Fatty acids in the de novo lipogenesis pathway and risk of coronary heart disease: the Cardiovascular Health Study¹–³

Jason HY Wu, Rozenn N Lemaitre, Fumiaki Imamura, Irena B King, Xiaoling Song, Donna Spiegelman, David S Siscovick, and Dariush Mozaffarian

ABSTRACT

Background: De novo lipogenesis (DNL) is an endogenous pathway whereby carbohydrates and proteins are converted to fatty acids. DNL could affect coronary heart disease (CHD) or sudden cardiac arrest (SCA) via generation of specific fatty acids. Whether these fatty acids are prospectively associated with SCA or other CHD events is unknown.

Objective: The objective was to investigate the relations of 4 fatty acids in the DNL pathway—palmitic acid (16:0), palmitoleic acid (16:1n-7), 7-hexadecenoic acid (16:1n-9), and cis-vaccenic acid (18:1n-7)—with incident CHD, including fatal CHD, nonfatal myocardial infarction (NFMI), and SCA.

Design: A community-based prospective study was conducted in 2890 men and women aged ≥65 y, who were free of known CHD at baseline and who were followed from 1992 to 2006. Cardiovascular disease risk factors and plasma phospholipid fatty acids were measured at baseline by using standardized methods. Incident CHD was ascertained prospectively and was centrally adjudicated by using medical records. Risk was assessed by using multivariable-adjusted Cox proportional hazards.

Results: During 29,835 person-years of follow-up, 631 CHD and 71 SCA events occurred. Both 18:1n-7 and 16:1n-9 were associated with a higher risk of SCA [multivariable-adjusted hazard ratio (95% CI) for the interquintile range: 7.63 (2.58, 22.6) for 18:1n-7 and 2.30 (1.16, 4.55) for 16:1n-9] but not of total CHD, fatal CHD, or NFMI. In secondary analyses censored to mid-follow-up (7 y) to minimize the effects of changes in concentrations over time, 16:1n-9 was also associated with a significantly higher risk of total CHD (2.11; 1.76, 2.54), including a higher risk of CHD death, NFMI, and SCA; 16:0 and 16:1n-7 were not associated with clinical CHD outcomes.

Conclusion: Higher plasma phospholipid 18:1n-7 and 16:1n-9 concentrations were prospectively associated with an elevated risk of SCA but not of other CHD events, except in secondary analyses. Am J Clin Nutr 2011;94:431–8.

INTRODUCTION

De novo lipogenesis (DNL) is an endogenous pathway for lipid synthesis, whereby carbohydrates and proteins are converted to fatty acids (1). The main fatty acid products of DNL include palmitic acid (16:0); palmitoleic acid (16:1n-7), cis-vaccenic acid (18:1n-7), stearic acid (18:0), and oleic acid (18:1n-9); myristic acid (14:0) is a possible minor product (Figure 1). 7-Hexadecenoic acid (16:1n-9) is another fatty acid related to this pathway that could be derived from partial β-oxidation of 18:1n-9 (2).

Previous interventional studies showed that DNL is up-regulated in humans by relatively extreme diets, influencing circulating concentrations of several specific fatty acids (4–9). However, the extent to which usual ranges of dietary and lifestyle exposures influence DNL or concentrations of these fatty acids is not established. In a prior cross-sectional analysis among free-living adults, factors known to stimulate DNL (eg, carbohydrate and alcohol intake) were independent positive predictors of plasma phospholipid 16:1n-7, which suggests that DNL may be activated by ranges of usual lifestyle habits (10). Whether this also applies to other fatty acids in the DNL pathway is unknown.

