Biomarkers of fatty acid exposure and breast cancer risk¹,²

Lenore Kohlmeier

ABSTRACT  Study of the effects of most individual biologically active dietary fatty acids on human disease requires the use of biomarkers of long-term intake in well-designed epidemiologic studies. Several small studies of tissue taken from women undergoing surgery for breast abnormalities have compared fatty acid profiles of women with ascertained metastatic breast cancer with those with other abnormalities. These studies, although often flawed in design and generally of inadequate statistical power to determine significant differences, provide some evidence. Human studies are generally consistent with animal models suggesting a protective effect of n-3 fatty acids, a detrimental effect of high n-6 fatty acids, and the possible importance of the ratio of these two classes of dietary fatty acids on both breast cancer incidence and recurrence. High intakes of monounsaturated fatty acid were also often negatively associated with breast cancer. The effects of trans fatty acids have rarely been studied, but there are some indications that they may enhance risk. In general, the study of individual fatty acids is in its infancy. Larger well-designed studies with diverse population and modern analyses of individual fatty acids are needed.  Am J Clin Nutr 1997;66(suppl):1548S–56S.

KEY WORDS  Mammary neoplasm, erythrocyte membranes, adipose tissue, breast cancer, n-6 fatty acids, n-3 fatty acids, trans fatty acids, biomarkers

INTRODUCTION

The possible association between fat consumption and breast cancer has been and continues to be the subject of active scientific debate (1–5). The origin of the interest in this area resides in concern about the magnitude of the devastation from this disease, the dearth of options through which breast cancer risk can be reduced, the early suggestions that dietary restriction in rodent models reduces mammary carcinogenesis (6), and the strength of the association between fat intakes and breast cancer mortality when examined from an ecologic perspective (7).

Ecologic studies indicate that women in countries with per capita disappearance data of 150 g fat·person⁻¹·d⁻¹ have a risk of dying from breast cancer that is fivefold greater than those from countries with 40 g fat·person⁻¹·d⁻¹. This has been extrapolated to suggest that a 40% reduction in breast cancer in the United States would be possible through a 50% reduction in fat intakes (8). Migration studies can also be interpreted in a fashion that lends credence to this idea. In women who move from countries that consume low amounts of dietary fat to those that are more affluent and consume richer diets, breast cancer risks increase by 60% within one generation (9). This increase, however, may be more due to the lack of soy in the diet than the gain of fat as a nutrient (10).

Most independent observations of breast cancer relations come from case-control studies. At least 12 studies have reported on the extent to which the fat intake of the diet is associated with the odds of developing a mammary neoplasm. These studies showed an overall significant trend between reported intake of dietary fat and risk of breast cancer, with the 20% of women who consumed the most fat having a 46% greater risk of the disease than did the women who consumed the least fat (11).

In cohort studies, the association is weaker: a recent meta-analysis of cohort studies with dietary data showed only a slight, nonsignificant effect of total energy-adjusted dietary fat consumption in postmenopausal women beyond the contribution of fat to energy intakes (12). Prentice (13) suggests that this may be due to measurement error. These studies all focus on fat intakes during adulthood. Diet may influence the developing mammary gland or the early stages of malignant transformation (14). Whether total fat intake in early life is important has not yet been addressed in well-designed studies for several reasons, including the lack of cohorts that begin dietary assessment early in life, problems in ensuring long-term funding for decades-long follow-ups, and the relative infrequency of the disease (which requires large sample sizes).

The finer points of these analyses are under discussion, including whether the cohort studies measure fat well enough for an effect to be seen, whether the methods being used to assess total fat consumption are biased such that a fat effect would be camouflaged by the more overweight individuals differentially underreporting their true consumption (15, 16), and whether attempts to focus on the role of total fat should exclude the potential role of fat in total energy intakes (17).

