# A Prospective Study of *Trans* Fatty Acids in Erythrocytes and Risk of Coronary Heart Disease

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***Background***—High consumption of *trans* fat has been linked to the risk of coronary heart disease (CHD). We assessed the hypothesis that higher *trans* fatty acid contents in erythrocytes were associated with an elevated risk of CHD in a nested case-control study among US women.

***Methods and Results***—Blood samples were collected from 32 826 participants of the Nurses' Health Study from 1989 to 1990. During 6 years of follow-up, 166 incident cases of CHD were ascertained and matched with 327 controls. Total *trans* fatty acid content in erythrocytes was significantly correlated with dietary intake of *trans* fat (correlation coefficient=0.44, $P<0.01$) and was associated with increased plasma low-density lipoprotein cholesterol ($P$ for trend =0.06), decreased plasma high-density lipoprotein cholesterol concentrations ($P$ for trend $<0.01$), and increased plasma low-density lipoprotein to high-density lipoprotein ratio ($P$ for trend $<0.01$). After adjustment for age, smoking status, and other dietary and lifestyle cardiovascular risk factors, higher total *trans* fatty acid content in erythrocytes was associated with an elevated risk of CHD. The multivariable relative risks (95% confidence intervals) of CHD from the lowest to highest quartiles of total *trans* fatty acid content in erythrocytes were 1.0 (reference), 1.6 (0.7 to 3.6), 1.6 (0.7 to 3.4), and 3.3 (1.5 to 7.2) ($P$ for trend $<0.01$). The corresponding relative risks were 1.0, 1.1, 1.3, and 3.1 ($P$ for trend $<0.01$) for a total of 18:1 *trans* isomers and 1.0, 1.5, 2.5, and 2.8 ($P$ for trend $<0.01$) for a total of 18:2 *trans* isomers.

***Conclusions***—These biomarker data provide further evidence that high *trans* fat consumption remains a significant risk factor for CHD after adjustment for covariates. **(*Circulation*. 2007;115:1858-1865.)**

**Key Words:** blood cells ■ coronary disease ■ fatty acids ■ women

*Trans* fat, primarily found in partially hydrogenated vegetable oils, is produced by the food industry to create solid fats from liquid oils. These fats increase the shelf life of food products and enhance the stability of frying oils. *Trans* fat accounts for 2 to 3% of total energy intake in US populations.[1] In a meta-analysis of 8 well-designed controlled trials, Mensink et al showed that, of all types of fatty acids, *trans* fatty acids had the strongest effect on raising the serum total cholesterol to high-density lipoprotein (HDL) ratio,[2] a known predictor of coronary heart disease (CHD) risk.[3] *Trans* fatty acids also increase lipoprotein (a)[4,5] and plasma triacylglycerol levels[6] and are associated with systemic inflammation, endothelial dysfunction,[7,8] and increased risks of type 2 diabetes,[9] all of which are previously demonstrated independent risk factors for CHD.

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Several prospective cohort studies have found a positive association between *trans* fat intake and the risk of CHD.[10–14] With data combined from 4 such studies,[11–14] Mozaffarian et al estimated a pooled relative risk of 1.23 (95% CI, 1.11 to 1.37) for every 2% energy from *trans* fat intake at baseline.[7] However, the effect size may be underestimated or overestimated because of measurement errors associated with dietary assessment. Biomarkers of *trans* fat intake have the advantages of freedom from reporting errors and the ability to assess different isomers of *trans* fatty acids; we therefore performed a nested case-control study to investigate the associations between *trans* fatty acid content in erythrocytes and risk of CHD.

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Received November 29, 2006; accepted January 24, 2007.

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*Circulation* **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.106.679985**

# Methods

## Study Population

The Nurses' Health Study was initiated in 1976 and enrolled 121 700 female registered nurses aged 30 to 55 years who lived in 11 US states. In 1989 and 1990, blood samples were collected from 32 826 women, among whom 167 cases of nonfatal myocardial infarction (MI) or CHD death were newly diagnosed between the time of blood drawing and June 1996. For each case, 2 controls matched for age (±1 year), smoking status (never, past, and current), fasting status at blood drawing (fasting for 10 hours or not), and date of blood drawing were randomly selected with risk-set sampling (ie, controls were selected from the rest of the nondiseased participants at the time of diagnosis of the cases). All cases and controls were free of diagnosed cancers and cardiovascular diseases at the time of blood drawing. After exclusion of 1 case and 7 controls that had missing data on blood fatty acid contents (as a result of contamination, dilution, or loss of blood samples), 166 cases and 327 controls were available for analysis.

## Assessment of Coronary Heart Disease

Medical records obtained from women who reported nonfatal MI in the biennial follow-up questionnaires were reviewed by study physicians who were blinded to the exposure status of participants. Nonfatal MI was confirmed if the World Health Organization criteria were met,[15] which require typical symptoms plus either diagnostic electrocardiographic findings or elevated cardiac enzyme levels. Deaths were identified by reports from next of kin, postal authorities, or by a search of the National Death Index. At least 98% of deaths among the Nurses' Health Study participants were identified.[16] CHD deaths were identified by examination of autopsy reports, hospital records, or death certificates, if CHD was listed as the cause of death. CHD deaths were then confirmed by a previous report of CHD and if there was no other more apparent or plausible cause of death. Unconfirmed CHD deaths were excluded from the present study. Total CHD was defined as nonfatal MI plus fatal CHD.

## Assessment of Other Factors

Information on the occurrence of diseases, medical history, and major lifestyle risk factors for CHD was collected at baseline by follow-up questionnaires. Information about history of hypertension, hypercholesterolemia, and diabetes was based on self-report. Diet has been assessed and updated every 4 years by validated semiquantitative food frequency questionnaires since 1980. A detailed description of the reproducibility and validity of the food frequency questionnaires has been published elsewhere.[17] Intake of nutrients and other lifestyle covariates were derived from the 1990 food frequency questionnaires and follow-up questionnaires, respectively. To minimize missing data, we carried forward the values obtained in previous questionnaires for values that were missing in the 1990 questionnaires.