Some evidence suggests that fatty acids in the DNL pathway could affect the risk of coronary heart disease (CHD) and sudden cardiac arrest (SCA) (11–14). We recently found that erythrocyte membrane concentrations of 16:0 and 16:1n-9 were positively associated with the risk of SCA, independent of other risk factors and fatty acids, in a population-based case-control study (14). Fatty acids are important cellular constituents that may directly affect biological processes relevant for the development of CHD or SCA. For example, in experimental studies, 16:0 induces endoplasmic reticulum stress, enhances cellular apoptosis, activates proinflammatory pathways, and impairs endothelial-dependent vasodilatation (15–20). The observed association between these fatty acids and the risk of SCA could also

¹ From the Division of Cardiovascular Medicine and Channing Laboratory, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA (DM); the Departments of Nutrition (DM), Epidemiology (JHYW, FI, DS, and DM), and Biostatistics (DS), Harvard School of Public Health, Boston, MA; the School of Medicine and Pharmacology, University of Western Australia, Perth, Australia (JW); the Department of Medicine, University of New Mexico, Albuquerque, NM (IBK); the Biomarker Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA (XS); and the Cardiovascular Health Research Unit (RNL and DSS) and Departments of Epidemiology (DSS) and Medicine (RNL and DSS), University of Washington, Seattle, WA.

² Supported by the National Heart, Lung, and Blood Institute with co-funding from the National Institutes of Health Office of Dietary Supplements (R01 HL 085710-01). The National Heart, Lung, and Blood Institute provided support for the Cardiovascular Health Study (contracts N01-HC-35129, N01-HC-45133, N01-HC-75150, N01-HC-85079 through N01-HC-85086, N01 HC-15103, N01 HC-55222, and U01 HL080295) and an additional contribution from the National Institute of Neurological Disorders and Stroke. A subset of additional fatty acid measurements was supported by a Searle Scholar Award (to DM).

³ Address correspondence to JHY Wu, 677 Huntington Avenue, Building 3-913, Boston, MA 02115. E-mail: jasonwu@hsph.harvard.edu.

Received January 12, 2011. Accepted for publication May 24, 2011. First published online June 22, 2011; doi: 10.3945/ajcn.111.012054.
supports its possible derivation from endogenously synthesized 18:1n and 16:1n stearic acid (18:0) and oleic acid (18:1n are not entirely clear, cell culture studies found that 7-hexadecenoic acid is a possible minor product of fatty acid synthesis. Whereas its metabolic origins are not entirely clear, cell culture studies found that 7-hexadecenoic acid (16:1n−7) could arise from the β-oxidation of 18:1n−9 (2). The fatty acid 16:1n−9 also increased in response to a low-fat, high-carbohydrate diet, which supports its possible derivation from endogenously synthesized 18:1n−9 (3).

reflect underlying DNL activation. Hepatic DNL increases fasting and postprandial triglyceride concentrations (6, 21), which are associated with other atherogenic mediators (22) and are independent risk factors for CHD (23). Elevated hepatic DNL also appears to increase fat deposition in the liver (24), which leads to hepatic insulin resistance and, if prolonged, development of nonalcoholic fatty liver disease (25), which is associated with an increased CHD risk (26, 27).

Few studies have investigated the relations of fatty acids in the DNL pathway with incident cardiovascular disease, with mixed results (11–13). None of these studies of total cardiovascular disease investigated 16:1n−9 or 18:1n−7. The relations of these fatty acids with risk of SCA have also not been evaluated prospectively. We therefore investigated independent lifestyle correlates of circulating plasma phospholipid fatty acids in the DNL pathway and the associations of these fatty acids with incident CHD and SCA in the Cardiovascular Health Study (CHS)—a community-based prospective cohort of older men and women.

**SUBJECTS AND METHODS**

**Study design and participants**

The CHS is a prospective cohort study funded by the National Heart, Lung, and Blood Institute to investigate risk factors for cardiovascular disease in community-based older adults (28, 29). The participants were randomly selected from Medicare eligibility lists from 4 US communities: Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Allegheny County, PA. A total of 5201 men and women were initially recruited in 1989–1990; an additional subcohort of 687 predominantly African Americans was recruited in 1992–1993. Individuals were eligible if they were aged ≥65 y, noninstitutionalized, expected to remain in their current community for >3 y, and not under active hospice or cancer treatment. Each center’s institutional review committee approved the study, and all participants gave written informed consent. For the current study, we measured plasma phospholipid fatty acids by using stored samples from 1992 to 1993 in 3736 participants, including 3238 participants with available blood samples and 498 participants with previous measures from a previous case-control study of myocardial infarction (MI) (30). All analyses accounted for this sampling within the cohort by using inverse-probability-of-sampling weights. We excluded 846 participants with prevalent CHD (history of MI, angina, or coronary revascularization) in 1992–1993 (the baseline for this analysis), which resulted in 2890 participants in the current analysis.