Dietary fat can be viewed as either a neutral carrier of energy contributing mainly to body fat mass, a vehicle of transport for fat-soluble nutrients or toxins (18), or as being directly biologically active. Although the field grows more interesting as our understanding of cell signaling, signal transduction, and DNA-regulating transcription factors increases, studies of dietary fat and breast cancer have just begun to address the ability of individual fatty acids to modulate carcinogenesis (19). In this

¹ From the Departments of Nutrition and Epidemiology, Schools of Public Health and Medicine, University of North Carolina, Chapel Hill.
² Address reprint requests to L Kohlmeier, Departments of Nutrition and Epidemiology, Schools of Public Health and Medicine, University of North Carolina, Chapel Hill, NC 27599-7400. E-mail: LenoreK@unc.edu.
context, essential fatty acids and fatty acids that might inhibit the activity of essential fatty acids are of particular interest. This article reviews the limited epidemiologic evidence of a relation between biomarkers of exposure to individual fatty acids as related to the risk of breast cancer and the human evidence of effects of fat intakes on circulating estrogen concentrations. The data based on subjective dietary records are presented elsewhere in this supplement (20).

DIETARY FAT AND ESTROGEN CONCENTRATIONS

One theory of diet and breast cancer suggests that dietary fat affects breast cancer risk through its influence on body fat and circulating estrogen concentrations (21). Breast cancer is undoubtedly hormonally related. Risk factors associated with reproduction, estrogen production, and exposure to estrogen present the most consistent associations known for breast cancer risk in epidemiologic studies (22). Most of what is known about differential breast cancer risk relates to behaviors that influence an individual's estrogen exposure. For this reason, dietary associations have often been examined in relation to their interfacing with sex hormones.

Dietary fat intake may affect breast cancer risk by its influence on body fat mass, which in turn might affect the total available estrogen concentrations. In general, studies in Western populations have shown that people who eat diets rich in fat lose control of their energy homeostasis and gain weight (23). Given the relative contribution, gram for gram, of fat energy to total energy intake, this is not surprising.

In premenopausal ovulating women, the contribution of estrogen synthesis from adipose tissue does not appear to significantly affect total circulating estrogen concentrations. Epidemiologic studies have not shown that excess body fat in premenopausal women increases their risk of breast cancer (24). However, in postmenopausal women, adipose fat is the primary source of circulating estrogen. Aromatase in the adipose tissue catalyzes the conversion of androsterone to estrone and testosterone to estradiol. The amount of activity of the P450 aromatase gene is a function of the mass of adipose tissue (25) and the age of the woman (26).

A few human studies have focused on estrogen concentrations and diet. Observational studies have shown that vegetarian women have lower circulating estrogen concentrations than do nonvegetarians and that the estrogen concentration correlates with the amount of saturated fat in the diet (27, 28). This has, however, been attributed partly to increased excretion of estrogens in the feces of vegetarians, which is the result of a greater dietary fiber content (29). Two studies examined the relation between the amount of fat in the diet and circulating estrogen concentrations, comparing the effects of diets low in fat (by Western standards, 15–20%) and high in fiber on circulating estrogen concentrations. Low-fat, high-fiber diets were shown to reduce estrogen concentrations significantly (in individuals with measurable estrogen concentrations) by 20–25% in a matter of weeks (30, 31). Attempts to separate the effects of fiber from those of fat have been made. Dietary fat alone, according to Goldin et al (31), results in increased androstenedione concentrations in women. In one study, reducing fat intake to 15–20% of energy without increasing dietary fiber was associated with a 20% reduction in estradiol concentrations after 6 mo in women who initially had measurable circulating estrogen (32). In summary, low-fat diets (<20% energy from fat) appear to be associated with lower circulating estrogen and estrogen precursor concentrations. This effect does not appear to be entirely a correlate of higher fiber intakes in the women consuming such low-fat diets.

BIOMARKERS OF FATTY ACID INTAKES

Few studies have examined the role of individual fatty acids consumed in the diet with respect to breast cancer risk. When this has been done, one of two approaches has been used to assess fatty acid exposure: either estimation of the habitual intakes of families of fatty acids on the basis of self-reported dietary intake measures, or measurement of biomarkers of intermediary and long-term exposure to individual fatty acids.

