Intake of \textit{trans} fat and incidence of stroke in the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort\textsuperscript{1–4}

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\textbf{ABSTRACT}

\textbf{Background:} Whether elevated intakes of \textit{trans} fatty acids (TFAs) increase the risk of stroke remains unclear. Except for the Women’s Health Initiative–Observational Study, most studies that directly assessed the association between TFA intake and stroke yielded null results.

\textbf{Objective:} The aim of this study was to investigate the association between TFA intake and stroke incidence.

\textbf{Design:} We prospectively investigated the association between TFA intake and stroke incidence in black and white men and women ($n = 17,107$) from the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. Participants were recruited between 2003 and 2007 from the continental United States and followed for incident stroke. Diet was assessed by using the Block 1998 food-frequency questionnaire. Cox regression was used to test whether energy-adjusted TFA intake in 1-SD increments was associated with incident stroke.

\textbf{Results:} During a median follow-up of 7 y, 479 strokes were identified, including 401 ischemic strokes. Sex modified the association between TFA intake and stroke ($P$-interaction = 0.06), and thus the results were stratified by sex. In fully adjusted models, a 1-SD (2-g/d) increase in TFA intake was associated with an increased risk of any stroke in men (HR: 1.13; 95% CI: 1.00, 1.28) but not in women (HR: 0.93; 95% CI: 0.79, 1.11). Similarly, our results showed an increased risk of ischemic stroke in men (HR: 1.13; 95% CI: 1.00, 1.28) but not in women (HR: 0.93; 95% CI: 0.77, 1.12).

\textbf{Conclusions:} We show that sex modifies the association between TFA intake and stroke; for every 2-g/d increase in TFA intake, there was a 14% increase in the risk of stroke in men but not in women. Our findings provide further evidence to support the concerted effort to minimize TFAs in the diet. \textit{Am J Clin Nutr} 2014;99:1071–6.

\textbf{INTRODUCTION}

The association between elevated intake of \textit{trans} fatty acids (TFAs)\textsuperscript{5} and risk of stroke remains controversial. The few studies that have explored this association yielded inconsistent results (1–6). For example, the Health Professionals Follow-Up Study (HPFS) reported a null association between TFA intake and the risk of ischemic stroke (4). Similarly, the Nurses’ Health Study (NHS) reported no association between TFA intake and the risk of ischemic or total stroke, but there was an inverse association with parenchymal hemorrhagic stroke (1). On the other hand, the Women’s Health Initiative–Observational Study (WHI-OS) reported a strong positive association between TFA intake and ischemic stroke (2, 6), and in the Cardiovascular Health Study the principal component with high TFA intake loading showed a strong positive association with total stroke (3). The generalizability of the aforementioned positive studies is limited given that the studies included either one sex group or participants who were ≥65 y old and a small number of non-white participants. Studies in the general population are warranted so as to understand the effects of TFA intake on stroke in different sex, race, and age groups.

Elevated TFA intake is associated with inflammation, atherogenic dyslipidemia, and endothelial dysfunction (7, 8); and all of these factors individually or in combination are associated with increased risk of atherogenesis, cardiovascular disease, and all-cause mortality (9–12). Ischemic stroke, which represents the majority of total stroke cases (13), is also an atherosclerotic disorder (14, 15). We hypothesized that elevated TFA intake may increase the risk of stroke and that the association may be modified by sex. We used data from the national population-based

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\textsuperscript{2}The contents of the current study are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the NIH.

\textsuperscript{3}Supported by a cooperative agreement (U01 NS041588) from the National Institute of Neurological Disorders and Stroke, NIH, Department of Health and Human Services. An investigator-initiated grant from General Mills supported the cost of coding data from the food-frequency questionnaires. Additional funding was provided by an investigator-initiated grant-in-aid from Amgen Corporation.

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\textsuperscript{5}Abbreviations used: HPFS, Health Professionals Follow-Up Study; NHS, Nurses’ Health Study; REGARDS, REasons for Geographic And Racial Differences in Stroke; TFA, \textit{trans} fatty acid; WHI-OS, Women’s Health Initiative–Observational Study.