**Blood sample collection and fatty acid analysis**

Individual plasma phospholipid fatty acids were measured as a percentage of total fatty acids by the Fred Hutchinson Cancer Research Center Biomarkers Laboratory (14). Blood was sampled after a 12-h fast and stored at −70°C before being shipped on dry ice for storage at −80°C. We assessed repeat measurements of the same stored samples in 38 subjects performed 10 y apart. Intercorelations of these repeated measures were moderate (r = 0.44 for 16:1n−9) to high (r = 0.72–1.00 for all other fatty acids in this article), which suggests that phospholipid fatty acids were reasonably stable under storage at −80°C, consistent with a previous report in another study (31), and the stability of laboratory measurements. Total lipids were extracted from plasma (32), and phospholipids were separated by using one-dimensional thin-layer chromatography. We followed LePage’s method to prepare transesterified fatty acid methyl esters (FAMEs) (33), which were analyzed by using gas chromatography–mass spectrometry at the US Department of Agriculture lipid laboratory in Peoria, IL. Pooled quality-control samples were run concurrently with study samples to confirm batch-to-batch variation, with CVs of 3% (16:0), 9% (16:1n−7), 6% (18:1n−7), and 23% (16:1n−9). Of the 7 potential fatty acids of interest (Figure 1), phospholipid concentrations of 16:0, 16:1n−7, 16:1n−9, and 18:1n−7 may more strongly reflect endogenous synthesis from DNL, because prior studies have shown 14:0 and 18:1n−9 to be moderately to strongly correlated with dietary sources (34, 35), and phospholipid 18:0 was not increased after a low-fat, high-carbohydrate diet (3). To focus on fatty acids that may more strongly reflect activated DNL, our prespecified primary exposures of interest for this analysis were 16:0, 16:1n−7, 16:1n−9, and 18:1n−7; 14:0, 18:0, and 18:1n−9 were evaluated in secondary analyses.

**Assessment of coronary heart disease**

The protocol for ascertainment of incident CHD events has been described (36). All CHD events were adjudicated by

---

**FIGURE 1.** The major saturated and monounsaturated fatty acids produced by the de novo lipogenesis (DNL) pathway, whereby acetyl-coenzyme A (acyl-CoA) is polymerized to form fatty acids. The initial major product of DNL is palmitic acid (16:0), which can be processed by Δ9 desaturation and/or elongation to palmitoleic acid (16:1n−7), cis-vaccenic acid (18:1n−7), stearic acid (18:0), and oleic acid (18:1n−9). Myristic acid (14:0) is another possible minor product of fatty acid synthesis. Whereas its metabolic origins are not entirely clear, cell culture studies found that 7-hexadecenoic acid (16:1n−7) could arise from the β-oxidation of 18:1n−9 (2). The fatty acid 16:1n−9 also increased in response to a low-fat, high-carbohydrate diet, which supports its possible derivation from endogenously synthesized 18:1n−9 (3).
a centralized Morbidity and Mortality Committee. MI was defined on the basis of cardiac enzymes, chest pain, and serial electrocardiogram changes. CHD deaths were defined as fatal MI or as fatal CHD events for which the participant had chest pain within 72 h of death or had a history of chronic CHD. From among the CHD deaths, SCA was defined based on review by a cardiologist as a sudden pulse-less condition with a cardiac origin in a previously stable individual that occurred out of the hospital or in the emergency room (37). These cases could not have a life-threatening noncardiac comorbidity or be under hospice or nursing home care. A second cardiologist conducted a blind review of a random sample of 70 potential SCA cases and found an interreviewer agreement of 88% and a k value of 0.74. Our primary outcome was incident CHD (fatal or nonfatal MI plus CHD death). We also separately evaluated SCA and other CHD subtypes, including nonfatal MI and total CHD death.

