Intake of trans fatty acids and risk of coronary heart disease among women

WALTER C. WILLET
MEIR J. STAMPFER
JOANN E. MANSON
GRAHAM A. COLDITZ
FRANK E. SPEIZER
BERNARD A. ROSNER
LAURA A. SAMPSON
CHARLES H. HENNEKENS

Trans isomers of fatty acids, formed by the partial hydrogenation of vegetable oils to produce margarine and vegetable shortening, increase the ratio of plasma low-density-lipoprotein to high-density-lipoprotein cholesterol, so it is possible that they adversely influence risk of coronary heart disease (CHD). To investigate this possibility, we studied dietary data from participants in the Nurses' Health Study.

We calculated intake of trans fatty acids from dietary questionnaires completed by 85 095 women without diagnosed CHD, stroke, diabetes, or hypercholesterolemia in 1980. During 8 years of follow-up, there were 431 cases of new CHD (non-fatal myocardial infarction or death from CHD). After adjustment for age and total energy intake, intake of trans isomers was directly related to risk of CHD (relative risk for highest vs lowest quintile 1.50 [95% CI 1.12-2.00], p for trend = 0.001). Additional control for established CHD risk factors, multivitamin use, and intakes of saturated fat, monounsaturated fat, and linoleic acid, dietary cholesterol, vitamins E or C, carotene, or fibre did not change the relative risk substantially. The association was stronger for the 69 181 women whose margarine consumption over the previous 10 years had been stable (1.67 [1.05-2.66], p for trend = 0.002). Intakes of foods that are major sources of trans isomers (margarine, cookies [biscuits], cake, and white bread) were each significantly associated with higher risks of CHD.

These findings support the hypothesis that consumption of partially hydrogenated vegetable oils may contribute to occurrence of CHD.


Introduction

Trans isomers of fatty acids are formed when liquid vegetable oils are partially hydrogenated to form margarine and shortening. These processed vegetable fats, which many people use instead of animal fats containing saturated fat and cholesterol because of health concerns, contain between 5% and more than 30% of the isomers. Trans isomers contribute to the hardness of the products; the carbon molecules on the two sides of a double bond provide a straight, closely packed configuration, whereas the cis isomers found in natural vegetable oils have a bent configuration. Trans fatty acids are also formed in the rumen of cattle and make up about 5% of dairy and beef fat; however, the predominant isomer is structurally distinct from those derived from vegetable oils. Trans isomers are estimated to constitute 5-6% of dietary fat consumed in the USA, but the proportion varies widely, depending on food choices.

There has been concern for some time that a high intake of trans isomers could adversely affect risk of coronary heart disease (CHD), partly because the straight configuration resembles that of saturated fats. Also, since many trans isomers are derived from the naturally occurring cis, cis-linoleic acid, a precursor of prostaglandins, they could have various effects on platelet activity and other important functions. Some, but not all, studies have shown moderate rises in serum total cholesterol with trans isomers.

The 1985 review by the Federation of American Societies for Experimental Biology concluded that available laboratory evidence was not sufficient to implicate trans fatty acids as a cause of CHD; however, further investigation was recommended. Concern has been heightened by reports that replacement of naturally occurring fatty acid with trans isomers can increase concentrations of low-density-lipoprotein (LDL) cholesterol and lower concentrations of high-density-lipoprotein (HDL) cholesterol (and J. Judd, unpublished) and lipoprotein(a), and lower concentrations of high-density-lipoprotein (HDL) cholesterol (and J. Judd, unpublished).

To investigate whether a higher intake of trans isomers is associated with an increased risk of CHD, we studied dietary data collected in 1980 from participants in the Nurses' Health Study in relation to subsequent risk of CHD.