Intakes of individual fatty acids in the diet are extremely difficult to estimate from reported dietary intakes. The reasons for this are many, ranging from the need for brand-name information to convert food consumption information into estimated nutrient intakes to the inconsistency of composition in the same foods over seasons of the year to changes in the food supply from year to year (33). Subjects are also often unable to relate information on the type of fat used in food preparation when they have not prepared the food themselves. Dietary intakes are subject to known biases, such as underreporting in general by more obese individuals and biased reporting of foods that are believed to be socially more or less acceptable (15). In addition, when they attempt to capture specific information on portions consumed, dietary measures are often qualitative rather than quantitative and are based on frequency of use without regard for absolute intakes, subject error, or measurement error.

Dietary fatty acid assessment is especially demanding. It requires information about the brand names of oils and margarines for determination of individual fatty acids as well as consideration of the amounts and types of fat consumed in baked goods and various cuts of meat. Furthermore, the fatty acid composition of foodstuffs is particularly volatile. The composition of margarines changes depending on the production and cost of the component oils, and the amounts of fatty acids from fish depend on the season and the species used. Finally, it is argued that if total energy is associated with disease, studies of fat intakes require energy adjustment if they are to be meaningful (34). To compensate for the limitations of dietary assessment of types of fat in the diet, other markers of fatty acid intake have been sought and used.

In contrast with self-reported diet, biomarkers of fatty acids provide quantitative measures of the relative availability of individual fats irrespective of food source. In addition, measures of exogenous fatty acids are not subject to confounding by total energy intake except under conditions of starvation. Biomarkers of fatty acids by definition reflect exposures at a different level than do intake estimates (35). Whereas reported dietary intakes attempt to capture an accurate reflection of the foods consumed that contribute these fats, biomarkers reflect bioavailable, postabsorptive amounts of these substances available in either in storage, in circulation, or at the target tissue of interest. The limitations of biomarkers reside in the relevance of the assessment time to the disease, the potential effects of
disease on the marker, and the effect of endogenous synthesis or metabolism on the measure. Biomarkers reflect an integrated measure of diet over time and the genetic factors that may influence metabolism of the fatty acid of interest.

Fatty acid profiles in serum, erythrocyte membranes, and adipose tissue have been used for this purpose. Each medium reflects a different time frame. Each of these markers, when used to assess exogenously produced fatty acids, provides estimates of prior intake unbiased by disease status, value judgments about desirable eating patterns, or body image.

Serum samples are much more likely to reflect acute dietary intakes of the past few hours or days or disturbed transport mechanisms (36). The fatty acid profile of serum triacylglycerols is likely to reflect the last meal eaten. Cholesterol esters and phospholipids are more likely to reflect the dietary intakes of the past few days.

Erythrocyte membranes, with half-lives of 120 d, reflect intakes over several weeks. They are sensitive indicators of change in the polyunsaturated fat composition of the diet (37, 38). For example, supplementation with fish oil resulted in measurable and maximal decreases in osmotic fragility within 14 d, along with corresponding increases in erythrocyte membrane concentrations of n−3 fatty acids. This occurred at the expense of linoleic acid (18:2n−6) and oleic acid (18:1) but not arachidonic acid (20:4n−6) (39). Sardine oil, rich in eicosapentaenoic acid (20:5n−3), administered at 2.7 g/d resulted in significant increases in 20:5n−3 in phospholipid acyl-chains within 4 wk and lowered erythrocyte membrane fluidity in diabetic subjects (40).

Adipose tissue is a relatively stable depot of triacylglycerols and fat-soluble substances (41). Its composition also reflects long-term dietary intakes of essential fatty acids. Almost all human fatty acids are derived from the diet. De novo biosynthesis contributes < 1 g of fatty acids per day (42). Fatty acids in adipose tissue are largely straight chain, containing an even number of carbon atoms. The chain lengths are generally between 14 and 20 carbons, with or without double bonds. In most people 18:1, palmitic acid (16:0), 18:2n−6, and palmitoleic acid (16:1) make up ~80% of the fat mass.

Half-lives of individual fatty acids in adipose tissue differ (43). For example, consumption of a diet with 0.5% of the fat as 20:5n−3 for 100 d did not induce a measurable increase in the 20:5n−3 content of adipose tissue (44), whereas a marked increase in the 18:2n−6 content of adipose tissue was detected only 2 wk after healthy women switched to a diet with an increased polyunsaturated fat content (45). In earlier studies, comparable changes were reported only after many months of increased 18:2n−6 intakes (46, 47). The average half-life of fatty acids in general has been estimated at 1–2 y (48).

