2.3. Other risk factors assessment

We collected information on demographic factors, medical conditions, dietary habits and lifestyle characteristics during a spouse interview, as previously described [5]. Dietary fat intake was estimated with the Northwest Lipid Research Clinic Fat Intake Scale [12], a qualitative measure that is correlated with dietary intake of saturated fat [5].

2.4. Statistical Analysis

Statistical analyses were carried out using STATA 11.0 (StataCorp, College Station, TX). To assess the association of fatty acids with SCA while taking into account the individual matching of cases and controls, we performed conditional logistic regression analyses. Fatty acid levels were included as continuous linear terms and odds ratios (estimates of relative risks) and 95% confidence intervals corresponding to a one standard deviation difference in fatty acid levels are presented. We also modeled the very long-chain fatty acids in quartiles and present the odds ratios for each upper quartile compared to the lowest quartile. Separate regressions were performed for each fatty acid, adjusted for age (years), current smoking

(yes/no), diabetes (yes/no), hypertension (yes/no), education beyond high school (yes/no), leisure-time physical activity (kcal/week), index of fat intake [5] (continuous linear), body weight (kg) and height (cm). We also performed analyses with further adjustment for erythrocyte levels of EPA+DHA and total *trans* isomers of linoleic acid (*trans*-18:2).

Data on the covariates included in the models were obtained from spouse interview for both cases and controls. Information on some of the covariates was missing on <3% of cases and <5% of controls. For the main analyses, missing values were imputed by multiple imputation, and five imputed datasets were combined using the method of Rubin[13] for estimating associations. Similar results were obtained when sensitivity analyses were restricted to those without missing values. To take into account multiple comparisons, we used a Bonferroni-corrected significance threshold of $0.05/17 \text{ tests} = 0.003$.

3. Results

The study included 265 SCA cases and 415 individually-matched controls. By design, the study subjects had no diagnosed heart disease before their index date. The study subjects were on average 58 years of age, 81% male, 91% White. Characteristics of cases and controls have been reported previously [10] and are provided as supplement table to this article (Table S1).

Table 1 shows the associations of incident SCA with 17 erythrocyte membrane fatty acids, not previously examined in the study. In analyses that accounted for the individual matching of controls to cases on age, gender and calendar year and were further adjusted for age, smoking, diabetes, hypertension, education beyond high school, leisure-time physical activity, fat intake, body weight and height, very long-chain saturated fatty acids (VLSFA) showed nominal associations with lower risk of SCA (Table 1). Higher levels of 20:0 (arachidic acid) corresponding to an increase of one standard deviation (SD) were associated with 24% lower risk of SCA (95% CI, 8%-38%). Similar point estimates were observed for the associations of 22:0 (behenic acid) and 24:0 (lignoceric acid) with SCA risk (Table 1). With further adjustments for levels of EPA+DHA and *trans*-18:2, the associations of 20:0 and 22:0 were slightly strengthened and reached the threshold significance for the conservative Bonferroni correction ($0.05/17 \text{ fatty acids tested} = 0.003$). The ORs corresponding to an increase of 1 SD were 0.70 (95% CI: 0.57–0.87, $p=0.001$) for 20:0, 0.71 (95% CI: 0.57–0.88, $p=0.002$) for 22:0 and 0.79 (95% CI: 0.63–0.98, $p=0.04$) for 24:0. When the VLSFA were modeled in quartiles, the highest quartile was associated with 49% (20:0), 52% (22:0) and 55% (24:0) lower risk of SCA compared to the lowest quartile (Table 2).

The VLSFA were correlated with each other ($r = 0.50$ between 20:0 and 22:0, $r = 0.54$ between 22:0 and 24:0 and $r = 0.24$ between 20:0 and 24:0). Exploratory analyses that included all three VLSFA in the same model resulted in attenuated odds ratios that were no longer significant (data not shown).

We explored covariates that might predict erythrocyte levels of VLSFA among the study controls. In both univariate and multivariate analyses, levels of VLSFA were not associated with prevalent diabetes, hypertension, body weight, education level, smoking status and

leisure-time physical activity (not shown). Levels of 22:0 and 24:0 were associated with an index of fat intake ($r = 0.20$), while 22:0 was inversely associated with EPA+DHA levels ($r = -0.30$) and with age ($r = -0.21$).

4. Discussion and conclusions

In this population-based case-control study, we report for the first time that higher erythrocyte levels of VLSFA, saturated fatty acids with 20 carbons or more, are associated with lower risk of incident SCA. The associations were independent of traditional risk factors and erythrocyte levels of n3 and *trans*-fatty acids. Higher levels of 20:0 or 22:0 corresponding to a 1 SD-difference were associated with about 30% lower risk of SCA.

The study results are in stark contrast with the previous report of higher risk of SCA associated with higher levels of palmitic acid (16:0) and other markers of *de novo* fatty acid synthesis in this study [9]. Altogether these findings illustrate the need to differentiate the health effects of VLSFA from that of 16:0.