Subjects and methods

The Nurses' Health Study began in 1976, when 121 700 US female registered nurses completed questionnaires about their medical history, including previous cardiovascular disease, diabetes, hypertension, high serum cholesterol concentrations, and parental myocardial infarction. We also included questions on menopausal status, the use of postmenopausal hormones, cigarette smoking, height, and weight. Every 2 years, follow-up questionnaires were sent so that information could be updated and newly diagnosed major illnesses identified. In 1980 we collected data on usual dietary intake by means of a semiquantitative food frequency questionnaire developed and validated specifically for this study. We identified 61 foods that allowed maximum discrimination among intakes of total, saturated, and monounsaturated fats, linoleic acid (the most abundant polyunsaturated fat), cholesterol, trans fatty acids, and other nutrients. For each food, a commonly used unit or portion size (eg, one egg or one slice of bread) was specified, and the subject was
Mean intake of trans fatty acids (g/day)

<table>
<thead>
<tr>
<th>Quintile of trans-fatty-acid intake</th>
<th>1 (n = 16,768)</th>
<th>2 (n = 17,112)</th>
<th>3 (n = 17,067)</th>
<th>4 (n = 17,123)</th>
<th>5 (n = 17,026)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intake of trans fatty acids (g/day)</td>
<td>2.4</td>
<td>2.2</td>
<td>2.0</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>% of group</td>
<td>Current smokers</td>
<td>28</td>
<td>29</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Parental MI before 60 yr</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Current postmenopausal hormone use</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Vigorous exercise ≥1 wk</td>
<td>50</td>
<td>46</td>
<td>43</td>
<td>41</td>
<td>35</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td>42</td>
<td>36</td>
<td>33</td>
<td>31</td>
<td>28</td>
</tr>
</tbody>
</table>

TABLE II—RELATIVE RISK OF CHD ACCORDING TO ENERGY-ADJUSTED TRANS-FATTY-ACID INTAKE IN 1980*

<table>
<thead>
<tr>
<th>Quintile of trans-fatty-acid intake</th>
<th>1 (n = 16,768)</th>
<th>2 (n = 17,112)</th>
<th>3 (n = 17,067)</th>
<th>4 (n = 17,123)</th>
<th>5 (n = 17,026)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative risk (95% CI) in quintile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of cases</td>
<td>89</td>
<td>89</td>
<td>70</td>
<td>86</td>
<td>106</td>
</tr>
<tr>
<td>Person-years follow-up</td>
<td>130,345</td>
<td>133,057</td>
<td>132,898</td>
<td>133,439</td>
<td>132,260</td>
</tr>
<tr>
<td>Relative risk with adjustment for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age only</td>
<td>1.0</td>
<td>1.15 (0.85-1.56)</td>
<td>1.03 (0.74-1.42)</td>
<td>1.16 (0.85-1.59)</td>
<td>1.50 (1.12-2.00)</td>
</tr>
<tr>
<td>Age and smoking</td>
<td>1.0</td>
<td>1.15 (0.85-1.56)</td>
<td>1.02 (0.74-1.42)</td>
<td>1.17 (0.86-1.60)</td>
<td>1.45 (1.08-1.94)</td>
</tr>
<tr>
<td>Standard risk factors*</td>
<td>1.0</td>
<td>1.12 (0.82-1.52)</td>
<td>0.97 (0.71-1.36)</td>
<td>1.12 (0.82-1.54)</td>
<td>1.35 (1.00-1.82)</td>
</tr>
<tr>
<td>Standard risk factors plus lipid*</td>
<td>1.0</td>
<td>1.15 (0.83-1.59)</td>
<td>1.03 (0.72-1.48)</td>
<td>1.22 (0.83-1.78)</td>
<td>1.57 (1.05-2.34)</td>
</tr>
<tr>
<td>Standard risk factors, lipid, and use of multivitamin</td>
<td>1.0</td>
<td>1.12 (0.81-1.55)</td>
<td>0.99 (0.69-1.43)</td>
<td>1.16 (0.80-1.70)</td>
<td>1.47 (0.96-2.20)</td>
</tr>
</tbody>
</table>

*Age, smoking, body-mass index, hypertension, alcohol intake, menopausal status, postmenopausal oestrogen use, energy intake, family history of myocardial infarction before age 60 yr. 