Dietary fatty acids can be completely used by muscles and liver for energy production (49). Polyunsaturated fatty acids can be converted into prostaglandins and other related compounds with high biological activity (50). In a state of positive energy balance (common to our society) fatty acids consumed and not used are conserved as triacylglycerols in adipose tissue. Because it is more energy efficient to use exogenously derived fatty acids than to synthesize new ones in the liver, these are largely maintained. Some of these fatty acids are structurally altered before storage, either through chain elongation or the partial desaturation of the fatty acid through the introduction of a double bond (51). Endogenous chain shortening and saturation of double bonds also contribute to structural changes of the fatty acids after absorption. The sum of these metabolic events determine which fatty acids are useful indicators of past diet and which are not.

The fatty acids found in human adipose tissue but not produced by human enzymes, which include the n−3 and n−6 classes of polyunsaturated fatty acids, trans fatty acids, and branched-chain fatty acids, are determined by absolute dietary intakes and metabolic alteration. Their adipose tissue concentrations are generally closely related to the amounts that are consumed habitually (52, 53). As seen in Table 1, the strength of association between diet and adipose fatty acids varies greatly, depending on the method of dietary assessment used to

### TABLE 1
Correlations between estimates of dietary intakes and concentrations of adipose tissue in fatty acids

<table>
<thead>
<tr>
<th>Adipose tissue fatty acids</th>
<th>Denmark</th>
<th>United States</th>
<th>Netherlands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.24</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>WDR</td>
<td>0.46</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.49</td>
<td>0.26</td>
<td>—</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.05</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td>WDR</td>
<td>0.19</td>
<td>0.09</td>
<td>—</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.26</td>
<td>0.15</td>
<td>—</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.44</td>
<td>0.15</td>
<td>0.68</td>
</tr>
<tr>
<td>WDR</td>
<td>0.57</td>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.63</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>18:2n−6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.44</td>
<td>0.13</td>
<td>0.70</td>
</tr>
<tr>
<td>WDR</td>
<td>0.51</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.57</td>
<td>0.43</td>
<td>—</td>
</tr>
<tr>
<td>18:3n−3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.12</td>
<td>0.07</td>
<td>—</td>
</tr>
<tr>
<td>WDR</td>
<td>0.36</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20:5n−3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.47</td>
<td>0.43</td>
<td>—</td>
</tr>
<tr>
<td>WDR</td>
<td>0.44</td>
<td>0.43</td>
<td>—</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.63</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>22:6n−3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>0.41</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>WDR</td>
<td>0.55</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Corrected WDR</td>
<td>0.80</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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1 FFQ, food-frequency questionnaire; WDR, two 7-d weighed diet records.
2 Tjønneland et al (54) 1993, Pearson correlation coefficients, n = 86.
5 Corrected for within-to-between-person variance fluidity.
6 Energy-adjusted FFQ.
7 19–24 h recalls, conducted at roughly monthly intervals.
8 24-h recall data corrected for measurement error.
determine intakes and the groups of fatty acids being examined. The only fatty acid showing relatively consistent results across methods and countries is 20:5n–3 (54). Saturated and monounsaturated fatty acids are less strongly related to dietary intakes (55, 57). The correlation coefficient for intake estimated from diet records and adipose tissue polyunsaturated fatty acid concentrations was extremely low, at 0.15 (53). This value is not corrected for the inherent measurement error that can attenuate observed associations. In a comparison of estimates based on fat biopsies and 14-d dietary records for a Danish population, Pearson correlation coefficients adjusted for measurement error reached 0.57 for 18:2n–6 and 0.80 for docosahexaenoic acid (22:6n–3) (53). A Dutch study, using multiple 24-h recalls over the course of 1 y, reported an adjusted coefficient of 0.77 for 18:2n–6 estimated from adipose tissue data compared with recall but did not examine correlations for n–3 fatty acids (58).