Assessment of other covariates

At the study visit in 1992–1993, participants completed standardized questionnaires on health status, medical history, cardiovascular disease risk factors, physical activity, and alcohol intake using validated questionnaires (28) and underwent clinical examination and laboratory evaluation (38). Information on usual dietary intake was available from reproducible and validated semiquantitative food-frequency questionnaires (FFQs) administered in 1989–1990 (39) and 1996–1997 (40). Because blood sampling was at the middle time point of these dietary assessments, we averaged responses on the 2 FFQs to minimize misclassification or used data from one FFQ when the other was not available (eg, only one FFQ was available for the subcohort recruited in 1992–1993) (41). Nutrient consumption levels were adjusted for total energy by using the multivariate nutrient density method, representing isocaloric substitution for fat (42).

Statistical analysis

The unadjusted interrelations between fatty acids were evaluated by using Spearman correlations (r). For the analyses of CHD risk, phospholipid fatty acids were modeled continuously according to the interquintile range (comparing the median of the first and fifth quintiles, ie, the 10th to 90th percentile). Additional analyses evaluated these fatty acids in categories (eg, quartiles), and restricted cubic splines showed relatively graded relations; therefore, we focused on the continuous analyses. Relations with incident CHD were evaluated by using multivariable-adjusted Cox proportional hazards, with time-at-risk until first diagnosis, death, or the latest adjudicated date of follow-up in 2006. The Cox proportional hazards assumption was tested by using Schoenfeld's residuals. To evaluate and correct for changes in fatty acid concentrations over time, serial phospholipid fatty acid measures were obtained in a subset of 100 participants at 6 y (1998–1999) and 13 y (2005–2006) after baseline. Within-individual fatty acid correlations were used to obtain regression dilution ratios and correct for regression dilution bias to obtain hazard ratios (HRs) based on the "usual" levels of each exposure variable by using methods established in prior analyses that related usual blood pressure (BP) and cholesterol values to cardiovascular disease risk (43, 44). Such methods correct the risk estimate, widen the CIs, and leave the statistical significance (P value) unchanged (43, 44).

The 13-y regression dilution ratios were 0.45 for 16:0, 0.53 for 16:1n–7, 0.29 for 16:1n–9, and 0.46 for 18:1n–7. Corresponding 6-y regression dilution ratios were 0.66, 0.72, 0.47, and 0.45, respectively, which were used for analyses limited to the midpoint of follow-up (7 y) to minimize the effects of changes in fatty acid concentrations and exposure misclassification with increasing duration of follow-up. These correlations were comparable with other within-individual correlations over time for widely used risk factors such as BP levels (45).

To minimize potential confounding, covariates were selected based on biologic interest, being well-established risk factors for CHD risk in older adults, or associations with exposures and outcomes in the current cohort. On the basis of these considerations and the goal of parsimony in covariate selection, 4 final multivariate models were fitted: 1) adjusted for age and sex 2), further adjusted for other CHD risk factors (race, education, income, smoking status, prevalent diabetes, hypertension, stroke, or transient ischemic attack) and lifestyle risk factors (body mass index, leisure-time physical activity, alcohol use, total fat intake, and total energy intake) 3), further adjusted for phospholipid concentrations of long-chain n–3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and trans fatty acids (total trans-18:1 and trans-18:2), and 4) further adjusted for factors that could be potential confounders or mediators (systolic BP, fasting HDL cholesterol, LDL cholesterol, triglycerides, C-reactive protein, fibrinogen, and incident angina as a time-varying covariate for total CHD, CHD death, and SCA). In secondary analyses we also further adjusted for phospholipid concentrations of 14:0. To assess the potential for reverse causation by undiagnosed CHD, we repeated the analyses after excluding participants with incident CHD in the first year of follow-up. Potential effect modification was investigated for age and sex by assessing the significance of multiplicative interaction terms by using Wald tests. Missing continuous covariates were imputed (all ≤8.2% missing) by single imputation with the use of baseline data, including age, sex, race, smoking status, alcohol use, education, physical activity, BMI, prevalent diabetes, and stroke as predictors. All categorical covariates had <0.1% missing and were not imputed. Results were similar after the exclusion of participants with missing values.