TNT intake of saturated fat, monounsaturated fat, and linoleic acid.
TABLE III—RELATIVE RISK OF CHD IN RELATION TO ENERGY-ADJUSTED TRANS-FATTY-ACID INTAKE AMONG WOMEN WITHOUT CHANGE IN MARGARINE CONSUMPTION 1970-80*

<table>
<thead>
<tr>
<th>RR (95% CI) in quintile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total trans isomers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomers from vegetable fats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomers from animal fats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*356 cases among 69 181 women, adjusted for age, smoking, body-mass index, hypertension, alcohol intake, menopausal status, postmenopausal oestrogen use, energy intake, dietary lipids, family history of myocardial infarction before age 60 yr, and multivitamin use.

Results

Among the 85 995 women followed for up to 8 years, there were 431 cases of CHD (324 non-fatal myocardial infarctions and 107 deaths from CHD) during 661 996 person-years of follow-up. The mean daily intake of total trans fatty acids in 1980 was 4.0 (SD 1.9) g or 5.8 (1.8)% of dietary fat. Median daily energy-adjusted trans-fatty-acid intake ranged from 2.4 g in the lowest quintile to 5.7 g in the highest (table I). Of the trans-isomer intake, 60% was from processed vegetable fats and 40% from animal sources, primarily beef and dairy fat.

Prevalence of current smoking, history of hypertension, parental myocardial infarction, and current postmenopausal oestrogen use did not vary significantly with intake of total trans fatty acids (table I). Although women with higher intakes of trans isomers tended to exercise less, their mean body-mass index was only slightly higher than that of women with lower intakes. As expected, intake of trans fatty acids was strongly associated with intake of other types of fat, especially monounsaturated fat and linoleic acid. These strong associations result partly from the inclusion of both cis and trans isomers in the calculations of monounsaturated fat and linoleic acid, as is standard practice. Intakes of carotene and dietary fibre and use of multivitamins were inversely associated with trans-fatty-acid consumption. In similar analyses for vegetable and animal sources of trans isomers, current cigarette smoking was less prevalent in groups with higher consumption of vegetable sources but increased with higher intake of animal sources (lowest vs highest quintile, 31% vs 28% for vegetable sources and 25% vs 34% for animal sources). As expected, intake of cholesterol and saturated fat were associated positively with animal trans-isomer intake and negatively with vegetable trans-isomer intake. Other variables were similarly related to intakes of animal and vegetable sources of trans isomers, except that the inverse relation with fibre intake was largely limited to animal sources.

When adjusted for age and total energy intake, intake of trans fatty acids was associated with a higher risk of CHD; the relative risk for the highest versus the lowest quintile was 1.50 (95% CI 1.12-2.00, p for trend = 0.001; table II). Adjustment for cigarette smoking, the strongest CHD risk factor in these data, decreased the relative risk only slightly. Further adjustment for other established CHD risk factors again slightly lowered the relative risk, but the trend with increasing intake remained significant.

The inclusion of other dietary fats in the analysis is very important because trans-isomer intake was strongly correlated with intake of monounsaturated fat and linoleic acid, both potentially protective against CHD. Additional adjustments individually for intake of monounsaturated fat or linoleic acid tended to increase the relative risk associated with intake of trans isomers. When linoleic acid, monounsaturated fat, and saturated fat were all added simultaneously to the model with established risk factors, the association between trans-isomer intake and CHD risk was a little stronger than that without these dietary lipids. Inclusion of multivitamin use, which was associated with lower risk of CHD, in the model with established risk factors and dietary lipids slightly reduced the association with trans isomers, but the trend remained significant. The 50% excess risk of CHD among women in the highest quintile of trans-isomer intake did not change appreciably and the tests for trends remained significant with further adjustments individually for intakes of carotene, vitamin E including supplements, vitamin C including supplements, dietary fibre, or cholesterol (data not shown). The inclusion of a term for vigorous physical activity did not affect the relative risks.