Imperfect correlations between the proportion of a particular fatty acid in the diet and in the sampled adipose tissue is explained partly by measurement error and partly by other dietary and nondietary influences. Alcohol consumption is one factor that may raise 16:1 and lower 18:2n–6 concentrations (59, 60). Adipose tissue turnover also appears to differ among femoral, gluteal, and abdominal sites (61, 62). Definitive data on site- and fatty acid–specific turnover times of individual fatty acids in human adipose tissue are not available. In a study of breast adipose tissue and breast cancer risk, adipose tissue from gluteal fat was compared with breast adipose tissue. The 18:2n–6 composition of the breast correlate (r = 0.983) with that of gluteal adipose tissue (63). Chajes et al (64) also found a strong association between 18:2n–6 in breast adipose tissue of women with breast cancer and the fatty acid profiles of these women in iliac fat. For other fatty acids, the relations were poor. In particular, mammary fat was higher in saturated and lower in monounsaturated and polyunsaturated fatty acids. Stearic acid (18:0) and 18:1 in breast and gluteal tissue were not correlated (64).

Dilution is also a concern when adipose tissue fatty acids are used as exposure markers. Two individuals consuming the same amount of 20:5n–3 would be expected to differ in adipose tissue concentration if they differ in body fat. A given dose of 20:5n–3 should be more diluted in a greater pool of body fat. This effect can be accounted for in epidemiologic studies by controlling for body mass index or adjusting the fatty acid concentrations to a total body burden. This can be estimated by multiplying concentration of a fatty acid per gram by the amount of body fat.

### BIOMARKER STUDIES OF INDIVIDUAL FATS AND BREAST CANCER

Nine studies used biomarkers of fatty acids in studies of women with and without breast cancer (63, 65–72) (Table 2). These are generally small studies, that used tissue samples from patients taken during biopsy for breast disorders. The studies were all done in the past decade, the earlier ones using technology allowing less differentiated analyses of the specific individual fatty acids present in the tissues studied. Some were conducted to study recurrence, others as case-control examinations of prediagnostic differences.

#### trans Fatty acids

trans Fatty acids are naturally occurring from some sources, but most found in the American diet have been introduced through hydrogenation of polyunsaturated fats. They compete

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**TABLE 2**

Serum or adipose tissue concentrations of fatty acid families in breast cancer patients

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Control population</th>
<th>n (cases/ controls)</th>
<th>Fatty acids&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohlmeier et al, 1997 (65)</td>
<td>Case-control</td>
<td>Mixed population</td>
<td>291/407</td>
<td>SFA (↑)</td>
</tr>
<tr>
<td>Simonsen et al, 1996 (66, 73)</td>
<td>Case-control</td>
<td>Mixed population</td>
<td>291/407</td>
<td>MUFA (↓)</td>
</tr>
<tr>
<td>Zhu et al, 1995 (67)</td>
<td>Case-control</td>
<td>Hospital-based, benign breast disease patients</td>
<td>pre 26/35, post 47/20</td>
<td>trans (↓)</td>
</tr>
<tr>
<td>Petrek et al, 1994 (68)</td>
<td>Case-control</td>
<td>Hospital-based biopsy patients</td>
<td>154/125</td>
<td>n–3 (↑)</td>
</tr>
<tr>
<td>Bougnoux et al, 1994 (69)</td>
<td>Case only (recurrence)</td>
<td></td>
<td>21/121</td>
<td>n–6 (↓)</td>
</tr>
<tr>
<td>London et al, 1993 (70)</td>
<td>Case-control</td>
<td>Hospital-based, benign breast disease patients</td>
<td>402/597</td>
<td>n–3:n–6 (↑)</td>
</tr>
<tr>
<td>Zaridze et al, 1990 (71)</td>
<td>Case-control</td>
<td>Hospital-based control patients (pre and post)</td>
<td>25/20</td>
<td>SFA (↓)</td>
</tr>
<tr>
<td>Eid and Berry, 1988 (63)</td>
<td>Case-control</td>
<td>Hospital-based, benign breast disease patients</td>
<td>37/27</td>
<td>MUFA (↑)</td>
</tr>
<tr>
<td>Caleffi et al, 1987 (72)</td>
<td>Case-control</td>
<td>Hospital-based</td>
<td>23/10</td>
<td>SFA (↑)</td>
</tr>
</tbody>
</table>

<sup>1</sup> SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; NA, not available; pre, premenopausal; post, postmenopausal.