## Blood Sample Collection and Fatty Acid Analysis

Within 24 hours of blood drawing, 97% of the samples arrived for analysis. Immediately upon arrival, the samples were centrifuged and divided into aliquots of plasma, erythrocytes, and buffy-coat fractions. These aliquots were stored in liquid nitrogen freezers at −130°C.

Fatty acids in erythrocytes and whole plasma were analyzed by gas-liquid chromatography. A detailed description of laboratory process has been reported elsewhere.[18] In gas-liquid chromatography analysis, the content of each fatty acid was expressed as a percentage of total fatty acids. Both technicians and laboratory personnel were blinded as to case-control status. Each case-control triplet was shipped in the same batch and analyzed in the same run. Within each triplet, samples were assayed by the same technicians in a random sequence under identical conditions. Laboratory control samples were run along with the case-control samples.

We quantified 7 *trans* isomers in erythrocytes and plasma. The average intra-assay coefficient of variation % of 18:1 *trans* isomers was 7.6% for erythrocytes and 8.0% for plasma. For 18:2 *trans* isomers, the values were 10.0% for erythrocytes and 6.9% for plasma.

Plasma total and HDL cholesterol was measured enzymatically. Low-density lipoprotein (LDL) cholesterol was determined by a homogenous direct method. The detailed description of laboratory methods and procedures has been published elsewhere.[3]

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

## Statistical Methods

Means and standard deviations were calculated for normally distributed continuous variables; medians and interquartile ranges were used otherwise. Proportions were calculated for categorical variables. Student *t* tests were used to evaluate the significance of associations between normally distributed continuous variables and CHD. Wilcoxon rank sum tests were used otherwise. $\chi^2$ tests of significance were used for categorical variables.

Conditional logistic regressions were used to estimate the relative risks of CHD associated with *trans* fatty acid contents. In nested case-control studies, odds ratios derived from conditional logistic regression models are unbiased estimates of hazard ratios or relative risks.[19] In the present study we focused primarily on erythrocyte *trans* fatty acid content because erythrocytes were more strongly correlated with long-term *trans* fat intake than plasma in our analysis (see Results). Cut points for quartiles of *trans* fatty acid contents were determined from the distribution of *trans* fatty acids among control participants. In addition to conditioning on the matching factors (by conditioning on the stratum indicator of matching), we further adjusted for established and potential risk factors for CHD, such as body mass index, physical activity, alcohol intake, parental history of MI before age 65 years, and other covariates. To examine potential confounding by fatty acids of other classes in the same blood fraction, we entered them into the multivariable models separately. Long-chain n-3 fatty acids, ie, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), and total n-6 fatty acids, which primarily consisted of linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), changed the relative risks of at least 1 category of total *trans* fatty acid content in erythrocytes by >15%. We therefore further adjusted for these fatty acids. Probability values for linear trend were calculated with an ordinal score based on the median value in each quartile of *trans* fatty acids entered into the models.

A secondary analysis examined the extent to which the association between *trans* fatty acids and the risk of CHD is explained by blood lipoprotein profiles by further controlling for the LDL to HDL ratio in the multivariable models. Potential confounding by other dietary risk factors (eg, folate, vitamin E supplement use, dietary fiber, and fruit and vegetable intake) was also assessed.

Multivariable linear regression was used to examine the linear trend of plasma lipoprotein parameters across quintiles of total *trans* fatty acid content in erythrocytes. Plasma lipoprotein parameters (such as plasma LDL and HDL cholesterol concentrations, and the LDL to HDL ratio) were entered into the model as dependent variables; quintiles of total *trans* fatty acid content, as well as age, smoking status, body mass index, and other covariates were entered as independent variables. Least-square means of biomarkers were calculated for each quintile. Robust estimators of variance for these means were calculated to allow for deviance from the assumption of normally distributed dependent variables.[20] Probability values for linear trend were estimated with the median value of each quintile of total *trans* fatty acid content entered into the model as an ordinal variable.

All probability values were 2-sided, and 95% CIs were calculated for relative risks and least-square means. Data were analyzed with the Statistical Analysis Systems software package, version 9.1 (SAS Institute, Inc., Cary, N.C.).

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**TABLE 1. Baseline Characteristics of Coronary Heart Disease Cases and Controls***

| Characteristics | Cases (N=166) | Controls (N=327) | *P* Value† |
|---|---|---|---|
| Age, y‡ | 60.6±6.0 | 60.4±6.1 | 0.77 |
| Body mass index, kg/m$^2$ | 26.9±5.9 | 25.4±4.5 | <0.01 |
| Physical activity, MET-hr | | | |
| Median | 10.6 | 13.4 | 0.07 |
| Interquartile range | 4.4 to 22.4 | 6.3 to 22.4 | |
| Diet, g/d | | | |
| *Trans* fat intake | 3.1±1.7 | 3.0±1.6 | 0.53 |
| Dietary fiber intake | 19.6±8.0 | 20.0±8.3 | 0.53 |
| Alcohol intake, g/d | | | |
| Median | 0.9 | 1.8 | 0.01 |
| Interquartile range | 0.0 to 3.5 | 0.0 to 6.7 | |
| Folate intake, μg/d | | | |
| Median | 378 | 407.0 | 0.52 |
| Interquartile range | 261 to 605 | 276 to 643 | |
| Fruit and vegetable intake, serving/d | | | |
| Median | 5 | 6 | 0.57 |
| Interquartile range | 4 to 8 | 4 to 8 | |
| Smoking status, %‡ | | | 0.99 |
| Current smoker | 32.5 | 31.9 | |
| Former smoker | 33.1 | 33.4 | |
| Never Smoked | 34.3 | 34.7 | |
| Medical history, % | | | |
| Diabetes | 21.1 | 6.7 | <0.001 |
| Hypertension | 59.0 | 29.4 | <0.001 |
| Hypercholesterolemia | 56.0 | 37.0 | <0.001 |
| Parental MI before age 65 years | 33.7 | 18.7 | <0.001 |
| Fasting status‡ | 60.2 | 55.4 | 0.30 |
| Postmenopausal | 92.8 | 90.2 | 0.35 |
| Postmenopausal hormone use§ | 57.8 | 58.6 | 0.86 |
| Cholesterol, mmol/L¶ | | | |
| HDL | 1.34±0.39 | 1.60±0.48 | <0.001 |
| LDL | 3.80±0.87 | 3.34±0.89 | <0.001 |
| Total | 6.25±1.06 | 5.78±1.02 | <0.001 |
| LDL to HDL ratio | 3.1±1.1 | 2.3±1.0 | <0.001 |