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SUBJECTS AND METHODS

The REGARDS cohort has been described in detail elsewhere (16). In brief, from January 2003 to October 2007, 30,239 black and white participants aged ≥45 y were recruited from the continental United States, with an oversampling of blacks and individuals from the “stroke belt” region (North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Alabama, Louisiana, and Arkansas), an area with higher stroke mortality compared with the rest of the United States. Potential participants were contacted through telephone and mail, and those who consented were interviewed by telephone to collect data on demographic and risk factors. During a subsequent in-home visit conducted 3–4 wk after the telephone interview, standardized protocols were used to collect blood samples, anthropometric measurements, and electrocardiogram and other risk factor data such as blood pressure. During the in-person assessment, self-administered questionnaires were left with the participants to complete and mail back. Participants (or their proxies) were contacted twice every year to determine their stroke and vital status. In the event of a suspected stroke, relevant hospital records were sought. Incident strokes were verified by using standardized protocols. Additional details are provided elsewhere (17). Deaths could be reported by proxies through telephone or mail. The vital status of participants lost during follow-up was determined by using the Social Security Administration vital status service for research and/or the National Death Index. Institutional review boards of all participating institutions approved the REGARDS study, and participants gave informed consent.

Dietary assessment

Dietary consumption was assessed by a self-administered Block 1998 food-frequency questionnaire (18). Pictures were given to participants to aid them in estimating their portion sizes. This instrument has been extensively validated in diverse populations in the United States and reliably estimates the intakes of nutrients including total fat, saturated fat, monounsaturated fat, polyunsaturated fat, and TFAs (19). Detailed information on TFA intake in the REGARDS study population is available in our previous publication (12).

Laboratory analyses

Fasting blood samples were collected and centrifuged; serum or plasma was then shipped overnight to a central laboratory at the University of Vermont for reprocessing and analysis (16, 20). Lipid concentrations were assayed by using colorimetric reflectance. High-sensitivity C-reactive protein was analyzed by particle-enhanced immunonephelometry (Abbott High-Sensitivity CRP; Dade Behring), and white blood cell count was determined by using an automated analyzer (Beckman Coulter).

Definitions and covariates

The main outcome variable was incidence of any stroke, whereas the secondary outcome was ischemic stroke. The main exposure variable was energy-adjusted TFA intake modeled in 1-SD increments. We also assessed the presence of a dose-response relation by using energy-adjusted TFAs in quintiles. Because of a small number of hemorrhagic strokes (n = 78), we did not test whether TFA intake is associated with incidence of hemorrhagic stroke. We considered the following covariates: age, sex, smoking status (never, past, or current smoker), race (black or white), region [stroke “buckle” (coastal plains of North Carolina, South Carolina, and Georgia), the rest of the stroke belt, or other], alcohol use (never, past, or current drinker), education (less than high school, high school, some college, or college graduate), waist circumference, physical activity, diabetes, ischemic heart disease, hypertension, heart failure, kidney failure, statin use (yes or no), regular aspirin use (yes or no), total energy intake, and intakes of saturated fat, monounsaturated fat, polyunsaturated fat, and protein. Physical activity was modeled in 3 categories (none, 1–3 times/wk, or ≥4 times/wk) on the basis of the question “How often do you exercise enough to work up a sweat?” Ischemic heart disease was defined as any self-report of myocardial infarction/heart attack, coronary angioplasty or bypass surgery, or electrocardiographic evidence of myocardial infarction (12). Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg at the baseline in-home visit or self-reported use of antihypertensive medication.

Statistical analyses

Cox proportional hazards regression was used to test the association between TFA intake and stroke incidence in 17,107 participants with complete data. Survival time was defined as the period between the in-home visit and stroke (first incidence), last follow-up, death, or data freeze (April 2013), whichever came first. To minimize correlations between nutrients and total energy intake, all nutrient variables were adjusted for total energy as previously described (21, 22). To test for trend, TFA intake was distributed into quintiles, and the quintile median value assigned to each individual in a given quintile. The resulting continuous TFA variable was used in Cox regression models to test for trend. HRs and 95% CIs were estimated before and after adjusting for various potential confounders. To maximize statistical power for testing the association between TFA intake and stroke, energy-adjusted TFA intake was divided by its SD and entered into the model as a continuous variable. As a result of evidence of a significant interaction between TFA intake and sex, and a confirmation of a previously documented interaction between age and race (17), separate analyses were conducted for men and women and analyses were adjusted for age × race interaction. In secondary analyses, the above models were refitted with ischemic stroke as the outcome variable. All models were checked for the proportionality assumption by using Schoenfeld residuals via the Loess smoothing algorithm. Main-effects P values <0.05 and interaction P values <0.10 were considered significant. All analyses were conducted in SAS version 9.2 (SAS Institute).