<sup>2</sup> Arrows indicate that mean difference is significant, p < 0.05; arrows in parentheses reflect mean differences between case and control subjects exceeding 10%, but not significant; dashes indicate no association.
with other polyunsaturated fats for the $\Delta 5$ and $\Delta 6$ desaturase enzymes along the pathway to eicosanoid production (74–76). The study of trans fatty acids is flawed by unreliable food-composition data and because the abundance of trans fatty acids in the food supply may not be stable over time. For example, it has been estimated that increased use of trans fatty acids in the preparation of French fries by fast-food restaurants during 1989 and the 1990s added 0.3 g per capita to daily trans fatty acid intake in the United States (33). Correlation coefficients between dietary trans fatty acid and adipose tissue trans fatty acid measures of $\approx 0.3$ were observed (53, 77).

The EURAMIC Study is one of the largest studies of breast cancer in which adipose tissue samples were used as a primary exposure measure for fat intake (78). It is also one of the few studies to use gluteal fat instead of breast adipose tissue for analyses and to recruit population-based healthy control subjects. In this study, which enhances variance by using a multicountry design, individual trans fatty acids in gluteal fat biopsy samples from 698 postmenopausal incident cases of primary breast cancer and controls showed a positive association with breast cancer (65). Women in the highest quartile of trans fatty acid stores had a breast cancer risk 40% greater than that of women in the lowest quartile. This effect was greatest in women with relatively low stores of polyunsaturated fats. The association is not attributable to differences in age, body mass index, exogenous hormone use, or socioeconomic status.

Another large study of adipose tissue trans fatty acids in women with breast cancer, this one with hospital-based control subjects, yielded consistent results for the trans fatty acid stores (70). American women with breast cancer were compared with control subjects at the same hospital with breast abnormalities. Although trans fatty acids showed no statistically significant dose-response relations with breast cancer, the odds ratio for trans fatty acids was $> 1.0$ in three quintiles, with a significant elevation of trans fatty acids in the second quintile. Dietary trans fatty acid intake estimates were also correlated with breast cancer, with odds ratios between 1.5 and 1.6 for all quintiles beyond the first but no indication of a dose-response beyond the second. The mean proportions of trans fatty acids in gluteal fat in these women were threefold higher than those seen in the European populations.

Petrek et al (68) studied women with of invasive breast cancer and compared their breast tissue fatty acid profiles with those of women in whom biopsies were performed and in whom any disease other than invasive breast cancer was diagnosed. In this study all of the odds ratios for higher quintiles of trans fatty acids were $< 1.0$.

These three studies present inconsistent results and combine many different trans fatty acids. Information on individual trans fatty acids, as measured by readily available methods with great specificity, would be useful. An increased risk of breast cancer from trans fatty acid intake cannot be ruled out on the basis of these studies. The size of the potential effect of trans and other fatty acids is strongly attenuated by large measurement error inherent in these earlier analyses.

### n-6 Fatty acids

Animal studies lead to the expectation that exposure to n-6 fatty acids increases breast cancer risk (79). The absolute amounts of n-6 fatty acids in adipose tissue were higher in case than control subjects in the EURAMIC Study, but this effect was not consistent across all centers in the study (66). Israeli women reportedly consume among the highest amounts of polyunsaturated fats in the world. Although differences were not significant, concentrations of 20:4n-6 and 18:0 in breast tissue were $\approx 10\%$ greater in 37 Israeli women with breast cancer than in 27 Israeli women with normal biopsy results, fibrocystic disease, or lipoma or who were mastopathy control subjects (63). Because the average age of women in a second control group of 27 women with fibroadenomas was 24 y younger than that of the case subjects, only the control group that is similar in age is considered.

Premenopausal and postmenopausal women were analyzed separately in a comparison of Finnish women with breast cancer and with benign breast disease (67). Despite this separation, the case subjects were 8–9 y older than the control subjects. The researchers analyzed triacylglycerol- and phospholipid-bound fatty acids from breast adipose tissue and also used food-frequency questionnaires to estimate dietary intakes of fatty acids. This study found no associations between n-6 fatty acid concentrations and disease status in either premenopausal or postmenopausal women. The study suffers, as do many of these biomarker studies, from small, hospital-based, convenience samples. It provides evidence that the strength and often direction of the relations differ between the dietary estimates of fatty acid intakes from food frequency questions and these measured amounts.