Factors that may stimulate DNL, chosen a priori based on previous studies (4) and including carbohydrate and protein intake, alcohol use, BMI as a surrogate measure of positive energy balance, and fasting insulin were evaluated by using multivariable-adjusted linear regression with each fatty acid in the DNL pathway as the dependent outcome. The predictors were evaluated continuously, and linear trend was tested by assigning participants the median value in quartiles of each factor treated as a continuous variable. All P values were 2-tailed (α = 0.05). The analyses were performed by using Stata 10.1 (Stata Corp, College Station, TX).

RESULTS

At baseline, the average age of the participants was 74 y (range: 65–97 y); 37% were men and 88% were white. Cardiovascular and other lifestyle risk factors are shown in Table 1. Consistent with an older US population, participants had diverse socioeconomic backgrounds and were on average overweight. The means and distributions of daily energy from carbohydrate (mean ± SD: 54 ± 7% of energy) and fat (mean ± SD: 31 ± 5% of energy) were also typical for US populations (46).
Levels of fatty acid biomarkers in the DNL pathway represented between 0.09–25.4% of total phospholipid fatty acids (Table 2). The highest values were seen for 16:0 (mean ± SD: 25.4 ± 1.6%), and the lowest values were seen for 16:1n−9 (mean ± SD: 0.09 ± 0.03%). Intercorrelations were generally positive but modest (r = 0.16–0.61); 18:0 was modestly inversely correlated with the other fatty acids (r = −0.15 to −0.44). The strongest correlations were observed between 14:0 and 16:1n−7 (r = 0.61), 16:0 and 16:1n−7 (r = 0.56), and 16:1n−7 and 18:1n−9 (r = 0.56).

During 29,835 person-years of follow-up, 631 incident cases of CHD occurred (22.4 per 1000 person-years), including 367 nonfatal MIs, 357 CHD deaths, and 71 SCAs among CHD deaths. In multivariate-adjusted analyses, none of the primary fatty acids of interest were significantly associated with total CHD (Table 3), nonfatal MI, or CHD death (data not shown). However, 18:1n−7 (interquintile HR: 7.63; 95% CI: 2.58, 22.6) and 16:1n−9 (interquintile HR: 2.30; 95% CI: 1.16, 4.55) were associated with an increased risk of SCA (data not shown). For example, the interquintile HRs (95% CIs) for 18:1n−7 and SCA were 7.38 (2.48, 22.0) and 6.20 (1.92, 20.0) for model 2 and model 3, respectively. When these 2 fatty acids were included simultaneously in the Cox-model, 18:1n−7 remained significantly associated with SCA (interquintile HR: 6.46; 95% CI: 0.01, 0.10) SD higher 18:1n−7, 0.18 (95% CI: 0.08, 0.36) SD higher 16:0, 0.12 (95% CI: 0.06, 0.22) SD higher 16:1n−9, and 0.16 (95% CI: 0.07, 0.26) SD higher 18:1n−7. The associations were linear across the range of carbohydrate, protein, and alcohol intakes (P for trend < 0.05).

