Adipose Tissue Trans Fatty Acids and Breast Cancer in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer

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Abstract

To investigate the relationship between trans fatty acids and postmenopausal breast cancer in European populations differing greatly in their dietary fat intakes, a case-control study using adipose tissue stores of trans fatty acids as a biomarker of exposure was conducted. Subjects included 698 postmenopausal incident cases of primary breast cancer and controls randomly drawn from local population and patient registries, ages 50-74. Concentrations of individual trans fatty acids in gluteal fat biopsies were measured in these women.

The adipose concentration of trans fatty acids showed a positive association with breast cancer. The covariate-adjusted OR was 1.40 (95% confidence interval: 1.02, 1.93) for the difference between the 75th and 25th percentiles of total adipose trans. The adjusted OR for trans in the lowest tertile of polyunsaturated fatty acid reached 3.6 (2.2, 6.1). These associations were not attributable to differences in age, body mass index, exogenous hormone use, or socioeconomic status. These findings suggest an association of adipose stores of trans fatty acids with postmenopausal breast cancer in European women. They require confirmation in other populations, with concomitant consideration of the potential roles of dietary saturated and monounsaturated fats.

Introduction

The hypothesis that dietary fat intake plays a role in the development of breast cancer is supported by migrant (1-3), case-control (4), and ecological studies (5) of diet and fat but not cohort studies (6-8). Inconsistencies among studies may be due in part to the physiological importance of individual fatty acids. Evidence is accumulating that consumption of specific types of fat may be important determinants of breast cancer risk (6, 9, 10).

PUFAs influence carcinogenesis in animal models. Although the mechanisms causing this effect remain unknown, metabolites of polyunsaturated fats have been shown to influence gene transcription (11, 12). The desaturation and chain elongation of these essential fatty acids to their long-chain polyunsaturated metabolites can be impaired by trans fatty acids. Trans fatty acids compete for desaturase enzyme sites and can reduce production of the 3-series eicosanoids (13-21). They have also been associated with chromosome breakage and spindle dysfunction in some experimental models (22). To the degree that perturbation of "normal" eicosanoid production affects carcinogenesis, the effect of a given increase in trans may depend on the level of polyunsaturated fat competing with it for binding sites. This study was designed to analyze the potential relationships between trans and polyunsaturated fat intakes in the occurrence of postmenopausal breast cancer.

Differences in measuring habitual intakes of individual fatty acids have inhibited research on specific fatty acids and cancer. Adipose tissue fatty acids provide a promising alternative to questionnaire-derived indices of long-term intake for fatty acids derived entirely or primarily from the diet, specifically trans and PUFAs (23-27). This study is unusual in that it collects adipose tissue as a biomarker of long-term trans fatty acid intakes in populations differing greatly in the nature of their dietary fat intakes.

The abbreviations used are: PUFA, polyunsaturated fatty acid; OR odds ratio; EURAMIC, European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer; SES, socioeconomic status.
Adipose Fatty Acids and Breast Cancer

The sum of all fatty acid that makes up most of the total polyunsaturated fat. C14:1 or 9 is a short-chain trans isomer peak detected in some subjects; C18:1 (tr6 or tr9) and (tr11 or cis9) include the monounsaturated trans isomers of the oleic acid family measured in this study, whereas C18:2 tr 9 or 12 represents the polyunsaturated trans isomers of the linoleic acid family. C18:2 au6 is linoleic acid, the cis (ω6) fatty acid that makes up most of the total polyunsaturated fat.

The sum of all trans fatty acids identified in footnote c.

### Patients and Methods

**Recruitment.** Centers in five European countries recruited incident cases of breast cancer from participating hospitals between 1990 and 1992. Recruitment involved all postmenopausal women (ages 50–74) newly diagnosed with primary breast cancer (ICD Code 174). Cases had ductal carcinomas with primary tumors less than 5 cm, axillary lymph nodes stage <N1, and no clinical indication of distant metastases.

Controls consisted of women with no history of breast cancer, recruited from the catchment areas of participating hospitals as described elsewhere (28). The sampling of controls was frequency-matched for age (5-year intervals). The study excluded all persons who in the past year had: (a) altered vitamin supplement use; (b) lost over 5 kg; or (c) received a recommendation from their physician for a change in diet (other than sodium or total calorie reduction). Individuals treated for alcohol or other chemical abuse, institutionalized persons, and those with major psychiatric disorders potentially compromising informed consent were also excluded. Informed consent was obtained for all subjects in accordance with the requirements of responsible committees on human experimentation.