The VLSFA, 20:0, 22:0 and 24:0, are an important component of sphingomyelins and ceramides [14], and may affect their biological activities [15]. The VLSFA content of sphingomyelin may influence the structure/function of lipid rafts, sphingomyelin-rich membrane domains where ion channels and signaling molecules are located; and lipid rafts in cardiomyocytes may play a role in cardioprotection against myocardial ischemia and reperfusion injury [16], processes involved in the pathogenesis of ventricular fibrillation and SCA [17, 18]. In addition, experimental evidence suggests the VLSFA content of ceramide has a profound influence on ceramide-induced apoptosis [15]. In cultured cells, only ceramide containing 16:0 promote apoptosis, and the balance between 16:0 and VLSFA containing ceramides appears to be the key factor for the induction of apoptosis [19]. Furthermore, in mice with heart-specific deletion of *SPTLC2* (serine-palmitoyl transferase 2), heart levels of ceramides with 20:0 and 24:0 are lower, and the heart shows more (not less) apoptosis [20]. Ceramide increases in the myocardium during experimental ischemia and reperfusion [21], therefore the VLSFA in ceramide may influence the process of apoptosis and the consequence of myocardial ischemia. Further studies are needed to explore potential protection from arrhythmia genesis from lipids containing VLSFA.

While we did not have detailed dietary information, the study suggests that dietary saturated fat in general influences circulating levels of VLSFA. The fatty acids 20:0, 22:0 and 24:0 are found in small quantities in peanuts and other nuts including macadamia and cashew nuts, and in canola oil [22]. The extent to which circulating VLSFA reflect dietary VLSFA is not well known, however, in a small dietary trial, macadamia nut intake substantially raised plasma levels of 20:0 and 22:0 [23]. Interestingly, nut consumption in the Nurse's Health Study was reported associated with lower risk of sudden cardiac death [24]. Whether circulating 20:0, 22:0 or 24:0 mediated the nut association is not known.

The VLSFA can be also be produced endogenously from 16:0, by a series of elongation steps. Three of the 6 mammalian elongases contribute to the elongation of 16:0; elovl6 produces 18:0 from 16:0; elovl1, elovl3 and elovl7 all have the ability to elongate 18:0 to 20:0 and 20:0 to 22:0, and elovl1 and elovl3 elongate 22:0 to 24:0 [25]. The elongation

process occurs in the endoplasmic reticulum, and is separate from *de novo* fatty acid synthesis, which generates 16:0 and occurs in the cytosol. While diet is known to influence *de novo* fatty acid synthesis [26–28], the factors, dietary and otherwise, that regulate the elongation to VLSFA are not known. Further studies are needed to examine the extent to which diet and metabolism contribute to circulating levels of 20:0, 22:0 and 24:0.

The other fatty acids examined in this study, 15:0 and 17:0, the n-9 fatty acids and n-6 fatty acids were not associated with risk. The fatty acids 15:0 and 17:0 are markers of dairy consumption, and the study suggests dairy consumption may not influence SCA risk. The n-9 fatty acids tend to be elevated in the context of essential fatty acid deficiency [29] which may be infrequent in our Western study population of middle aged adults. The n-6 fatty acid linoleic acid is the major polyunsaturated fatty acid in the diet, and membrane levels are a good biomarker of intake [30, 31]. Meta-analyses have reported greater intake of linoleic acid is related to lower risk of coronary heart disease [32, 33]. The current study suggests that higher levels of linoleic acid and its longer chain derivatives, unlike n-3 polyunsaturated fatty acids, are not associated with lower risk of SCA. We also examined DPA (22:5n3), an n-3 fatty acid that appears to have a metabolic origin rather than diet [34]. The study suggests that unlike EPA and DHA, DPA is not associated with risk of SCA.

The strengths of the study include the use of population-based cases and controls, the objective assessment of fatty acid levels in erythrocyte membranes and adjustment of results for other known risk factors. To address the possibility that cases might have changed their diet or lifestyle as a consequence of poor health leading to SCA, we restricted the case-control study to cases with no history of clinically-recognized heart disease and no life-threatening co-morbidities. While the study spanned 15 years during which dietary trends may have occurred, cases and individually-matched controls were recruited concurrently, and samples within each pair of cases and matched controls were submitted to gas chromatography in the same batch.

Due to the observational nature of the study, the possibility of residual confounding cannot be eliminated. The use of surrogate respondents in the study may have introduced some misclassification in assessment of potential confounders, however, the exposure of interest was measured objectively. Participation rate in the controls was 55 % and the odds ratios could be biased if controls who declined participation in the study had different fatty acid patterns than the controls who participated. In spite of this limitation, previously reported findings in this study population on dietary intake and cell-membrane levels of n-3 PUFA and the risk of SCA have been replicated in prospective cohort studies [4, 35].