### Table 1

Baseline characteristics of 2890 US men and women in the Cardiovascular Health Study (1992–1993)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>74 ± 51</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>37</td>
</tr>
<tr>
<td>White race (%)</td>
<td>88</td>
</tr>
<tr>
<td>Educational level (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>26</td>
</tr>
<tr>
<td>High school graduate</td>
<td>28</td>
</tr>
<tr>
<td>&gt; High school</td>
<td>46</td>
</tr>
<tr>
<td>Income (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;$16,000/y</td>
<td>37</td>
</tr>
<tr>
<td>$16,000–$35,000/y</td>
<td>37</td>
</tr>
<tr>
<td>&gt;$35,000/y</td>
<td>26</td>
</tr>
<tr>
<td>Smoking habit (%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>48</td>
</tr>
<tr>
<td>Former</td>
<td>43</td>
</tr>
<tr>
<td>Current</td>
<td>9</td>
</tr>
<tr>
<td>Drug-treated diabetes (%)</td>
<td>15</td>
</tr>
<tr>
<td>Drug-treated hypertension (%)</td>
<td>41</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>136 ± 21</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Physical activity (kcal/wk)</td>
<td>1450 ± 1727</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>127 ± 33</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>54 ± 14</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>142 ± 83</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>5.0 ± 9.5</td>
</tr>
<tr>
<td>Total energy intake (kcal/d)</td>
<td>1998 ± 572</td>
</tr>
<tr>
<td>Total fat (% of energy)</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Saturated fat (% of energy)</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Alcohol use (%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>54</td>
</tr>
<tr>
<td>≤2 drinks/wk</td>
<td>29</td>
</tr>
<tr>
<td>&gt;2 drinks/wk</td>
<td>17</td>
</tr>
</tbody>
</table>

¹ Mean ± SD (all such values).

**Figure 2**

For example, replacing 5% of energy from fat with carbohydrate was associated with 0.06 (95% CI: 0.01, 0.10) SD higher 16:0, 0.07 (95% CI: 0.03, 0.12) SD higher 16:1n−7, 0.07 (95% CI: 0.02, 0.12) SD higher 16:1n−9, and 0.12 (95% CI: 0.08, 0.16) SD higher 18:1n−7. Replacing 5% of energy from protein was similarly associated with 0.2 (95% CI: 0.11, 0.29) SD higher 16:0, 0.13 (95% CI: 0.04, 0.22) SD higher 16:1n−7, 0.18 (95% CI: 0.06, 0.29) SD higher 16:1n−9, and 0.16 (95% CI: 0.07, 0.26) SD higher 18:1n−7. For alcohol, an additional drink per day was associated with 0.26 (95% CI: 0.11, 0.41) SD higher 16:0, 0.29 (95% CI: 0.09, 0.49) SD higher 16:1n−7, and 0.1 (95% CI: 0.03, 0.17) SD higher 18:1n−7. The associations were linear across the range of carbohydrate, protein, and alcohol intakes (P for trend < 0.05).
TABLE 2

Spearman correlation coefficients for plasma phospholipid fatty acids in the de novo lipogenesis pathway in 2890 men and women

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>16:0</th>
<th>16:1n−7</th>
<th>16:1n−9</th>
<th>18:1n−7</th>
<th>14:0</th>
<th>18:0</th>
<th>18:1n−9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of total phospholipid fatty acids</td>
<td>25.4 ± 1.6</td>
<td>0.50 ± 0.21</td>
<td>0.09 ± 0.03</td>
<td>1.30 ± 0.20</td>
<td>0.28 ± 0.07</td>
<td>13.4 ± 1.1</td>
<td>7.62 ± 1.12</td>
</tr>
<tr>
<td>Palmitoleic acid, 16:1n−7</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Hexadecenoic acid, 16:1n−9</td>
<td>0.03</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-Vaccenic acid, 18:1n−7</td>
<td>−0.003</td>
<td>0.16</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic acid, 14:0</td>
<td>0.39</td>
<td>0.61</td>
<td>0.23</td>
<td>−0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stearic acid, 18:0</td>
<td>−0.44</td>
<td>−0.22</td>
<td>−0.15</td>
<td>−0.38</td>
<td>−0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid, 18:1n−9</td>
<td>0.32</td>
<td>0.56</td>
<td>0.39</td>
<td>0.23</td>
<td>0.30</td>
<td></td>
<td>−0.28</td>
</tr>
</tbody>
</table>

1 All correlations were significant (P < 0.0001, t test), except for the correlations of 16:0 with 16:1n−9 (P = 0.11) and 16:0 with 18:1n−7 (P = 0.89).
2 Correlations between all fatty acids in the de novo lipogenesis pathway are shown here for completeness; however, all other primary analyses focused on 16:0, 16:1n−7, 16:1n−9, and 18:1n−7, which were selected a priori because they may more strongly reflect endogenous synthesis (see Subjects and Methods).
3 Values are means ± SDs.