Zaridze et al (71) examined erythrocyte membranes for differences in five fatty acids in phospholipids between women with breast cancer and those with minor complaints. Descriptive information on the age and weight of the women was not provided. Contrary to expectations, Zaridze et al found lower concentrations of 18:2n-6 (in premenopausal women) and 20:4n-6 (in postmenopausal women) in erythrocyte membrane phospholipids in the women with breast cancer.

London et al (70) noted no significant association across this family of fatty acids, largely on the basis of contribution of 18:2n-6. Contributions of two minor components, 18:3n-6 and 20:3n-6, were on average higher for case subjects (odds ratios: 1.4 and 1.1, respectively).

Bougnoux et al (69) collected data on 121 women with breast cancer in whom adipose tissue adjacent to the tumor was available, pathology and staging of the disease was complete, and follow-up was possible. These women were followed for an average of 31 mo, during which 21 recurrences were observed. The relative risk of metastasis was estimated to be 1.7 for women with high 20:4n-6 or nervonic acid concentrations in their adipose tissue. These point estimates are consistent with expectations from animal studies but were not significant in a study of this size.

A small study of subcutaneous fatty acids compared fatty acid concentrations in breast tissue of 23 British women undergoing biopsy or surgery for breast cancer with those of 20 women with benign breast conditions and 10 hospital-based control subjects undergoing surgery for nonmalignant and non-breast-related conditions. The study suffers from lack of statistical power, lack of hospital-based control subjects, and the fact that the case subjects were 10 y older than the control subjects. No odds ratios were calculated and no significant differences were noted. However, the mean concentration of 18:2n-6 was 7% greater and the concentration of 20:4n-6 was 23% greater in the case subjects than in the control
n–3 Fatty acids

There is strong evidence of a chemoprotective effect of dietary n–3 fatty acids in animal models of chemically induced carcinogenesis in the presence of diets rich in n–6 fatty acids (76, 79). Seven hospital-based case-control studies examined these associations in women at risk of breast cancer. London et al (70) reported nearly identical median percentages of long-chain n–3 fatty acids (primarily 22:6n–3, with a small 20:5n–3 component) in gluteal adipose tissue from postmenopausal case and control subjects with nonproliferative breast disease. All quintiles of 20:5n–3 yielded odds ratios < 1, but the downward trend was not significant. Petrek et al (68) examined abdominal and mammary adipose tissue in New York women with breast cancer and in control subjects undergoing breast biopsy and reported that no significant case-control differences in n–3 fatty acids in either tissue. Zhu et al (67) found postmenopausal Finnish women with breast cancer to have lower concentrations of 20:5n–3 and docosapentaenoic acid (22:5n–6) in triacylglycerols of mammary adipose tissue than did women with benign breast disease. Phospholipid n–3 fatty acids did not differ significantly between the case and control subjects. Docosahexaenoic acid (22:6n–3) in phospholipids, but not in triacylglycerols, of case subjects was also lower in the postmenopausal women. In premenopausal women, women with and without breast cancer showed no difference in concentrations of n–3 fatty acids (67). In the study of Israeli women, α-linolenic acid (18:3n–3) concentrations in the breast tissue of case subjects were greater than in control subjects when the diet was extremely rich in n–6 fatty acids (63). The EURAMIC Study found a nonsignificant negative association between breast cancer incidence and n–3 fatty acid concentrations of adipose tissue (66).

Despite the small number of case subjects (n = 21) in the biomarker study of breast cancer recurrence (69), significant associations were noted between 16:0, 18:3n–3, and 22:6n–3 concentrations and risk of recurrence. Each of these appeared to protect against recurrence. These researchers found that the strongest determinants of metastases were low 18:3n–3 concentrations in the adipose breast tissue and tumor size. Overall, this provides weak evidence for the protection against invasive breast cancer in women by n–3 fatty acids.

