Cell Membrane Trans-Fatty Acids and the Risk of Primary Cardiac Arrest

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Background—The relation of trans-fatty acid intake to life-threatening arrhythmias and primary cardiac arrest is unknown.

Methods and Results—We investigated the association of trans-fatty acid intake, assessed through a biomarker, with the risk of primary cardiac arrest in a population-based case-control study. Cases, aged 25 to 74 years, were out-of-hospital cardiac arrest patients attended by paramedics in Seattle, Washington from 1988 to 1999 (n=179). Controls, matched to cases by age and sex, were randomly identified from the community (n=285). Participants were free of previous clinically diagnosed heart disease. Blood was obtained at the time of cardiac arrest (cases) or at the time of an interview (controls) to assess trans-fatty acid intake. Higher total trans-fatty acids in red blood cell membranes was associated with a modest increase in the risk of primary cardiac arrest after adjustment for medical and lifestyle risk factors (odds ratio for interquintile range, 1.5; 95% CI, 1.0 to 2.1). However, trans isomers of oleic acid were not associated with risk (odds ratio for interquintile range, 0.8; 95% CI, 0.5 to 1.2), whereas higher levels of trans isomers of linoleic acid were associated with 3-fold increase in risk (odds ratio for interquintile range, 3.1; 95% CI, 1.7 to 5.4).

Conclusions—These findings suggest that dietary intake of total trans-fatty acids is associated with modest increase and trans isomers of linoleic acid with a larger increase in the risk of primary cardiac arrest. These associations need to be confirmed in future studies that distinguish between trans isomers of linoleic acid and trans isomers of oleic acid.

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Key Words: heart arrest ■ fatty acids ■ epidemiology

Sudden cardiac death is the leading cause of death from coronary heart disease (CHD), with about half of the cases occurring in persons without clinically diagnosed heart disease. Although the prevention of sudden cardiac death in the community remains a challenge, there is growing evidence that the fatty acid composition of the diet can influence the risk of life-threatening arrhythmias and sudden cardiac death. Whether trans-fatty acids influence the risk of sudden cardiac death is unknown.

Diet enriched in saturated fatty acids increase the risk of ventricular fibrillation and sudden cardiac death in primates. Because trans-fatty acids resemble saturated fatty acids in their biophysical properties, we hypothesized that higher levels of dietary trans-fatty acids, assessed by a biomarker, would be associated with an increased risk of primary cardiac arrest (sudden cardiac death). Trans isomers of linoleic acid (trans-18:2) represents 25% of total trans-fatty acids in adipose tissue, suggesting that they contribute to trans fatty acid intake. Trans-18:2 fatty acids, which have one cis double bond and one trans double bond, have different biophysical properties than trans-fatty acids with a single trans double bond (trans-18:1). For these reasons, we also investigated whether trans-18:2 fatty acids might differ from trans-18:1 in their association with primary cardiac arrest (PCA). To examine these questions, we conducted a population-based case-control study.

See p 669

Trans-fatty acids are unsaturated fatty acids found in partially hydrogenated oils, beef, and dairy products. Trans-fatty acids differ from most naturally occurring unsaturated fatty acids by the presence of one or more double bonds in the trans configuration instead of the usual cis configuration. Like saturated fatty acids, fatty acids with trans double bonds pack more tightly than cis fatty acids, allowing the processing of oils into margarine. Population studies suggest an increased risk of CHD with higher dietary intake of total trans-fatty acids.

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Methods

Study Subjects
Cases were out-of-hospital PCA patients attended by paramedics in Seattle and suburban King County, Washington, between October 1988 and June 1999. We defined PCA as a sudden pulseless condition in the absence of evidence of a noncardiac cause of cardiac arrest. Cases were identified from emergency service incident reports. In addition to incident reports, we reviewed death certificates, medical examiner reports, and autopsy reports, when available, to exclude patients with cardiac arrest attributable to a noncardiac cause.

We restricted PCA cases to married residents of King County, Washington, between the ages of 25 and 74 years. Cases for whom the paramedics were unable to draw a blood sample at the time of the arrest were excluded. We have previously shown that the distributions of risk factors among cases with and without blood samples were similar.16 Because the focus of the study was on persons who appeared healthy until their cardiac arrest, we excluded cases with a history of chronic disease or life-threatening comorbidities. We also excluded users of fish-oil supplements, because fish oil use would affect red blood cell (RBC) membrane fatty acid composition.

We were able to contact 89% of the spouses of identified cases. The spouses of 205 eligible cases (86%) participated in an in-person interview (n=196) or a telephone interview (n=9), for an overall response rate of 77%. Additionally, 26 cases were excluded because their blood could not be analyzed.