Because the relation of trans-fatty-acid intake to incidence of CHD could be distorted by alteration of diet in women who suspected they were at higher risk, we excluded at baseline those with previous angina, myocardial infarction, stroke, diabetes, or hypercholesterolaemia. To investigate further the possibility that some women might have suspected early, undiagnosed symptoms of CHD and thus substituted margarine for butter intake, we did an analysis excluding women who in 1980 reported that their margarine intake had greatly changed in previous 10 years (table III).

The association of trans-isomer intake with risk of CHD was somewhat stronger in this analysis; after adjustment for established risk factors and dietary lipids the relative risk (highest vs lowest quintile of trans-isomer intake) was 1.67 (95% CI 1.05-2.66). When intake of trans fatty acids was expressed as a percentage of total energy intake rather than adjusted for total energy intake by regression analysis, there was a similar significant relation (figure).
The possibility that early symptoms of incipient CHD distorted the relation was further examined by dividing the follow-up into two 4-year periods (1980–84 and 1984–88). Positive trends with intake of trans isomers in 1980 were seen in both periods, but the association was slightly stronger during the second period (highest vs lowest quintile, relative risk = 1.55 for 1980–84 and 2.34 for 1984–88 in analyses adjusted for established risk factors and dietary lipids).

Trans fatty acids formed in the partial hydrogenation of vegetable oils differ structurally from those found in fat from ruminants; we therefore examined these sources separately in relation to risk of CHD (table III). The positive overall association was entirely accounted for by partially hydrogenated vegetable fats. By contrast, a non-significant inverse association was observed for trans isomers from animal fats.

To elucidate these relations further, we identified the fats that contributed most to differences in total trans isomer intake among study participants. By stepwise regression with energy-adjusted total trans isomer intake as the dependent variable, each food was entered as a predictor variable. The four most important determinants were margarine, beef, pork, or lamb as a main dish, cookies (biscuits), and white bread (total $r^2 = 0.82$). We then examined each food in relation to risk of CHD by including them in models with control for age and other CHD risk factors (table IV). Women who ate four or more teaspoons of margarine (20 ml) per day were at higher risk of CHD (relative risk 1.66 [1.10–2.49]) than women who ate margarine less than once per month (p for trend = 0.02).

When we examined associations for margarine in tub form or stick form separately, similar positive trends were seen for both (data not shown). Consumption of beef, pork, or lamb as a main dish was not significantly related to risk. However, consumption of cookies and white bread was significantly associated with higher risk of CHD. Intake of butter was not an important source of trans isomers, was not significantly associated with risk of CHD.

**Discussion**

In this large prospective cohort study we observed a positive association between intake of trans isomers of fatty acids and risk of CHD that was not accounted for by known risk factors, including other measured dietary variables. The positive association with total intake of trans isomers was due to intake of partially hydrogenated vegetable fats rather than isomers from ruminant sources.

One reason for concern that trans-isomer intake may increase the risk of CHD has been that consumption has greatly increased since the beginning of the century, at the same time as a major rise in CHD mortality. Since the mid 1960s, mortality from CHD has declined, as has intake of trans isomers as a percentage of total vegetable fat intake; the average absolute intake is also likely to have declined because both the trans-isomer composition of all fats produced in the USA and total fat intake per person decreased during this time. The reductions in intake are due largely to shifts towards softer margarines, which tend to have higher cis, ω-6 fatty acids and lower trans-isomer content, and less hydrogenation of vegetable shortenings.