In summary, we report for the first time an association of higher erythrocyte membrane levels of VLSFA with lower risk of incident SCA. These novel findings support the need for further investigation of the biologic effects and determinants of circulating VLSFA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SCA	sudden cardiac arrest
VLSFA	very long-chain saturated fatty acids

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Highlights

- Higher levels of erythrocyte membrane saturated fatty acids with 20 or more carbons (very long-chain saturated fatty acids) are associated with lower risk of SCA
- After adjustment for clinical, demographic and life-style risk factors, and levels of n-3 and *trans*-fatty acids, higher levels of 20:0 corresponding to 1 SD are associated with 30% lower SCA risk (13%–43%, $p=0.001$).
- Higher levels of 22:0 corresponding to 1 SD are associated with 29% lower SCA risk (12%–43%, $p=0.002$).
- Higher levels of 24:0 corresponding to 1 SD are associated 21% lower SCA risk (2%–37%, $p=0.04$).

Table 1

Association of 17 erythrocyte membrane fatty acids with incident sudden cardiac arrest (SCA)

Fatty acid	Mean (SD), % total fatty acids		OR ^a (95% CI)	p-value
	Cases	Controls		
20:0	0.37 (0.06)	0.38 (0.05)	0.76 (0.62–0.92)	0.005
22:0	1.66 (0.25)	1.71 (0.25)	0.78 (0.63–0.96)	0.02
24:0	4.60 (0.70)	4.74 (0.59)	0.74 (0.60–0.92)	0.005
15:0	0.12 (0.04)	0.12 (0.04)	0.93 (0.79–1.11)	0.43
17:0	0.33 (0.06)	0.33 (0.06)	0.84 (0.68–1.02)	0.08
17:1n9	0.95 (0.18)	0.98 (0.15)	0.92 (0.76–1.12)	0.41
20:1n9	0.22 (0.05)	0.22 (0.04)	1.03 (0.88–1.22)	0.69
22:1n9	0.07 (0.03)	0.07 (0.03)	1.10 (0.90–1.35)	0.35
24:1n9	4.04 (0.63)	3.99 (0.07)	0.83 (0.66–1.05)	0.12
18:2n6	9.27 (1.27)	9.10 (1.11)	1.14 (0.96–1.35)	0.12
20:2n6	0.25 (0.04)	0.24 (0.04)	1.29 (1.06–1.57)	0.01
22:2n6	0.06 (0.03)	0.06 (0.03)	1.14 (0.85–1.52)	0.39
18:3n6	0.05 (0.02)	0.05 (0.02)	0.89 (0.71–1.11)	0.29
20:3n6	1.55 (0.35)	1.51 (0.34)	1.18 (0.98–1.42)	0.08
20:4n6	14.00 (1.21)	13.78 (1.22)	1.14 (0.93–1.39)	0.20
22:4n6	3.62 (0.78)	3.63 (0.81)	1.06 (0.82–1.38)	0.64
22:5n3	2.15 (0.31)	2.22 (0.29)	0.95 (0.79–1.14)	0.60

^aOdds ratios for the association of 1-SD higher levels of each fatty acid with SCA risk, with adjustments for age (years), gender, current smoking (yes/no), diabetes (yes/no), hypertension (yes/no), education beyond high school (yes/no), leisure-time physical activity (kcal), index of fat intake [5] (continuous), body weight (kg) and height (cm).

Table 2
Association of erythrocyte 20:0, 22:0 and 24:0 with incident sudden cardiac arrest (SCA).

	Q1	Q2	Q3	Q4	p-trend
A. 20:0					
Median ^a	0.32	0.36	0.39	0.43	
Model 1 ^b	REF	0.63 (0.38–1.05)	0.53 (0.30–0.92)	0.51 (0.29–0.89)	0.02
Model 2 ^b	REF	0.61(0.36–1.02)	0.45 (0.25–0.81)	0.42 (0.23–0.77)	0.004
B. 22:0					
Median ^a	1.45	1.63	1.74	1.97	
Model 1 ^b	REF	0.73 (0.43–1.22)	0.54 (0.31–0.94)	0.48 (0.27–0.85)	0.008
Model 2 ^b	REF	0.72 (0.41–1.24)	0.49 (0.27–0.87)	0.35 (0.19–0.65)	<0.001
C. 24:0					
Median ^a	4.03	4.54	4.87	5.36	
Model 1 ^b	REF	0.59 (0.34–1.02)	0.68 (0.39–1.18)	0.45 (0.25–0.83)	0.03
Model 2 ^b	REF	0.58 (0.33–1.03)	0.71 (0.40–1.27)	0.52 (0.27–0.98)	0.10

^aMedian levels among controls, % of total fatty acids

^bOdds ratio and 95% CI using the same adjustments as in Table 1 (Model 1); and with additional adjustments for erythrocyte levels of EPA+DHA and *trans*-18:2 (Model 2).