**Data Collection.** To maintain comparability, all centers used a common questionnaire for collection of health, demographic, and lifestyle information. s.c. adipose tissue was taken from the buttock by needle aspiration (29). Samples were collected directly into Vacutainer adapters and immediately placed on dry ice or in liquid nitrogen. They were stored at −70°C and transported at −56°C. After saponification and acidification, the free fatty acids were extracted with hexanol and methylated. Gas-liquid chromatography (Carlo Erba; HRGC 5300 Mega Series), with split injection, was conducted in Zeist using a 30-m long DB-23 column, inside diameter of 0.253 mm, phase layer 0.25 μ, and helium as carrier gas, in a temperature-programmed run. Coefficients of analytic variation ranged from 13 to 38%, with the majority of the within-run variance attributable to the rarest individual trans fatty acids.

**The Data Analysis.** Total trans fatty acids consisted of the sum of following isomers: 14-carbon monounsaturate with a trans bond at the 9 position; 18-carbon monounsaturate (oleate) with trans 6, 9, or 11; and 18-carbon polyunsaturate (linoleate) with trans 9 or 12 bonds. Our instrumentation precluded differentiation of oleate trans 6 from trans 9 isomers. Trans 11 and cis 6 oleates were also combined. No other trans isomer peaks could be determined. Statistical analyses were conducted using SAS version 6.10.

Potential confounders or effect modifiers were examined by stratification and logistic regression analysis (30). A standard set of covariates including age, body mass index, center, current smoking, former smoking, alcohol use, postmenopausal hormone use, and socioeconomic status resulted from this process.

ORs are reported for logistic regression models containing the standard covariate set along with adipose tissue percentages of fatty acids, unless otherwise noted. Crude and adjusted estimates were also calculated separately for each center. All ORs presented represent estimates of the difference in risk between the 25th and 75th percentiles of fatty acid levels within the total study population, unless otherwise noted.

### Results

A total of 698 women from five European countries contributed information to this study. Of the eligible cases, 86% participated in the study, as did 41% of the eligible controls. Participation among controls differed by center and recruitment method. It was highest with hospital-based control recruitment and lowest among those centers using resident registration lists for targeting the control group. Details are presented in an earlier publication on this population (31). The analyses are based on adipose tissue aspirations from 209 cases and 407 control women.

Adipose tissue percentages of total trans among controls varied 4-fold among centers (Table 1). The Spanish center showed the lowest mean trans values (0.34%), and Northern Ireland and the Netherlands the highest (1.5–1.6% trans fatty acids). The oleate trans 6 and 9 peak made up the bulk of the total trans fatty acid. Mean levels of the major oleate trans isomer group and total trans were higher in cases than controls, as seen in Table 2.

Cases and controls did not differ significantly in respect to age or body mass index. Multivariate models revealed body
Body mass index, defined as weight (in kilograms) divided by the square of height (in meters), was used to assess the risk of breast cancer in relation to quintile analysis, despite fat, however. Socioeconomic status, alcohol intake, and family history had minimal effect on the risk estimates for first childbirth as the strongest of the conventional risk factors for breast cancer, with the exception of socioeconomic status (SES) as a three-level categorical variable, pastmenopausal breast cancer risk among countries using biomarkers of long-term dietary risk factors for breast cancer in cases and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 291)</th>
<th>Controls (n = 407)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>SD</td>
<td>Means</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>62.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Body mass index*</td>
<td>27.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Mother with breast cancer (%)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Age at first childbirth, excluding nulliparae (yr)</td>
<td>26.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Low SES (%)</td>
<td>48.6</td>
<td>47.1</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>No. of cigarettes (cigs/d)</td>
<td>2.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Former smokers (%)</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>30.8</td>
<td>67.5</td>
</tr>
<tr>
<td>Hormone use (%)</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>14:1 trans 9 (%)’</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>18:1 trans 11 or cis 6 (%)’</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>18:1 trans 6 or 9 (%)’</td>
<td>1.02</td>
<td>0.64</td>
</tr>
<tr>
<td>18:2 trans (%)’</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>Total trans fatty acid (%)’</td>
<td>1.30</td>
<td>0.75</td>
</tr>
<tr>
<td>Total PUFA (%)</td>
<td>14.89</td>
<td>4.51</td>
</tr>
</tbody>
</table>

* Body mass index, defined as weight (in kilograms) divided by the square of height (in meters).