*Plus-minus values are mean±SD. Percentages are based on nonmissing data. MET-hr indicates metabolic equivalent-hours. Data are from the Nurses' Health Study, 1990.

†*P* value estimates are based on Student *t* test for variables expressed as mean±SD, Wilcoxon rank sum test for variables expressed as medians, or Pearson $\chi^2$ test for variables expressed as percentages.

‡Matching factors.

§Proportions are based on the number of postmenopausal women.

¶HDL cholesterol data were missing for 7 women; LDL cholesterol data were missing for 26 women. To covert values from mmol/L to mg/dL, multiply by 38.6698.

## Results

Table 1 shows the baseline characteristics of study participants. Women diagnosed with CHD events had a higher body mass index, drank less alcohol, were less physically active, were more likely to have a parental history of MI, and had less favorable plasma lipoprotein parameters than did controls.

**TABLE 2. Baseline *Trans* Fatty Acid Content in Erythrocytes of Study Participants***

| Fatty Acid† | Cases (N=166) | Controls (N=327) | *P* Value‡ |
|---|---|---|---|
| t 16:1n-7 | 0.13±0.03 | 0.14±0.03 | 0.53 |
| t 18:1n-12 | 0.33±0.10 | 0.30±0.09 | <0.001 |
| t 18:1n-9 | 0.52±0.17 | 0.48±0.16 | <0.01 |
| t 18:1n-7 | 0.40±0.10 | 0.38±0.11 | 0.05 |
| Total 18:1 *trans* isomers | 1.25±0.34 | 1.16±0.35 | <0.01 |
| 9t,12t 18:2n-6 | 0.13±0.05 | 0.12±0.05 | 0.05 |
| 9c,12t 18:2n-6 | 0.15±0.04 | 0.14±0.04 | <0.01 |
| 9t,12c 18:2n-6 | 0.10±0.03 | 0.10±0.03 | 0.22 |
| Total 18:2 *trans* isomers | 0.38±0.11 | 0.36±0.10 | 0.02 |
| Total *trans* fatty acids | 1.78±0.44 | 1.66±0.43 | <0.01 |

*Fatty acid contents are expressed as a percentage of total fatty acids in erythrocytes. Plus-minus values are mean±SD.

†t denotes *trans* configuration; c denotes *cis* configuration. Total *trans* fatty acids include 16:1n-7, 18:1, and 18:2 *trans* isomers listed in the table, plus 14:1n-5, 20:1n-9, and 20:2n-6 *trans* isomers, for which the contents are low in erythrocytes (mean<0.01).

‡*P* value estimates are based on Student *t* test.

Baseline erythrocyte contents of *trans* isomers are shown in Table 2. On average, *trans* isomers in erythrocytes accounted for only a small proportion of total fatty acids (total content for cases was 1.78% and for controls was 1.66%). The 18:1 isomers accounted for most of the *trans* isomers found in erythrocytes (70% in both cases and controls); *trans* 18:1n-9 was the most abundant 18:1 *trans* isomer in erythrocytes. For 18:1 *trans* isomers, CHD cases had significantly higher contents of 18:1n-12 and 18:1n-9 *trans* isomers than did controls. With respect to the 18:2 *trans* isomers, significant differences were found only for 9*cis*,12*trans* 18:2n-6.

The distribution of covariates across quartiles of total *trans* isomers in erythrocytes among controls is shown in Table 3. Erythrocyte *trans* fatty acid contents were inversely correlated with alcohol and folate intake. Weak inverse correlations were found between erythrocyte *trans* fatty acid content and fiber, fruit, and vegetable intake.

*Trans* fatty acids in erythrocytes reflected dietary *trans* fat intake as measured by the 1990 food frequency questionnaires; a Spearman partial correlation coefficient ($r_s$) of 0.44, which was adjusted for age, fasting status, and other covariates ($P<0.01$), was observed between them. The correlation between *trans* fat intake and plasma total *trans* fatty acid contents was weaker ($r_s=0.30$, $P<0.01$).

Higher total *trans* fatty acid content in erythrocytes were associated with nonsignificantly increased plasma LDL cholesterol (*P* for trend =0.06), significantly decreased plasma HDL cholesterol concentrations (*P* for trend <0.01), and significantly increased plasma LDL to HDL ratio (*P* for trend <0.01) (Figure 1).