RESULTS

Of the 30,239 participants in the REGARDS study, we excluded individuals with a history of stroke at baseline (n = 919) and those without data from the Block food-frequency
TFA intake and total stroke

During a median (25th, 75th percentile) follow-up of 6.8 (5.4, 8.1) y, a total of 479 incident strokes were identified, including 401 ischemic strokes (Table 2). There was a significant interaction between TFA intake and sex (P = 0.06) and between age and race (P = 0.02) in relation to stroke risk. In unadjusted stratified analyses, there was a significant association between TFA intake and total stroke in men (HR: 1.20; 95% CI: 1.08, 1.33) but not in women (HR: 0.97; 95% CI: 0.83, 1.13). In models adjusted for age, race, age × race interaction, smoking, region, alcohol use, education, waist circumference, level of physical activity, diabetes, ischemic heart disease, hypertension, heart failure, kidney failure, statin use, regular aspirin use, total energy and energy-adjusted saturated fat, monounsaturated fat, polyunsaturated fat, and protein (fully adjusted models), the association remained significant in men (HR: 1.14; 95% CI: 1.02, 1.28) and nonsignificant in women (HR: 0.93; 95% CI: 0.79, 1.11). All models satisfied the proportionality assumption.

In dose-response analyses with TFA distributed into quintiles, we observed a significant (P < 0.05) trend of increased total stroke with increasing TFA intake in the unadjusted model for men but not for women (see Supplemental Table 1 under “Supplemental data” in the online issue). In fully adjusted models, there was no evidence for a significant trend for men or women.

TFA intake and ischemic stroke

Similar to total stroke, there was a significant interaction between TFA intake and sex (P = 0.08) and between age and race (P = 0.09) in relation to ischemic stroke risk. In unadjusted analyses, there was a significant association between TFA intake and incidence of ischemic stroke in men (HR: 1.19; 95% CI: 1.06, 1.34) but not in women (HR: 0.96; 95% CI: 0.81, 1.14). After adjustment for covariates, the association remained significant in men (HR: 1.13; 95% CI: 1.00, 1.28) but not in women (HR: 0.93; 95% CI: 0.77, 1.12). All models satisfied the proportionality assumption.

We also conducted dose-response analyses with TFA intake distributed into quintiles. Similar to total stroke, we observed a significant trend (P < 0.05) of increased ischemic stroke with increasing TFA intake in the unadjusted model for men. In women and in fully adjusted models for men, the trend was nonsignificant (see Supplemental Table 2 under “Supplemental data” in the online issue).

DISCUSSION

In this study, the relation between TFA intake and stroke incidence was modified by sex; for every 2-g/d increase in TFA intake, there was a 14% increase in risk of any stroke in men but not in women. This effect modification by sex was also observed when analyses were restricted to ischemic stroke, which contributed 84% of all adjudicated stroke incidences, in line with results from previous studies (13, 14). It is possible that TFA intake may have unequal effects on stroke subtypes, and findings from the NHS suggest that elevated TFA intake is associated with decreased risk of parenchymal hemorrhagic stroke (1); unfortunately, we could not test for the association between TFA intake and parenchymal or total hemorrhagic stroke in the current study because of the small number of hemorrhagic strokes (n = 78).

To our knowledge, this is the first study to show an interaction between TFA intake and sex with regard to stroke incidence. Most studies that investigated the association between TFA intake and stroke (see Supplemental Table 3 under “Supplemental data” in the online issue) were based on exclusively male or female cohorts, making it impossible to test for the interaction (1, 2, 4), whereas the only other study that comprised both men and women did not report testing for this interaction (3). In addition, despite much evidence showing regional differences in TFA intake and stroke incidence (23, 24), none of the previous studies adjusted for region. Our results, based on a national sample of blacks and whites and adjusted for region of the country, showed that elevated intake of TFAs is associated with increased risk of total and ischemic stroke in men but not in women.