Monounsaturated fats

A protective effect of olive oil consumption is supported by some dietary studies in southern European populations (73, 80–84). In most biomarker studies, monounsaturated fat concentrations are presented as means for case and control subjects. No consistent effect was noted for all types of monounsaturated fats. There did appear to be slightly lower concentrations of 16:1 and 18:1 in cases in three of the studies (63, 69, 71). Analyses of the EURAMIC Study suggest that an 18:1 effect is seen only in olive oil–consuming countries, which may mean that some other component of the olive oil is the active ingredient (73). The possibility of metabolic conversion to monounsaturated fatty acids results in adipose tissue stores of monounsaturated fatty acids not being as informative of prior exposure as stores of some other fatty acids.

Ratio of n–3 to n–6 fatty acids

If the mechanism of protective action of n–3 fatty acids is through competitive inhibition with n–6 fatty acids, then the ratio of n–3 to n–6 fatty acids should be of primary interest. The EURAMIC Study is the only study to examine this ratio in adipose tissue, where it was more significantly associated with disease than was the percentage of n–3 or n–6 fatty acids alone (66). Increasing the ratio of n–3 to n–6 appears to be protective. However, a switch to n–6 polyunsaturated fatty acids at the expense of saturated fat often appears favorable as well, which is at odds with the apparent deleterious association for n–6 fatty acids independent of n–3 fatty acids.

Recurrence and metastasis

Most studies of this type are designed to examine preventive effects of these fatty acid exposures on breast cancer occurrence, not metastases. In one of the few studies providing data on metastases, clinically aggressive tumors contained lower concentrations of phosphatidylethanolamine polyunsaturated fat and less 18:0 in their phosphatidylcholine (85). The single study designed to study recurrence was previously discussed (69); the strongest finding was of a protective effect of n–3 fatty acids.

Mechanisms of action

Several of possible mechanisms of action of individual fatty acids are reviewed throughout this report. Those most relevant to breast cancer risk are summarized below. It has been suggested that tumor cell membrane phospholipids might influence tumor growth through a modulating effect on mitogenic signal transaction (86). Another theory is that n–3 long-chain polyunsaturated fats interfere with cell proliferation through the formation of oxidation products (87).

Long-chain n–3 fatty acids competitively inhibit the Δ5 and Δ6 desaturase pathways necessary for conversion of 18:2n–6 to 20:4n–6. The metabolites of n–3 fatty acids produce eicosanoids that differ or oppose those produced via the n–6 fatty acid–eicosanoid pathway. Thus, eicosanoid-regulated growth and metabolism may be reduced, favoring suppression of tumor cell growth.

DISCUSSION

Biomarker studies of fatty acids and breast cancer provide the best measure of long-term intakes of fatty acids; these studies are generally strong in exposure measures but weak in design. Many of the studies have inadequate statistical power to detect even a large effect. Most are limited by their use of hospital-based women with other diseases of the breast or symptoms to represent the population at large. The use of breast adipose tissue raises questions regarding the potential effect of localized tumors on fatty acid stores. Local tumor cells may use fatty acids, which would bias biomarker studies of long-term exposures in cases.
Few of the studies of breast cancer using biomarkers of fatty acids control adequately for confounding factors in their analyses. Major breast cancer risk factors were rarely addressed in the analyses. Some of the studies did not even conduct statistical analyses of the data. This lack of control for effects modifying breast cancer risk could unpredictably affect risk estimates. In addition, studies conducted during the past decade on individual fatty acids used an earlier generation of technology, which was insensitive to small quantities of individual fatty acids and had large coefficients of variation. None of the authors attempted to adjust for measurement error inherent in the analyses of fatty acids present in low concentrations.

Given these limitations, the human studies referred to in this article are generally consistent with the expectations from animal studies. In general, n-6 fatty acid concentrations were positively associated with risk. This association was significant in two of the studies presented (65, 72) and in the expected direction in another two (63, 69). The less flawed studies were in this latter group. n-3 Fatty acids were less consistently associated with a protective effect. This may be due to the difficulties in measuring these smaller components or the importance of the balance of intake of the two families, as suggested in one study. Knowledge would be greatly enhanced by a few large population-based case-control studies that use adipose tissue from a site other than breast as a biomarker of trans fatty acid intake as well as modern gas chromatography and mass spectroscopy methods to ensure the precision of the measurements.

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