Table 4 shows the relationship between baseline *trans* fatty acid contents in erythrocytes and the risk of total CHD. Total *trans* fatty acid content was significantly associated with the risk of CHD after conditioning on the

**TABLE 3. Distribution of Cardiovascular Risk Factors According to Quartile of Erythrocyte Total *Trans* Fatty Acid Contents Among Controls at Baseline***

| | Quartile of Total *Trans* Fatty Acids in Erythrocytes | | | | |
|---|---|---|---|---|---|
| | 1 (n=81) | 2 (n=82) | 3 (n=82) | 4 (n=82) | *P* for Trend |
| Total *trans* fatty acids, mean, %† | 1.17 | 1.50 | 1.72 | 2.23 | <0.0001 |
| Fatty acids, mean, % | | | | | |
| Total 18:1 *trans* isomers† | 0.78 | 1.02 | 1.21 | 1.61 | <0.0001 |
| Total 18:2 *trans* isomers† | 0.26 | 0.33 | 0.37 | 0.47 | <0.0001 |
| Body mass index, mean, kg/m$^2$ | 25.1 | 25.6 | 25.6 | 25.3 | 0.81 |
| Physical activity, median, MET-hr | 16.0 | 14.4 | 10.6 | 12.2 | 0.59 |
| Diet | | | | | |
| *Trans* fat intake, mean, g/d | 2.5 | 2.7 | 3.1 | 3.6 | <0.0001 |
| Dietary fiber intake, mean, g/d | 21.1 | 20.5 | 20.1 | 18.5 | 0.05 |
| Alcohol drinkers, % | 74.1 | 62.2 | 57.3 | 48.8 | <0.001 |
| Alcohol intake among drinkers, median, g/d | 9.3 | 4.7 | 3.7 | 2.1 | <0.0001 |
| Folate intake, median, $\mu$g/d | 426 | 440 | 418 | 334 | 0.02 |
| Fruit and vegetable intake, median, serving/d | 6 | 5 | 6 | 5 | 0.03 |
| Medical history, % | | | | | |
| Diabetes | 7.4 | 4.9 | 8.5 | 6.1 | 0.98 |
| Hypertension | 35.8 | 22.0 | 37.8 | 22.0 | 0.26 |
| Hypercholesterolemia | 34.6 | 35.4 | 39.0 | 39.0 | 0.48 |
| Parental MI before age 65 years | 17.3 | 18.3 | 18.3 | 20.7 | 0.59 |
| Postmenopausal | 90.1 | 87.8 | 92.7 | 90.2 | 0.72 |
| Postmenopausal hormone use‡ | 57.5 | 61.1 | 57.9 | 58.1 | 0.95 |
| Cholesterol, mean, mmol/L§ | | | | | |
| LDL | 3.24 | 3.37 | 3.38 | 3.38 | 0.40 |
| HDL | 1.77 | 1.57 | 1.55 | 1.49 | <0.001 |
| LDL to HDL ratio (mean) | 2.1 | 2.4 | 2.4 | 2.5 | 0.02 |

*Percentages are based on nonmissing data.

†Total 18:1 *trans* isomers include 18:1n-12, 18:1n-9, and 18:1n-7 *trans* isomers. Total 18:2 *trans* isomers include 9t,12t 18:2n-6, 9t,12c 18:2n-6, and 9c,12t 18:2n-6 *trans* isomers. Total *trans* fatty acids include 16:1n-7, 18:1, and 18:2 *trans* isomers, as well as 14:1n-5, 20:1n-9, and 20:2n-6 *trans* isomers, for which the contents are low in erythrocytes (mean<0.01).

‡Proportions are based on the number of postmenopausal women.

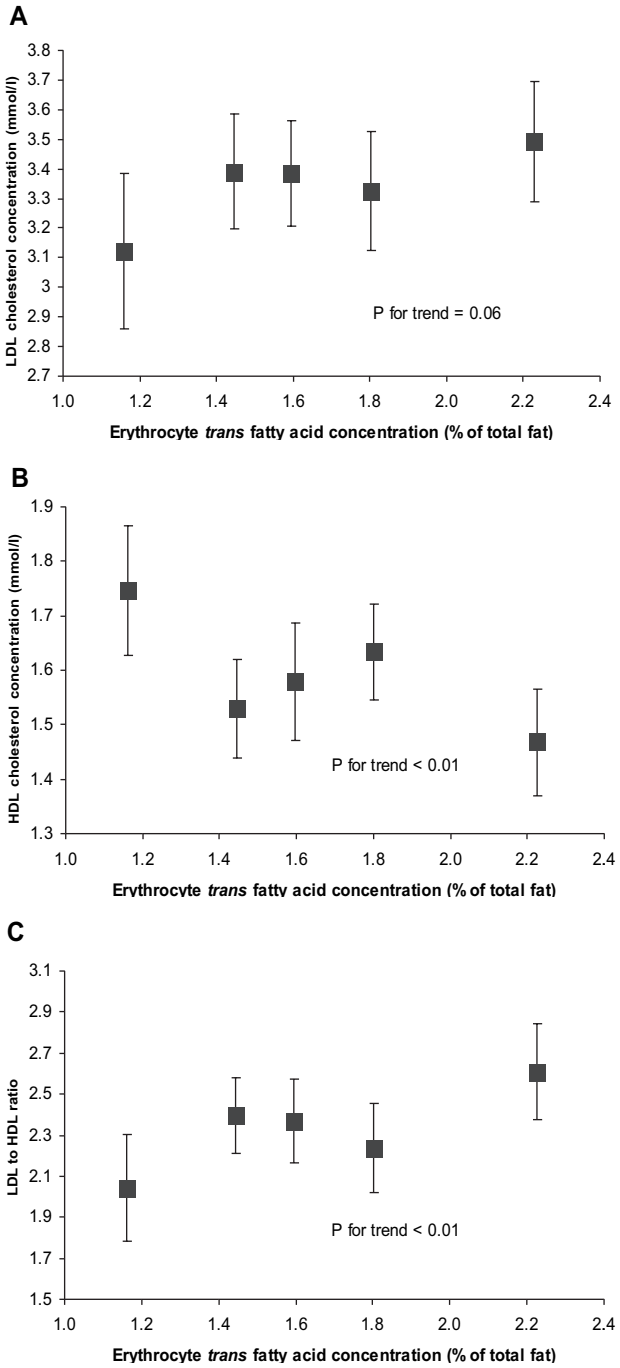
§Data of HDL were missing for 5 women; data of LDL were missing for 17 women. To covert values from mmol/L to mg/dL, multiply by 38.6698.