0.05 for all). BMI and plasma insulin were not major independent predictors.

DISCUSSION

In this large prospective cohort study among older US adults, circulating concentrations of 18:1n−7 and 16:1n−9 were associated with an increased risk of SCA, and for 16:1n−9, possibly also total CHD. In contrast, 16:0 and 16:1n−7 were not related to the risk of CHD or SCA.

In a previous population-based case-control study, we found positive associations of erythrocyte membrane 16:1n−9 concentrations with risk of SCA, independent of established risk factors and other fatty acids (14). The multivariable-adjusted odds ratio (95% CI) for each 1-SD higher concentration of 16:1n−9 was 1.9 (1.3, 2.8); for 16:0 the odds ratio was 1.4 (1.1, 1.7). The current investigation extends these previous observations by showing an association of plasma phospholipid 16:1n−9 with SCA in a different population and using a prospective cohort design. If causal, the association of this fatty acid with SCA suggests a possible role or influence on cardiac electrophysiology and risk of ventricular arrhythmias. Experimental studies have shown electrophysiologic effects of other relatively minor fatty acids, such as eicosapentaenoic acid, potentially related to incorporation...
into lipid rafts and modulation of membrane ion channel function (47). Our findings support the need for experimental studies to examine whether 16:1n–9 could similarly affect these or other arrhythmia-related pathways.

We also found a 7-fold higher risk of SCA across the interquintile range of 18:1n–7 concentrations. In our prior retrospective case-control study, erythrocyte membrane 18:1n–7 was not significantly associated with the risk of SCA, although the risk estimate and CIs did not exclude the possibility of modestly higher risk: for each higher SD, the odds ratio was 1.15 (95% CI: 0.98, 1.34). The current analysis did not confirm the previous seen association between 16:0 and risk of SCA (47). Conversely, 16:0 is one of the precursors to 18:1n–7 (Figure 1). Collectively, our current and prior analyses highlight the potential association between this metabolic pathway (16:0–18:1n–7) and the risk of SCA, which requires further elucidation. Other differences in our previous and current analysis could also have contributed to the differing results for 16:0 compared with 18:1n–7, including the study design (retrospective case-control compared with prospective cohort), type or timing of fatty acid measurement (erythrocyte membrane concentrations within 30 min of SCA compared with fasting plasma phospholipid concentrations in generally healthy individuals at baseline), lack of statistical power, or chance. Notably, in both our previous (14) and current results, 14:0, 18:0, and 18:1n–9 were not associated with risk of SCA.

Phospholipid 16:1n–9 concentrations were also associated with incidence of total CHD in analyses restricted to the first half (7 y) of follow-up. These findings should be interpreted with caution and could have been due to chance. In contrast, the statistical interaction with time was highly significant, and the relatively low correlation of repeated measurements of 16:1n–9 indicated high temporal and analytic variability over time that could produce such findings. Thus, the lack of association between 16:1n–9 concentrations at baseline and CHD risk observed in the latter half of follow-up could be a result of random misclassification (errors) of 16:1n–9 concentrations over time, attenuating associations toward the null. Our findings support the need for serial measurements of fatty acid concentrations in later time points to ascertain possible effects of changes in 16:1n–9 and for investigation of associations of 16:1n–9 concentrations and CHD risk in other cohorts.