For each case, control subjects matched on age (within 7 years) and sex were randomly selected from the community by the sampling technique of random-digit dialing.17 Ninety-seven percent of known residential households contacted were successfully screened to determine if residents were eligible for the study. We obtained blood samples and spouse interviews from 309 eligible control subjects (61%), for an overall response rate of 59%. Additionally, 24 controls were excluded because their blood could not be analyzed. Controls followed the same eligibility criteria as the cases.

Red Blood Cell Membrane Fatty Acid Measurements
Paramedics obtained blood specimens from the cases in the field 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 minutes of the cardiac arrest. Blood specimens were obtained from controls at the time of the interview. The University of Washington Human Subjects Review Committee approved the protocol.

Blood specimens were processed18 and submitted to gas chromatography16 according to published methods. Technicians blinded to case and control status conducted the laboratory analyses. Quality control included the use of pooled RBCs and internal standards.

Using gas chromatography, we measured nine trans-fatty acids in RBC membrane samples: five trans isomers of oleic acid, collectively referred to as trans-18:1 (12 trans-18:1, 11 trans-18:1, 10 trans-18:1, 9 trans-18:1, and mix of 6 to 8 trans-18:1); two trans isomers of linoleic acid, collectively referred to as trans-18:2 (9 cis, 12 trans-18:2 and 9 trans, 12 cis-18:2); and two trans isomers of palmitoleic acid, collectively referred to as trans-16:1 (7 trans-16:1 and 9 trans-16:1). We did not identify any conjugated trans isomer of linoleic acid. We summed data on the individual trans-fatty acids to obtain the RBC membrane levels of total trans-fatty acids, trans-18:1, trans-18:2, and trans-16:1. Fatty acid levels were expressed as percentages of total fatty acids.

Validity of Red Blood Cell Trans-Fatty Acid Measurement
To validate the measurement of RBC membrane level of trans-fatty acids as a biomarker of dietary intake of trans-fatty acids, we developed a 17-item food frequency questionnaire. For each food consumed, spouses as proxy respondents for cases and controls were asked to estimate usual serving size and frequency of consumption during the prior month of 17 processed foods, previously found to contribute the most to dietary intake of trans-fatty acids in a previous study.16 Using the United States Department of Agriculture’s Special Purpose Table No. 1,18 we calculated an index of total trans-fatty acid dietary intake from the trans-fatty acids consumed in the 17 foods. Among 111 controls, RBC membrane total trans-fatty acid levels were related to the index of total trans-fatty acid intake (r=0.5, P<0.001).

Relation of Red Blood Cell Membrane Levels of Trans-18:1 and Trans-18:2 Fatty Acids to Intake of Specific Foods
We used the spouse dietary data for 111 controls to perform linear regression analyses using RBC membrane levels of trans-18:1 and 9-trans-18:2 isomers as dependent variables and consumption frequencies of the 17 foods listed in the questionnaire as predictors. Higher consumption of commercially available pizza, fried chicken, and cookies were independent predictors of higher RBC membrane levels of trans-18:2 fatty acids (model Rsq=0.33). In contrast, higher intake of margarine, donuts, and cookies were independent predictors of higher RBC membrane levels of trans-18:1 fatty acids (model Rsq=0.26), suggesting that different foods might possibly contribute to the higher intake of trans-18:2 and trans-18:1 fatty acids.

Detailed Nutrient Assessment
On the basis of 4 days of food records completed by 55 controls themselves, RBC membrane levels of total trans-fatty acids were not related to total caloric intake (r=0.1, P=0.5) and to percentage of energy from saturated fat (r=0.1, P=0.3) after adjustment for red cell membrane levels of long-chain n-3 polyunsaturated fatty acids and linoleic acid.

Assessment of Other Risk Factors
We collected information on demographic factors, medical conditions, lifestyle characteristics, and dietary habits during the in-person interview or, in the case of 9 case-control pairs, the telephone interview. Dietary saturated fat intake was assessed with the Northwest Lipid Research Clinic Fat Intake Scale, an index that correlates with saturated fat intake.10

Statistical Methods
Statistical analyses were carried out using STATA 6.0. We compared the distribution of risk factors among cases and controls using t test for continuous variables and χ2 test for categorical variables. We compared risk factor distribution across tertiles of trans-fatty acid levels among controls using ANOVA. We assessed the associations of trans-fatty acids with other fatty acids among controls from Pearson correlation coefficients.