matching factors (model 1). In comparison to the women whose erythrocyte total *trans* fatty acid contents were in the lowest quartile, women in the highest quartile had a relative risk of total CHD of 2.7 (95% CI, 1.5 to 5.0; *P* for trend <0.01). After adjustment for established risk factors for CHD (model 2), the association was unchanged. After further adjustment for long-chain n-3 and total n-6 fatty acids (model 3), the association was strengthened (relative risk between extreme quartiles, 3.3; 95% CI, 1.5 to 7.2; *P* for trend <0.01). Similarly, in all 3 models, total 18:1 *trans* isomers and total 18:2 *trans* isomers were both significantly associated with an elevated risk of CHD (*P* for trend <0.01 in model 3). Adjustment for other fatty acids, such as $\alpha$-linolenic acid and saturated and monounsaturated fatty acids, did not change these associations. Further adjustment for intake of fiber, folate, vitamin E supplement, fruits, and vegetables did not alter these associations. To examine whether these findings might be caused by changes in diet subsequent to the diagnosis of diabetes, we excluded participants who were diagnosed with diabetes at baseline. Results were basically unchanged (data not shown). Associations for individual 18:1 and 18:2 *trans* isomers were similar to the associations for total 18:1 and 18:2 *trans* isomers, respectively.

Because 18:2 *trans* isomers are present at lower content and narrower variation than 18:1 *trans* isomers, to make a fair comparison we also estimated the relative risks for the same unit of increment in each class of *trans* isomer. For each 0.25% increment of content in erythrocytes, total 18:1 *trans* isomers were associated with an adjusted relative risk of 1.3 (95% CI, 1.1 to 1.6; *P*<0.01); total 18:2 isomers were associated with an adjusted relative risk of 2.2 (95% CI, 1.2 to 4.2; *P*=0.01).

To examine whether associations could be entirely explained by blood lipoproteins that may be in the causal pathway between *trans* fat intake and the risk of CHD, we




Relationship between erythrocyte *trans* fatty acid content and plasma lipid parameters among controls at baseline. Least-square means and 95% CIs of plasma lipoprotein parameters plotted against medians for each quintile of total *trans* fatty acid content in erythrocytes (%), adjusted for age at blood draw, smoking status (never, past, current), body mass index (<25, 25 to 29, ≥30), physical activity (≤8.1 metabolic equivalents [METs], 8.2 to 18.6 METs, ≥18.6 METs), alcohol intake (0 g/d, 1 to 4 g/d, 5 to 14 g/d, ≥15 g/d), parental history of MI before age 65 years (yes, no), and fasting status (yes, no). ■ indicate least-squares means of lipids; bars indicate 95% CIs for these means. A, LDL cholesterol; B, HDL cholesterol; C, LDL to HDL ratio.

further adjusted for the LDL to HDL ratio in the multivariable models (data not shown). Associations were attenuated after such adjustment. For example, the relative risk of CHD between extreme quartiles of total *trans* fatty acid content was attenuated from 3.3 to 2.2 (95% CI, 0.9 to 5.4; *P* for trend =0.07). Similarly, the association for total 18:1 *trans* isomers was also attenuated, although a significant trend was still observed. The relative risk between extreme quartiles of total 18:1 *trans* isomers was reduced from 3.1 to 2.2 (95% CI, 1.0 to 5.2; *P* for trend =0.02) after such adjustment. This suggests that part of the adverse effect of *trans* fat is mediated through changes in lipoprotein levels.

Plasma *trans* fatty acid content was also positively associated with the risk of CHD in multivariable analysis, although the linear trend was less clear. The relative risks (95% CI) for the second to the fourth quartile of plasma total *trans* fatty acid content were 1.3 (0.6 to 3.0), 2.5 (1.2 to 5.1), and 1.4 (0.7 to 3.2), respectively.

## Discussion

In this nested case-control study, we observed significant positive associations between erythrocyte *trans* fatty acid content and the risk of CHD. The risk among women in the highest quartile of erythrocyte *trans* fatty acid content was 3 times that of women in the lowest quartile after adjustment for established cardiovascular risk factors and other classes of fatty acids in erythrocytes. The plasma LDL to HDL ratio, which was significantly correlated with erythrocyte *trans* fatty acid contents, only partially explained these associations.

Substantial evidence from prospective cohort studies supports a positive association between dietary intake of *trans* fat and risk of CHD. In a pooled analysis of 4 cohort studies,[10–14,21] Mozaffarian et al estimated a pooled relative risk of 1.23 (95% CI, 1.11 to 1.37) for every 2% energy from *trans* fat intake at baseline.[7] The association was stronger in the Nurses' Health Study when repeated measures of diet were analyzed: multivariable relative risk of CHD was 1.33 (95% CI, 1.04 to 1.70) associated with 2% energy from *trans* fat intake.[12] Hu et al demonstrated that the use of repeated measures of diet strengthened the observed association as compared with the analysis that used baseline measures only, probably as a result of reduction in measurement error by use of the cumulative average of diet.[22]