Our analyses of lifestyle factors suggest that usual ranges of habitual behaviors could stimulate DNL, as evidenced by higher circulating concentrations of fatty acids in the DNL pathway. Although direct dietary consumption and other potential factors, such as incorporation or metabolism, may also influence the concentrations of these fatty acids, our findings are consistent with those of previous short-term metabolic studies (days to weeks) that showed changes in circulating concentrations of these fatty acids after relatively extreme dietary interventions that would stimulate DNL (3, 8, 9). In our cohort, dietary factors associated with higher fatty acid concentrations included higher carbohydrate and protein intakes (in place of fat) and alcohol use, even across a relatively low range of intake (90% of participants consumed ≤1 drink/d). The findings for alcohol are consistent with those of a previous observational study in middle-aged men (48) and with those of a metabolic study in which administration of 24 g alcohol (~1.7 drinks) acutely increased VLDL 16:0 values from 2% to 30% (49). Conversely, whereas positive energy balance stimulates DNL, and hyperinsulinemic subjects had higher DNL in clinical studies (50, 51), BMI and fasting insulin (90th percentile = 146 pmol/L) were not associated with fatty acids in the DNL pathway in our study. The effects of positive energy balance may not have been captured by BMI in our cohort as a surrogate marker for adiposity, which is prone to misclassification in older adults because of a variable loss of lean muscle mass, and insulin may only affect DNL at higher levels.

Overall, our results suggest that DNL could be occurring and could be influenced by usual ranges of dietary and alcohol exposures in free-living older individuals. These associations need to be investigated in future studies using direct measures of DNL activity. If confirmed, these findings could have potential public health relevance because prevailing dietary guidelines have produced a shift in overall energy intake from fat to carbohydrate (52). This increased carbohydrate intake has also included large amounts of fructose, which have a more direct and pronounced inductive effect on DNL than do other sugars or starch (53).

The current study had several strengths. Exposure was assessed by means of objective biomarkers in a large number of participants of both sexes in a well-established prospective cohort study. The prospective cohort design reduced selection and recall bias, and exclusion of persons with known CHD at baseline reduced the potential bias from reverse causation (presence of disease influencing fatty acids). Furthermore, the exclusion of subjects with CHD events in the first year of follow-up did not alter the observed associations. Standardized assessment of multiple covariates allowed careful multivariable adjustment to reduce the influence of confounding. Low loss to follow-up and comprehensive review and
centralized adjudication of outcomes decreased the potential for missed or misclassified outcomes and allowed a separate evaluation of SCA.

Several limitations are of note. Residual confounding as a result of unrelied or imprecisely measured confounders could not be ruled out. The CHS included older adults; therefore, our results may not be generalizable to younger populations. Conversely, the use of community-based recruitment increased the generalizability of our findings, and absolute CHD risk is particularly high later in life, which increased the relevance of our findings. The association of 16:1n-9 with CHD risk was attenuated over time—a finding that may have been due to misclassification over time or to chance. A single baseline measurement of fatty acids will misclassify long-term exposure, which likely causes an underestimation of the true relations, especially for fatty acids with high temporal and analytic variability (eg, 16:1n-9). The different contributions of DNL, direct dietary consumption, or other metabolic processes (eg, incorporation) to the plasma phospholipid concentrations of these fatty acids also remain to be elucidated.

In summary, higher phospholipid 18:1n-7 and 16:1n-9 value were associated with higher SCA risk. Dietary carbohydrate, protein, and alcohol intakes, even at usual ranges of population exposures, were positive independent predictors of circulating concentrations of fatty acids in the DNL pathway. These findings highlight the need for experimental studies of the potential mechanistic link between SCA and 18:1n-9 and the conditions that increase the concentrations of these fatty acids.

We thank the CHS participants. A full list of participating CHS investigators and institutions can be found at http://www.chs-nhlib.org. JHYW gratefully acknowledges Research Fellowship support from the National Heart Foundation of Australia and the International Atherosclerosis Society.

The authors’ responsibilities were as follows—JHYW: study concept and design, statistical analysis, interpretation of the data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; RNL: funding, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; FL: statistical advice, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; IBK: funding, laboratory analysis, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; DS: funding, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; DSS: funding, acquisition of the data, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission; and DM: study concept and design, statistical advice, funding, drafting of the manuscript, interpretation of the data, critical revision of the manuscript for important intellectual content, and approval of the final manuscript for submission. The supporting agencies had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, and approval of the manuscript. The authors did not declare any conflicts of interest.

REFERENCES
25. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via