Our most recent analyses indicate that the trans-isomer content of margarines in the USA ranges between 7% and 24% of fatty acids, and that of vegetable shortening is about 15% (P. Slacks, personal communication). Also, production of partially hydrogenated cooking oils stopped in 1985–88. Although the intake of trans isomers generally followed the rise and subsequent decline of CHD in the USA during this century, data on time trends are difficult to interpret because many other factors vary simultaneously. In 1989–90 the US fast-food industry largely changed from use of beef tallow (3–5% trans isomers) to partially hydrogenated vegetable fats (about 30% trans isomers). Thus, we found that the trans-isomer content of French fried potatoes from McDonald's and Burger King restaurants was between 24% and 35% of fatty acids. Pressures to avoid beef tallow and tropical oils are resulting in the substitution of partially hydrogenated vegetable fats in other products too.

In the only previous analytical study of trans-fatty-acid intake and risk of CHD the trans-isomer content of adipose fat was higher in people who died from ischaemic heart disease than among people dying from other causes. However, the study design did not allow for the control of other CHD risk factors. Moreover, in the part of the UK where that study took place, such isomers derived from partially hydrogenated marine oils used for margarine. Although replication of that finding suggests that it is not an important source of trans isomers, was not significantly associated with risk of CHD.
may account for these findings. Trans isomers seem to increase plasma lipoprotein a concentrations.8,9

Trans fatty acids are closely related structurally to linoleic acid, and thus could interfere with important biological functions. Trans isomers may influence essential-fatty-acid metabolism by impairing the activity of Δ6Δ5 desaturase, thus reducing the amount of arachidonic acid available, by interfering with other steps in the synthesis of eicosanoids from essential-fatty-acid precursors, and by becoming incorporated into membrane phospholipids and altering physical structure and function.21 In vitro, collagen-induced platelet aggregation is inhibited less by trans than by cis isomers;24 thrombogenesis may therefore be increased.

Our data on consumption of specific foods in relation to risk of CHD show that the association with trans-isomer intake is not derived from a single food or eating pattern; the use of margarine (as opposed to butter) is regarded by many as healthy eating behaviour. By contrast, consumption of cookies and white bread is widely considered less healthy. Wefound no association between red meat intake and CHD despite the fact that positive responses to this question were strongly associated with colon cancer risk.25 This lack of association could be due to the distinct structure of the primary trans isomer in ruminant fat, trans-vaccenic acid, which has a double bond in the 11 as opposed to the 9 position in elaidic acid, the main trans isomer in partially hydrogenated vegetable oils.2 In human fibroblast cultures, elaidic acid inhibits Δ6 desaturase and fatty-acid synthesis to a greater degree than does trans-vaccenic acid.40 On the other hand, the trans-isomer content of beef fat is low and most of the fatty acids are monounsaturated; these compounds have beneficial effects on blood lipids41 and the susceptibility of LDL-cholesterol to oxidative modification.28

Many sources of potential bias are limited by the prospective nature of this study. Nevertheless, it is possible that women with high intakes of trans isomers were at increased risk of CHD for other unknown reasons. A higher intake of animal sources of trans isomers was related to a general pattern of unhealthy behaviours, whereas it was the intake of vegetable sources that was associated with higher risk of CHD. We do not have evidence that the observed association is due to a general relation with less health-conscious behaviour. More direct data on the relation of intake of trans fatty acids and risk of CHD might be obtained from a randomised trial, but this does not seem feasible. Thus, findings from controlled metabolic studies, which indicate potentially adverse effects of these isomers, as well as other observational studies, will be important. Our study cannot distinguish the influence of the many specific isomers formed by the partial hydrogenation of vegetable oils, which include cis isomers with the double bonds in altered positions as well as various trans-isomers. Although this study included only women, the fact that blood lipids are related to intake of trans isomers similarly in men and in women suggests that the findings are likely to apply to men too.

Our findings must add to concern that the practice of partially hydrogenating vegetable oils to produce solid fats may have reduced the anticipated benefits of substituting these oils for highly saturated fats, and instead contributed to the occurrence of CHD.

REFERENCES