Biomarkers of dietary intake are often believed to be more reliable than traditional dietary measurements because they are not subject to reporting errors.[17] Several retrospective case-control studies have examined the relationship between *trans* fatty acid contents in human blood or tissues and CHD risk, but results have been inconsistent.[18,23–28] Small sample size,[26] inadequate biomarkers,[27] and uncontrolled confounding by dietary factors[25] may explain the discrepancy among these studies. Our study is consistent, however, with 4 more recent and well-designed case-control studies.[18,23,24,28] Lemaitre et al observed moderate associations between erythrocyte or plasma phospholipid *trans* fatty acid content and increased risks of sudden death and fatal CHD.[24,28] Similarly, in a Costa Rican population Baylin et al detected a positive association between adipose tissue *trans* fatty acid contents and an elevated risk of non-fatal MI.[18] Clifton et al showed that, after *trans* fat was eliminated from margarines sold in Australia, the positive associations observed between *trans*

**TABLE 4. Relative Risk (95% CI) of Coronary Heart Disease Associated With *Trans* Fatty Acid Content in Erythrocytes***

| | Quartile of *Trans* Fatty Acid Content (%) | | | | |
|---|---|---|---|---|---|
| *Trans* Fatty Acid† | 1 (Lowest) | 2 | 3 | 4 (Highest) | P for Trend‡ |
| Total *trans* fatty acids | | | | | |
| Mean (range) | 1.17 (0.76 to 1.36) | 1.50 (1.37 to 1.59) | 1.72 (1.60 to 1.87) | 2.23 (1.88 to 3.42) | |
| Model 1 (matching factors) | 1.0 | 1.8 (1.0 to 3.4) | 1.7 (0.9 to 3.1) | 2.7 (1.5 to 5.0) | <0.01 |
| Model 2 (multivariable) | 1.0 | 1.6 (0.7 to 3.4) | 1.4 (0.7 to 3.0) | 2.7 (1.3 to 5.6) | 0.01 |
| Model 3 (model 2 plus n-3 and n-6 fatty acids) | 1.0 | 1.6 (0.7 to 3.6) | 1.6 (0.7 to 3.4) | 3.3 (1.5 to 7.2) | <0.01 |
| Total 18:1 *trans* isomers | | | | | |
| Mean (range) | 0.77 (0.48 to 0.93) | 1.03 (0.94 to 1.10) | 1.21 (1.11 to 1.32) | 1.62 (1.33 to 2.68) | |
| Model 1 (matching factors) | 1.0 | 1.3 (0.7 to 2.4) | 1.5 (0.8 to 2.7) | 2.4 (1.4 to 4.3) | <0.01 |
| Model 2 (multivariable) | 1.0 | 1.1 (0.5 to 2.3) | 1.2 (0.6 to 2.5) | 2.5 (1.2 to 5.0) | <0.01 |
| Model 3 (model 2 plus n-3 and n-6 fatty acids) | 1.0 | 1.1 (0.5 to 2.4) | 1.3 (0.6 to 2.7) | 3.1 (1.5 to 6.7) | <0.01 |
| Total 18:2 *trans* isomers | | | | | |
| Mean (range) | 0.25 (0.14 to 0.28) | 0.31 (0.29 to 0.34) | 0.38 (0.35 to 0.41) | 0.50 (0.42 to 0.78) | |
| Model 1 (matching factors) | 1.0 | 1.2 (0.6 to 2.2) | 2.1 (1.2 to 3.9) | 2.2 (1.2 to 4.1) | <0.01 |
| Model 2 (multivariable) | 1.0 | 1.5 (0.7 to 3.2) | 2.3 (1.1 to 5.0) | 2.2 (1.0 to 4.8) | 0.03 |
| Model 3 (model 2 plus n-3 and n-6 fatty acids) | 1.0 | 1.5 (0.7 to 3.4) | 2.5 (1.1 to 5.7) | 2.8 (1.2 to 6.3) | <0.01 |

*A total of 166 cases and 327 controls are included for analyses. Mean (range) of each quartile of *trans* fatty acid content in erythrocytes are based on the distributions among controls. The lowest quartile is the reference group. Model 1 is conditioned on the matching factors: age at blood draw, smoking status (never, past, current), fasting status (yes, no), and time of blood drawing. On the basis of model 1, multivariable model 2 is further controlled for body mass index (<25 kg/m$^2$, 25 to 29 kg/m$^2$, ≥30 kg/m$^2$), postmenopausal status (yes, no), postmenopausal hormone use (never, past, current), physical activity (≤8.1 metabolic equivalents [METs], 8.2 to 18.5 METs, ≥18.6 METs), alcohol intake (0 g/d, 1 to 4 g/d, 5 to 14 g/d, ≥15 g/d), parental history of MI before age 65 years (yes, no), history of hypertension (presence, absence), history of hypercholesterolemia (presence, absence), and history of diabetes (presence, absence). On the basis of model 2, multivariable model 3 is further controlled for long chain n-3 fatty acids and total n-6 fatty acids (both in quartiles) in erythrocytes.

†Total 18:1 *trans* isomers include 18:1n-12, 18:1n-9, and 18:1n-7 *trans* isomers. 18:2 *trans* isomers include 9t,12t 18:2n-6, 9t,12c 18:2n-6, and 9c,12t 18:2n-6 *trans* isomers. Total *trans* fatty acids include 16:1n-7, 18:1, and 18:2 *trans* isomers, as well as 14:1n-5, 20:1n-9, and 20:2n-6 *trans* isomers, for which the contents are low in erythrocytes (mean<0.01).