We used conditional logistic regression to obtain odds ratios (estimates of relative risks) of PCA associated with increasing levels of RBC membrane trans-fatty acids. Statistical significance was assessed with the likelihood ratio test. In the main analyses, trans-fatty acids were included as continuous terms. Odds ratios and 95% CIs corresponding to the interquintile range of the control distribution were then calculated from the regression estimates. Quadratic terms were not included, because they did not improve the fit of any of the models. We also present odds ratios associated with upper quartiles of trans-18:2 fatty acid levels obtained from a model with indicator variables for the quartiles, using the lowest quartile as reference.

Information was missing on smoking (1% of controls), hypertension (2% of cases, 1% of controls), education (1% of cases), diabetes (0.5% of cases), weight (3% of cases, 7% of controls), and family history (9% of cases, 6% of controls). Fourteen percent of all subjects had 1 missing value on one of these covariates and 1% had 2 missing values. The missing values were imputed using a multiple imputation method.19 Results obtained with imputed missing values are presented in this study. Similar results were obtained when matched case-control pairs without missing values were examined.

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Results

Given the matching, mean age and sex distribution were similar in cases and controls (Table 1). As expected, other traditional risk factors for PCA, such as present smoking, diabetes, hypertension, and family history of myocardial infarction or sudden cardiac death, were more prevalent in cases than in controls (Table 1). In addition, cases were less likely to engage in leisure-time physical activity.

Mean RBC trans-fatty acid levels were higher in cases than controls. In contrast, mean levels of long-chain n-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were higher in controls (Table 2).

RBC membrane levels of total trans-fatty acids were not related to age, sex, hypertension, diabetes, or smoking (Table 3). However, they were inversely related to alcohol consumption and education (Table 3), inversely related to RBC membrane levels of DHA+EPA \((r=-0.39, P<0.001)\), and positively associated with RBC membrane levels of linoleic acid \((r=0.33, P<0.001)\). The associations of RBC membrane levels of trans-18:2 fatty acids with risk factors were similar except for age (61 years in the lowest tertile of trans-18:2 versus 56 years in other tertiles, \(P=0.0013\), weight (from lowest to highest tertile: 81.3 kg, 82.5 kg, 84.3 kg, \(P=0.03\)), and RBC levels of linoleic acid, which were not related to levels of trans-18:2 fatty acids \((r=0.05)\).

After adjustment for traditional PCA risk factors, RBC membrane levels of total trans-fatty acids were associated with a modest increase in risk of PCA (Table 4). An increase in total trans-fatty acids from 1.59% to 2.45% of total fatty acids, the interquintile range among controls, was associated with an odds ratio of 1.47 (95% CI, 1.01 to 2.13). However, the association was reduced by adjustment for RBC membrane levels of DHA+EPA (Table 4).

Additional adjustments for alcohol consumption, caffeine consumption, RBC membrane levels of linoleic acid, and other major fatty acids did not change the results. Subject characteristics, including sex, age, hypertension, diabetes, smoking, family history of myocardial infarction or sudden death, weight, and DHA+EPA levels, did not modify the association of trans-18:2 fatty acids with PCA (not shown).

Discussion

In this population-based study, higher levels of total trans-fatty acids in RBC membranes were associated with a modest 1.5-fold increase in risk of PCA after adjustment for tradi-
tional risk factors. However, examination of the main classes of trans-fatty acids revealed that trans isomers of oleic acid (trans-18:1) and trans isomers of linoleic acid (trans-18:2) were not equivalent. Although trans-18:1 fatty acids showed no association with PCA, an increase in RBC membrane trans-18:2 fatty acids corresponding to the interquintile range was associated with nearly 3-fold increase in risk of PCA. The association was independent of traditional risk factors, membrane levels of other fatty acids, and levels of trans-18:1 fatty acids.

The strengths of this study include the use of population-based cases and controls, the objective assessment of trans-fatty acid intake using a biomarker, and adjustment of results for other known risk factors. To address the possibility that cases might have changed their diet as a consequence of poor health leading to PCA, we restricted the study to cases with no history of clinically recognized heart disease and no life-threatening comorbidities.

This study has several limitations. We did not measure all aspects of diet, and the possibility of residual confounding cannot be eliminated. In particular, we may have incompletely adjusted for saturated fat intake. However, among control subjects who completed a 4-day food record, we did not find evidence of a relation of RBC membrane trans-fatty acids with saturated fat intake. It is therefore unlikely that differences in saturated fat intake can fully account for the observed association between trans-18:2 and PCA. For 15% of the study subjects, 1 or 2 covariate values were imputed; however, similar study results were obtained with subjects without any missing values. The use of surrogate respondents inevitably introduced some misclassification in assessment of potential confounders; however, the exposure of interest was measured objectively. Participation rate in the controls was 60%, and the odds ratios associated with higher levels of trans-18:2 fatty acids could be overestimated if controls who declined participation in the study ate more foods containing trans-18:2 than the controls who participated.