Several differences in distribution between cases and controls were observed. The use of the continuous model was supported by evidence of a significant dose response trend in quintile analyses (data not shown). The ORs for quintiles of trans fatty acids were similar to those noted from the continuous model for the 25th and 75th percentiles of concentration. PUFA were also associated with risk. The inclusion of total PUFA, and a trans-PUFA interaction term in the same model increased the OR for trans considerably (OR, 5.87; 95% confidence interval, 2.45–14.05). Inclusion of the trans-PUFA interaction term significantly improved the model (P < 0.005 for change in log likelihood).

Stratification for tertiles of polyunsaturated fat revealed that the most significant effect of trans fatty acids is to be seen at the lowest tertile of polyunsaturated fat. The result of an analysis stratified by tertiles of PUFA, presented in Fig. 1 and Table 2, indicates that the effect of trans fatty acids is largely confined to those individuals with the lowest levels of polyunsaturated fat. Risk was estimated to be 3.6-fold greater for people in the lowest tertile of polyunsaturates after adjustment for confounding factors. In the highest tertile of polyunsaturates, the estimates approach 1.

Calculation of ORs and confidence limits for individual trans fatty acids (data not shown) revealed that oleate trans (elaidic acid) carried the greatest OR (1.45) after adjustment for covariates.

The center-specific crude estimates for trans fatty acid were above 1 for four of the five centers (Table 4). Although these estimates vary substantially, they fall within a range consistent with sampling variation. The logit-based test for heterogeneity yielded a \( \chi^2 \) value of 7.7, \( P > 0.10 \). Adjustment for covariates increased estimates in the three centers with elevated initial ORs.

To explore the possible impact of the disease process on trans fatty acid levels in cases, relationships between adipose tissue fatty acids, nodal status, and tumor size were explored. Nodal status (three levels) and tumor size (four levels), two indicators of disease severity and progression, correlated poorly (Pearson's \( r = 0.10; P > 0.19 \)) with total trans. This suggests that stores of trans fatty acids did not increase with advancing disease, which might have resulted in spurious associations. Correlations of these markers of severity with total polyunsaturates and total monounsaturates were even weaker.

**Discussion**

The EURAMIC study was designed to enhance study power to detect true associations by capitalizing on dietary variation across Europe and the gradient in postmenopausal breast cancer risk among countries using biomarkers of long-term dietary variation.
intakes. As a large biomarker-based case-control study, its data are well suited to explore the hypotheses that trans fatty acid stores are associated with breast cancer and that a relative abundance of PUFA may "wash out" a trans-breast cancer association. Both of these hypotheses received support in this study.

The plausibility of the study's findings hinge upon acceptance of adipose tissue stores as a valid measure of long-term exposure. Adipose tissue represents a stable, long-term reservoir that integrates exposure levels over time. Use of adipose stores to measure exposure precludes recall bias. The alternative exposure measure in examining this hypothesis would involve traditional dietary assessment. This approach would not only, in a case-control context, be subject to error inherent in subjective reports of dietary intake, but it would also suffer from the problem of determining brand name usage of margarine, savory snacks, and baked goods over time and would be limited by the fact that food composition has dramatically changed in recent history in this regard, making it nearly impossible to estimate the aggregate of intakes over a number of years or the last decade (27, 32, 33). Reported correlation coefficients for the percentage of trans fat estimated from dietary intake data and adipose tissue range from 0.3 to 0.5, suggesting that they are measuring very different exposure periods (24, 34).

The half lives of individual fatty acids in adipose tissues are estimated to exceed 2 years. These estimates stem from studies of major fatty acids, including linoleic acid, the predominant polyunsaturate (35, 36). Trans fatty acids share transport mechanisms and oxidation rates with cis monenoic fatty acids of equal chain length (37) and could reasonably share half-lives as well.

Weight change could compromise the validity with which adipose tissue reflects long-term intake. The potential impact of rate of fat accretion and weight loss on adipose tissue fatty acid levels (38) was addressed by the exclusion of women with recent weight loss exceeding 5 kg and adjustment of results for body mass index. The possibility that the disease process caused the elevated levels of trans fatty acids among cases appears unlikely given that both tumor size and nodal status showed negligible correlation with trans concentrations.

Laboratory evidence that trans fatty acids may possess carcinogenic properties, detailed earlier, provides biological plausibility for the observed results. Evidence specific to cancer incidence in vivo derives from studies in which trans fatty acids were fed to rodents along with known carcinogens. In the absence of essential (polyunsaturated) fatty acids, greater incidences of intestinal and nonintestinal carcinomas have been observed in rats fed trans oleate compared to chow-fed controls (39). In the presence of adequate dietary polyunsaturated fat, trans fatty acid feeding produces inconsistent results (40).