‡Estimates of *P* value for linear trend are based on linear scores derived from the medians of quartiles of *trans* fatty acid content among controls.

fatty acid contents in adipose tissue and the risk of nonfatal MI were diminished.[23]

The fact that 18:1 *trans* isomers have different biophysical characteristics from 18:2 *trans* isomers[29] leads to the hypothesis that these 2 classes of *trans* fatty acids may have different associations with CHD risk. Except for *trans* 16:1n-7, which exists exclusively in ruminant fats, 18:1 and 18:2 *trans* isomers exist in both partially hydrogenated oils and ruminant fats.[30] In partially hydrogenated oils, elaidic acid (*trans* 18:1n-9) is the major *trans* isomer, whereas, in ruminant fats, vaccenic acid (*trans* 18:1n-7) is the major *trans* isomer.[31] Thus, in the US diet, 18:1 *trans* isomers are the dominant form of *trans* fatty acids.[31] In the present study, the erythrocyte 18:1 *trans* isomers were also more abundant than 18:2 *trans* isomers. However, when *trans* fatty acid content was expressed as a continuous variable for the same amount of increment in erythrocyte content, 18:2 *trans* isomers were associated with a higher relative risk of CHD than 18:1 *trans* isomers. In the Costa Rican population, which had higher dietary intake of 18:2 *trans* fat but relatively lower intake of 18:1 *trans* fat than our population, the 18:2 *trans* isomers in adipose tissue were associated with higher relative risks of CHD than 18:1 *trans* isomers.[18] Two US studies have also found detrimental relationships between 18:2 *trans* isomers and cardiovascular end points.[24,28] Therefore, although 18:2 *trans* fat accounts for only a small proportion of total *trans* fat intake in US, its potential adverse effects should not be overlooked.

Because humans cannot synthesize *trans* fatty acids, *trans* isomers in human tissues represent their dietary intake levels, although different tissues may reflect long-term intake with various precisions. The associations with CHD appeared to be stronger for erythrocyte *trans* fatty acid contents than for plasma contents. In the present study, erythrocyte *trans* fatty acid content was more strongly correlated with dietary *trans* fat intake than plasma. Thus, erythrocyte *trans* fatty acid content may be a better biomarker of long-term *trans* fat intake than plasma content, probably because erythrocytes have a longer half-life than plasma lipid fractions. An alternative explanation is that fatty acid contents in plasma lipid fractions, particularly chylomicrons, reflected very recent diet more than longer-term diet when the blood samples of our participants were considered nonfasting.

Of many potential mechanisms through which *trans* fatty acids may increase the risk of CHD, the effect of *trans* fatty acids on plasma lipoprotein profiles has been most extensively studied in clinical dietary trials. Mensink et al summarized results from 8 clinical trials that specifically examined the effect of 18:1 *trans* isomer intake in a meta-analysis.[2] Results indicated that, of all classes of fatty acids, *trans* fatty acids had a strong effect raising serum LDL cholesterol concentrations and were the only class of fatty acids that did not raise HDL cholesterol concentrations in the process of

carbohydrate replacement. As a result, *trans* fatty acids had the strongest effect on raising total cholesterol to HDL ratio, which is a stronger predictor of CHD risk than total cholesterol.[3] In the present study, erythrocyte total *trans* fatty acid contents were significantly correlated with the plasma LDL to HDL ratio. After further adjustment for this ratio, the association between *trans* fatty acids and CHD risk was only partially attenuated. This is consistent with previous observations that CHD risk associated with *trans* fat intake was higher than that predicted based on change in lipoprotein profiles.[2,32] *Trans* fatty acids may increase the risk of CHD through other pathways, such as an increase in lipoprotein(a)[4,5] and blood triacylglycerol concentrations[6] and interference with essential fatty acids metabolism and eicosanoids balance by inhibition of delta-6-desaturase.[33,34] Furthermore, *trans* fat intake has been shown to be associated with systemic inflammation, endothelial cell dysfunction, and insulin resistance.[7,8] By their incorporation into the phospholipids in cell membranes, *trans* fatty acids may also alter the membrane function and decrease the membrane fluidity in a manner similar to saturated fat.[35]

The prospective design of the preset study is a noteworthy strength; the occurrence of disease could not influence biomarker contents in erythrocytes and plasma. Moreover, we adjusted for major lifestyle and dietary risk factors for CHD and other classes of fatty acids in erythrocytes. To address whether these associations could be caused by changes of diet subsequent to the diagnosis of diabetes, we reanalyzed the data after exclusion of diabetic participants. Results did not change appreciably.

The present study is also subject to several limitations. Single baseline measurements of erythrocyte *trans* fatty acid content will not perfectly reflect long-term dietary intake, which is the variable of interest. However, because the occurrence of CHD would not affect these biomarkers, and because blood samples were analyzed in a way that case-control status could not influence the measurements, any measurement errors are likely to be random and therefore would attenuate the association with CHD risk. Because we examined the associations for individual *trans* isomers simultaneously, the possibility of false-positive results could not be fully excluded, although these analyses were planned a priori, and these associations were all significant. Lastly, in observational studies residual confounding can never be entirely ruled out.

In summary, erythrocyte *trans* fatty acid content was associated with an increased risk of CHD among US women. These associations could be only partially explained by plasma lipoprotein parameters. The present study provides further evidence of the potential adverse effects of *trans* fat intake on cardiovascular health. *Trans* fat intake has been substantially reduced in European countries, whereas intake in the US is relatively stable.[36] Elimination of partially hydrogenated oils and other sources of *trans* fat from diet is likely to make an important contribution to the goal of reducing the burden of cardiovascular diseases.

## Sources of Funding

The present study was supported by research grants CA49449, CA42182, HL24074, HL34594, and CA87969 from the National Institutes of Health. Dr. Hu is a recipient of American Heart Association Established Investigator Award.

## Disclosures

None.