In agreement with our results, findings from the NHS, a women-only cohort with a mean age of 46 y at baseline, showed no association between TFA intake and incidence of ischemic or total stroke (1). However, recent results in older women in the WHI-OS (mean baseline age of 64 y) showed a strong positive association between elevated TFA intake and incidence of ischemic stroke (2). Because advancing age may directly increase the risk of stroke or enhance potent stroke risk factors such as hypertension (13, 25–27), the observed association in the WHI-OS may, in part, be a reflection of higher risk of stroke in older women. Findings from the Cardiovascular Health Study, which included men and women (≥65 y old) and in which TFA was included in a principal component model, indirectly support findings of a positive association between TFA intake and ischemic stroke (3). Contrary to findings of a positive association in men in the REGARDS cohort, results from the HPFS, a men-only cohort, showed no association between TFA intake and ischemic stroke (4). However, results from the HPFS may not be surprising given that the study was based on health professionals who, compared with the general population, are more knowledgeable about the risk factors for stroke and who reported lower intakes of TFAs (~4.4 g/d compared with 8.6 g/d in the top quintiles of HPFS and REGARDS, respectively) (28).

Whether the observed disparity in risk of total or ischemic stroke by sex is a result of differences in the metabolism of TFAs in men compared with women, or simply a reflection of significantly higher intakes of TFAs in men compared with women,
**TABLE 1**

Distribution of potential confounders by quintiles of energy-adjusted trans fat intake in the REGARDS study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quintiles of trans fat intake</th>
<th>P value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>trans</em> Fat (% of energy)</td>
<td>1 (<em>n</em> = 3422)</td>
<td>2 (<em>n</em> = 3421)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>64.6 ± 9.0</td>
<td>64.5 ± 9.1</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>73</td>
<td>64</td>
</tr>
<tr>
<td>Region (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race (% white)</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>Education (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91.7 ± 14.8</td>
<td>93.9 ± 15.5</td>
</tr>
<tr>
<td>IHD (%)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Heart failure (%)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Kidney failure (%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Statin users (%)</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>MAI (%)</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>WBC (×10&lt;sup&gt;3&lt;/sup&gt; cells/L)</td>
<td>5.50 (4.53, 7.60)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5.57 (4.63, 8.62)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>104 (77, 146)</td>
<td>108 (80, 153)</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.62 (0.88, 4.52)</td>
<td>2.06 (0.91, 4.86)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>125.8 ± 16.0</td>
<td>125.8 ± 16.4</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.7 ± 9.6</td>
<td>75.8 ± 9.3</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>1658 ± 624</td>
<td>1621 ± 654</td>
</tr>
<tr>
<td>Protein (g/d)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>58.8 ± 14.8</td>
<td>59.3 ± 13.9</td>
</tr>
<tr>
<td>Carbohydrates (g/d)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>187 ± 47.2</td>
<td>187 ± 39.3</td>
</tr>
<tr>
<td>MUFAs (g/d)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>15.1 ± 5.7</td>
<td>16.5 ± 5.2</td>
</tr>
<tr>
<td>Saturated fat (g/d)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>16.5 ± 5.4</td>
<td>18.3 ± 5.0</td>
</tr>
<tr>
<td><em>trans</em> Fat (g/d)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2.8 ± 0.6</td>
<td>4.0 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>1</sup>The REGARDS study recruited 30,239 participants; 8447 participants were excluded for missing food-frequency questionnaire data, 214 for missing data on mortality, 919 for history of stroke at baseline, and 3552 for missing data on covariates, which left 17,107 participants for the current analysis. DBP, diastolic blood pressure; hsCRP, high-sensitivity C-reactive protein; IHD, ischemic heart disease; REGARDS, REasons for Geographic And Racial Differences in Stroke; SBP, systolic blood pressure; WBC, white blood cell count.

<sup>2</sup>The number of participants missing values was 5442 for WBC, 7 for SBP, and 8 for DBP.

<sup>3</sup>Differences between values in different quintiles were tested by using chi-square tests for categorical variables and 1-factor ANOVA or Kruskal-Wallis tests for continuous variables.

<sup>4</sup>Mean ± SD (all such values).

<sup>5</sup>Median; 25th and 75th percentiles in parentheses (all such values).

<sup>6</sup>Adjusted for total energy intake.
Fat intake was modeled in 1-SD increments (1 SD = 2.13 g/d). REGARDS, REasons for Geographic And Racial Differences in Stroke.