The modest association of total trans-fatty acid levels with PCA when adjusting for traditional risk factors only is comparable to the association of total trans-fatty acid intake

<table>
<thead>
<tr>
<th>Tertile of Red Blood Cell Total Trans-Fatty Acids*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean (SD)</td>
<td>58.7 (9.2)</td>
</tr>
<tr>
<td>Sex, %</td>
<td>82.4</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>8.8</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>13.5</td>
</tr>
<tr>
<td>Family history of CHD, %</td>
<td>44.7</td>
</tr>
<tr>
<td>Weight, kg, mean (SD)</td>
<td>83.3 (16.8)</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>9.0</td>
</tr>
<tr>
<td>Leisure-time physical activity, kcal, mean (SD)</td>
<td>1468 (1516)</td>
</tr>
<tr>
<td>Alcohol users, %</td>
<td>78.0</td>
</tr>
<tr>
<td>Alcohol consumption among drinkers, g/day, mean (SD)</td>
<td>25.4 (37.2)</td>
</tr>
<tr>
<td>High school graduates, %</td>
<td>80.2</td>
</tr>
</tbody>
</table>

*Median (range) of total trans-fatty acid levels in tertiles were expressed as % of total fatty acids: 1.52 (0.98–1.75) in I, 1.94 (1.75–2.14) in II, and 2.51 (2.15–3.54) in III.

<table>
<thead>
<tr>
<th>Interquintile Range, %</th>
<th>OR Corresponding to the Interquintile Range (95% CI)</th>
<th>Trans-18:1 and Trans-18:2 Assessed Simultaneously</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total trans-fatty acids</td>
<td>0.86</td>
<td>1.47 (1.01–2.13)</td>
</tr>
<tr>
<td>Trans-18:1 fatty acids</td>
<td>0.81</td>
<td>1.41 (0.97–2.06)</td>
</tr>
<tr>
<td>Trans-18:2 fatty acids</td>
<td>0.08</td>
<td>2.97 (1.79–4.91)</td>
</tr>
</tbody>
</table>

*Traditional risk factors were age, smoking (never, past, current), history of diabetes (yes/no), treated hypertension (yes/no), education beyond high school (yes/no), family history of MI or sudden death (yes/no), and the continuous covariates weight, height, index of dietary saturated fat intake, and energy expended in leisure-time physical activity.

DHA+EPA indicates docosahexaenoic acid + eicosapentaenoic acid.
with CHD reported in several cohort studies.\textsuperscript{13–15} A pooled estimate of the adjusted relative risk of CHD (CHD death and nonfatal myocardial infarction) associated with an increase of 2\% in energy in total trans-fatty acid intake is 1.25 (95\% CI, 1.11 to 1.40).\textsuperscript{15} Intake of total trans-fatty acids was estimated from a food frequency questionnaire in these studies, and whether trans-18:2 fatty acids differed from trans-18:1 fatty acids was not reported.

There is little published information on the possible association of trans-fatty acids with PCA. In an autopsy-based case-control study of sudden cardiac death with 66 cases, adipose tissue trans-fatty acids were not associated with risk of cardiac arrest.\textsuperscript{20} However, the low prevalence of hypertension and diabetes in the cases, known PCA risk factors, suggests that these autopsy cases were not representative of the cases in the population as a whole.

The mechanism by which trans-fatty acids might influence the risk of PCA is largely unknown. Trans-fatty acids have similarities to saturated fatty acids, and saturated fatty acids have been shown to increase the risk of life-threatening arrhythmias in primates.\textsuperscript{4–7} Whether trans-fatty acids in general and trans-18:2 fatty acids in particular influence the risk of arrhythmias in primates has yet to be investigated.

Trans-fatty acids are made during partial-hydrogenation and frying of vegetable oils\textsuperscript{21,22} and by bacterial action in ruminants’ stomachs.\textsuperscript{23} The trans isomer composition of processed foods varies with oil type and the specifics of partial hydrogenation. In a subset of controls, higher consumption of commercially available pizza, fried chicken, and cookies predicted 33\% of the variation in RBC membrane trans-18:2 levels, suggesting that these common processed foods might rise trans-18:2 intake. Consumption of margarine was a predictor of trans-18:1 levels, together with donuts and cookies, but did not predict trans-18:2 levels, suggesting the possibility that margarine might contribute to total trans-fatty acid intake but not trans-18:2. This finding needs confirmation by feeding studies.

In conclusion, we observed a modest association of RBC membrane levels of total trans-fatty acids with PCA and a strong positive association of membrane trans-18:2 fatty acids with PCA. These associations need to be confirmed in future studies that distinguish between trans-18:1 and trans-18:2 fatty acids.

Acknowledgments

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