## References

1. Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT. Estimated intakes of trans fatty and other fatty acids in the US population. *J Am Diet Assoc*. 99:166–174, 1999; quiz 175–166.
2. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr*. 2003;77:1146–1155.
3. Shai I, Rimm EB, Hankinson SE, Curhan G, Manson JE, Rifai N, Stampfer MJ, Ma J. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. *Circulation*. 2004;110:2824–2830.
4. Nestel P, Noakes M, Belling B, McArthur R, Clifton P, Janus E, Abbey M. Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. *J Lipid Res*. 1992;33:1029–1036.
5. Sundram K, Ismail A, Hayes KC, Jeyamalar R, Pathmanathan R. Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *J Nutr*. 1997;127:514S–520S.
6. Katan MB, Zock PL, Mensink RP. Trans fatty acids and their effects on lipoproteins in humans. *Annu Rev Nutr*. 1995;15:473–493.
7. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med*. 2006;354:1601–1613.
8. Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, Willett WC, Hu FB. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr*. 2005;135:562–566.
9. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med*. 2001;345:790–797.
10. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med*. 1997;337:1491–1499.
11. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. Dietary fat and risk of coronary heart disease in men: cohort follow-up study in the United States. *Bmj*. 1996;313:84–90.
12. Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the Nurses' Health Study. *Am J Epidemiol*. 2005;161:672–679.
13. Oomen CM, Ocke MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet*. 2001;357:746–751.
14. Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol*. 1997;145:876–887.
15. Rose GA, Blackburn H, Gillum RF, Prineas RJ. *Cardiovascular Survey Method.* 2nd ed. Geneva, Switzerland: World Health Organization; 1982.
16. Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, Hennekens CH. Test of the National Death Index. *Am J Epidemiol*. 1984;119:837–839.
17. Willett WC. *Nutritional Epidemiology.* 2nd ed. New York: Oxford University Press; 1998.
18. Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H. High 18:2 trans-fatty acids in adipose tissue are associated with increased risk of nonfatal acute myocardial infarction in Costa Rican adults. *J Nutr*. 2003;133:1186–1191.
19. Rothman KJ, Greenland S. *Modern Epidemiology.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998.
20. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42:121–130.
21. Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet*. 1993;341:581–585.
22. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of

approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol*. 1999;149:531–540.
23. Clifton PM, Keogh JB, Noakes M. Trans fatty acids in adipose tissue and the food supply are associated with myocardial infarction. *J Nutr*. 2004; 134:874–879.
24. Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copass MK, Cobb LA, Siscovick DS. Cell membrane trans-fatty acids and the risk of primary cardiac arrest. *Circulation*. 2002;105:697–701.
25. Pedersen JI, Ringstad J, Almendingen K, Haugen TS, Stensvold I, Thelle DS. Adipose tissue fatty acids and risk of myocardial infarction: a case-control study. *Eur J Clin Nutr*. 2000;54:618–625.
26. Roberts TL, Wood DA, Riemersma RA, Gallagher PJ, Lampe FC. Trans isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. *Lancet*. 1995;345:278–282.
27. van de Vijver LP, van Poppel G, van Houwelingen A, Kruyssen DA, Hornstra G. Trans unsaturated fatty acids in plasma phospholipids and coronary heart disease: a case-control study. *Atherosclerosis*. 1996;126: 155–161.
28. Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, Tracy RP, Siscovick DS. Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the Cardiovascular Health Study. *Circulation*. 2006;114:209–215.
29. Emken EA. Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. *Annu Rev Nutr*. 1984;4:339–376.
30. Trans fatty acids and coronary heart disease risk. Report of the expert panel on trans fatty acids and coronary heart disease. *Am J Clin Nutr*. 1995;62:655S–708S; discussion 518–626.
31. Parodi PW. Distribution of isomeric octadecenoic fatty acids in milk fat. *J Dairy Sci*. 1976;59:1870–1873.
32. Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. *N Engl J Med*. 1999;340: 1994–1998.
33. Hill EG, Johnson SB, Lawson LD, Mahfouz MM, Holman RT. Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. *Proc Natl Acad Sci U S A*. 1982;79:953–957.
34. Kinsella JE, Bruckner G, Mai J, Shimp J. Metabolism of trans fatty acids with emphasis on the effects of trans, trans-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. *Am J Clin Nutr*. 1981;34:2307–2318.
35. Roach C, Feller SE, Ward JA, Shaikh SR, Zerouga M, Stillwell W. Comparison of cis and trans fatty acid containing phosphatidylcholines on membrane properties. *Biochemistry*. 2004;43:6344–6351.
36. Willett WC. Trans fatty acids and cardiovascular disease-epidemiological data. *Atheroscler Suppl*. 2006;7:5–8.

## CLINICAL PERSPECTIVE

Although previous cohort studies have consistently shown that high intake of *trans* fat was associated with an elevated risk of coronary heart disease (CHD), the association might be underestimated or overestimated because of the measurement errors of dietary instruments used in the cohort studies. *Trans* fatty acids in erythrocytes or plasma, which are biomarkers of dietary *trans* fat intake, are believed to be more objective in the measurement of long-term intake of *trans* fat. The current study examined the association between biomarkers of *trans* fatty acid intake and CHD risk among US women. In the present study, we demonstrated that high content of *trans* fatty acids, which included both 18:1 and 18:2 *trans* isomers, in erythrocytes was associated with an increased risk of CHD. In line with metabolic studies, we observed positive associations between these biomarkers and low-density lipoprotein cholesterol levels and inverse associations for high-density lipoprotein cholesterol levels. The elevated risk of CHD was only partially attenuated after adjustment of the blood lipid parameters, which suggests that *trans* fat intake may increase CHD risk through other mechanisms. The present study provides further evidence that higher consumption of *trans* fatty acids is associated with increased risks of CHD.

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# A Prospective Study of *Trans* Fatty Acids in Erythrocytes and Risk of Coronary Heart Disease

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*Circulation.* 2007;115:1858-1865; originally published online March 26, 2007;
doi: 10.1161/CIRCULATIONAHA.106.679985
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539