<table>
<thead>
<tr>
<th>Model</th>
<th>Overall</th>
<th>Men</th>
<th>Women</th>
<th>Overall</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.17 (1.08, 1.27)</td>
<td>1.20 (1.08, 1.33)</td>
<td>0.97 (0.83, 1.13)</td>
<td>1.16 (1.06, 1.27)</td>
<td>1.19 (1.06, 1.34)</td>
<td>0.96 (0.81, 1.14)</td>
</tr>
<tr>
<td>2</td>
<td>1.08 (0.99, 1.18)</td>
<td>1.14 (1.03, 1.27)</td>
<td>0.96 (0.82, 1.12)</td>
<td>1.07 (0.97, 1.18)</td>
<td>1.14 (1.01, 1.27)</td>
<td>0.95 (0.81, 1.13)</td>
</tr>
<tr>
<td>3</td>
<td>1.07 (0.97, 1.18)</td>
<td>1.14 (1.02, 1.28)</td>
<td>0.93 (0.79, 1.11)</td>
<td>1.06 (0.96, 1.18)</td>
<td>1.13 (1.00, 1.26)</td>
<td>0.93 (0.77, 1.12)</td>
</tr>
</tbody>
</table>

The REGARDS study recruited 30,239 participants; 8447 participants were excluded for missing food-frequency questionnaire data, 214 for missing data on mortality, 919 for history of stroke at baseline, and 3552 for missing data on covariates, which left 17,107 participants for the current analysis. trans Fat intake was modeled in 1-SD increments (1 SD = 2.13 g/d). REGARDS, REasons for Geographic And Racial Differences in Stroke.

Results were obtained by using Cox regression analyses. Model 1: unadjusted; model 2: adjusted for sex, age, and smoking status; model 3: adjusted as for model 2 plus race, age × race interaction, region, alcohol use, education, waist circumference, level of physical activity, diabetes, ischemic heart disease, hypertension, baseline stroke, heart failure, kidney failure, statin use, regular aspirin use, total energy and energy-adjusted saturated fat, monounsaturated fat, polyunsaturated fat, and protein.

The United States and other Western countries have set policies to reduce TFA intake in an attempt to avoid the deleterious health effects of elevated TFA intake (29–31). Elevation TFA intake is known to increase inflammation, dyslipidemia, and endothelial dysfunction in both men and women (7, 8). These mechanisms are thought to enhance the process of atherosclerosis, which is considered to be the main underlying disorder in ischemic stroke (14, 15). However, it is important to note that the number of ischemic strokes in men and women in this study was 221 and 180, respectively, whereas the number of total strokes was 265 and 214, respectively. The lower absolute number of strokes in women in the REGARDS cohort together with lower intake of TFAs in this group could have reduced the statistical power to detect the association in this group. Thus, the WHI-OS, which gave us more power to test the association between elevated TFA intake and stroke incidence, especially in the context of an interaction between TFA intake and sex.

We acknowledge a number of limitations. First, the intake of TFAs was self-reported, leading to potential inaccuracies associated with self-reported estimates. However, this approach has been validated in diverse populations in the United States and has been shown to provide reliable estimates of the intake of TFAs and other nutrients (19, 37, 38). Second, because we used baseline values to determine the association between TFA intake and incidence of stroke, the associations observed in this study may be imprecise given the decline in TFA exposure during follow-up (32, 33). Nevertheless, this approach has been used in many studies and has been shown to give reliable results (7, 37, 38).

In conclusion, our results suggest that elevated TFA intake is associated with increased risk of ischemic and total stroke in men but not in women. For every 2-g/d increase in TFA intake, there was a 14% increase in risk of stroke in men. Our findings provide further evidence to support the concerted effort to minimize TFAs in the diet. There is need for studies with larger numbers of stroke events to confirm these findings and to determine whether the increased risk observed for ischemic stroke also exists for hemorrhagic stroke and other stroke subtypes.

We thank the investigators and staff of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at http://www.regardsstudy.org.

The authors’ responsibilities were as follows—EKK: conceived the study idea and designed the analysis plan; PDM: conducted statistical analysis; JNK: drafted the manuscript; and all authors: participated in data interpretation, data acquisition, and critical revision of the manuscript for important intellectual content and approved the final draft of the manuscript. Amgen did not have any role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation or approval of the manuscript. None of the authors declared a conflict of interest.

REFERENCES