Two other studies have examined adipose tissue trans fatty acids in women with breast cancer, one yielding results consistent with ours. American women with breast cancer were compared to controls presenting at the same hospital with breast abnormalities (41). Although trans fatty acids showed no statistically significant dose-response relationships with breast cancer, the OR for adipose trans fatty acids did exceed 1.0 in three quintiles, with a statistically significant elevation in the second quintile. Trans fatty acid intake estimated from questionnaires yielded ORs between 1.5 and 1.6 for all quintiles beyond the first. In a study of women undergoing breast biopsy at a New York hospital, mammary adipose tissue in women diagnosed with breast cancer contained slightly (but not statistically significantly) lower percentages of trans fatty acids than tissue from women with noncancerous conditions (42). Lack of direct adjustment for most established risk factors complicates interpretation of these results.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Center-specific models of breast cancer by trans and PUFAs*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Berlin</td>
</tr>
<tr>
<td>N*</td>
<td>16/103 (13/86)</td>
</tr>
<tr>
<td>Total trans %</td>
<td>1.74 (0.85-3.55)</td>
</tr>
<tr>
<td>Total trans % with covariates</td>
<td>3.97 (0.41-3.82)</td>
</tr>
<tr>
<td>Total PUFAs % with covariates</td>
<td>0.84 (0.19-3.77)</td>
</tr>
</tbody>
</table>

* Center-specific fatty acid odds ratios for model containing variables listed in first column, accompanied by 95% confidence interval in parentheses. ORs are based on the difference between 75th and 25th percentiles (see footnote b of Table 3 for units).
* N, number of observations for cases/controls in model containing only trans fatty acid as independent variable followed (in parentheses) by number for model containing full covariate package as well.
* Covariates: age in years, body mass index, cigarettes/day currently smoked, ex-smoker status, SES as three-level categorical variable, estrogen supplementation, and current alcohol consumption (in grams/week), except ex-smoker status omitted for Malaga due to inadequate numbers.
The lower response rate for controls than cases presents a potential source of selection bias. The response rates in this study were calculated based on theoretically potential controls, without a reduction of the denominator for those not reachable, and are comparable to those achieved using Department of Motor Vehicle registration lists for control recruitment. The lowest response rate among controls, and the greatest case-control difference in response, occurred in Zurich (92% versus 22% response for cases versus controls). The best response rates occurred in Malaga (97% versus 91%). It is encouraging that the relationship between trans fatty acids and breast cancer risk was consistent across all centers with good participation rates. In fact, the trans fatty acid OR was greater than 1 in all centers except Zurich and reached its maximum in Malaga. This indicates that if selection did bias the results, the likely direction of that bias was toward the null. The persistence of the observed associations despite inclusion of smoking and drinking habits, body mass index, and SES in the regression model argues against differences in lifestyle or health habits between cases and controls as a source of the association. The addition of parity, age at first childbirth, family history of breast cancer, age at menarche, and age at menopause to the model had a negligible impact on the pooled results [e.g., OR for total trans 1.41 (1.02–1.92) with these additional covariates, versus 1.40 (1.02–1.92) without them]. The observed results thus do not appear attributable to confounding by any of the above-mentioned risk factors.

Adipose tissue concentrations of trans fatty acids, polyunsaturates, or both may be simply an indicator of some other dietary or lifestyle factor affecting breast cancer risk or may in fact reflect a risk directly associated with consumption of these isomers at the level currently available in Western diets. The proportion of these fatty acids in adipose tissue reflects the distribution of other fatty acids. This can occur either through excess intake or through altered metabolism of other fatty acids. Thus, an alternative explanation for our findings might be that, because levels of trans, PUFAs, or both are associated with saturated fat intakes, this is fatty acid family that is of primary importance. Because saturated fat intakes cannot be estimated well from adipose tissue, their potential effects cannot be controlled for in this study. Epidemiological studies based on traditional dietary assessment do not, however, lend support to this hypothesis.

Bearing these caveats in mind, we find an association between adipose stores of trans isomer fatty acids and the occurrence of breast cancer. This is apparently the first report of a significant association of this nature in women. The relationship appears to be heavily influenced by polyunsaturated fat stores. These findings require confirmation in other populations, with concomitant consideration of the potential roles of dietary saturated and monounsaturated fats.

Acknowledgments

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References


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