Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China[1–3]

Jackilen Shannon, Irena B King, Rachel Moshofsky, Johanna W Lampe, Dao Li Gao, Roberta M Ray, and David B Thomas

ABSTRACT

Background: The role of individual fatty acids in the development and progression of breast cancer is unclear. Although in vitro and animal experiments have supported an inverse association between intake of long chain n−3 fatty acids [primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid] and breast cancer risk, findings from population studies are inconsistent. Recent studies have also shown associations between the ratio of saturated to monounsaturated fatty acids (SI) and breast cancer risk. The SI reflects the activity of several genes involved in lipid metabolism, including fatty acid synthase and steroyl coenzyme-A desaturase, that have been shown to be overexpressed in breast cancer.

Objective: The purpose of this analysis was to determine the association between erythrocyte fatty acid concentrations and breast cancer risk among women participating in a randomized trial of breast self-examination in Shanghai, China.

Design: We conducted a case-control study. Erythrocyte fatty acid concentrations were determined in specimens from 322 women with histologically confirmed breast cancer and 1030 frequency age-matched control women.

Results: We report a significant direct association among palmitic, γ-linolenic, palmitoleic, and vaccenic acids and risk of breast cancer. Total n−3 fatty acids, EPA, and the SI for palmitic to palmitoleic acid were associated with significantly lower risk of breast cancer.

Conclusion: Our results support a protective effect of n−3 fatty acids on breast cancer risk and provide additional evidence for the importance of evaluating the ratio of fatty acids when evaluating diet and breast cancer risk. *Am J Clin Nutr* 2007;85:1090–7.

KEY WORDS Case-control study, fatty acids, breast cancer

INTRODUCTION

Data from migrant studies support the contention that, in addition to the known risk factors, other factors, including diet, are likely to play an important role in determining risk of breast cancer (1). One of the first dietary factors suggested to alter breast cancer risk was total dietary intake of fat. However, the results of numerous large, prospective studies have raised questions about this hypothesis while bringing to the forefront several newer hypotheses focusing on specific types of fats or fatty acids (2, 3).

In vitro and animal experiments suggest varying and sometimes opposing effects of individual fatty acids on expression of genes involved in multiple biologic pathways, including inflammation, lipid metabolism, and oxidative stress (4–9). Findings from numerous case-control and cohort studies have inconsistently reported an association between questionnaire-based assessment of fatty acid intake and breast cancer risk. Results from studies of fatty acid concentrations in adipose tissue, erythrocytes, and serum were somewhat more consistent.

In a recent meta-analysis, Saadatian-Elahi et al (10) described 11 case-control and 3 cohort studies that had been published between 1966 and 2002. Results from that analysis confirmed the inconsistency of findings from case-control studies but suggested that data from the cohort studies support a significant inverse association between total n−3 fatty acids and breast cancer risk, and the suggestion of an inverse association for the specific fatty acids, eicosapentaenoic acid (EPA) and docosohexanoic acid (DHA).

Since publication of that meta-analysis, others have suggested that the ratios of fatty acids in erythrocyte membranes or adipose tissue may be of greater importance than individual fatty acids. Recently, Bougnoux et al (11) found that a composite measure of a low ratio of n−6 to n−3 fatty acid (n−6:n−3) and a high concentration of monounsaturated fatty acids (MUFAs) was associated with reduced risk of breast cancer. Chajes et al (12) and Pala et al (13) address this concept through the use of a saturation index (SI), which represents ratios of the 2 most common saturated fatty acids in tissues and MUFAs that are direct metabolites of these saturated fatty acids. Thus, the ratios of 16:0 to 16:1n–7 and 18:0 to18:1n–9 correspond to SI (n–7) and SI (n−9), respectively. The reciprocal of these ratios may in part reflect activity of δ-9 desaturase. δ-9 Desaturase is a product of the steroyl coenzyme-A desaturase gene family and has been shown to be overexpressed in several cancers, including breast cancer (14–17). Fatty acid synthase (FAS), which directs the synthesis of

[1] From the Center for Research on Occupational and Environmental Toxicology, Oregon Health and Science University, Portland, OR (JS and RM); the Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA (IBK, JWL, RMR, and DBT); and the Department of Epidemiology, Zhong Shan Hospital Cancer Center, Shanghai, China (DLG).

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[3] Address reprint requests to J Shannon, Center for Research on Occupational and Environmental Toxicology, Oregon Health and Science University, 3181 Southwest Sam Jackson Park Road, L606, Portland, OR 97239. E-mail: shannoja@ohsu.edu.

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palmitic acid (16:0) when the cell is in a starvation state, is also frequently overexpressed in breast cancer and other cancers (18–21). Pala et al (13) found higher SI (n−9) in erythrocytes to be strongly predictive of postmenopausal breast cancer in an Italian cohort study. In another cohort study by Chajes et al (12) in northern Sweden, the higher SI (n−9) in serum phospholipids was also found to be associated with lower risk of breast cancer. In the current study, we use the fatty acid composition of red blood cells (RBCs) to address the association among individual fatty acid concentrations, the SI, n−3:n−6, and breast cancer risk in a case-control study in Shanghai, China.

SUBJECTS AND METHODS

Study subjects

Study subjects were selected from participants in a previously described randomized trial of breast self-examination (BSE) in Shanghai, China (22). The participants were all women who were born between 1925 and 1958, were permanent residents of Shanghai, and were either current or retired employees of the Shanghai Textile Industry Bureau (STIB). Between 1989 and 1991, all women in the cohort were administered a baseline questionnaire to collect information on the main demographic and reproductive risk factors for breast cancer. All women were actively followed through July 2000 for benign breast disease and breast cancer. From 1995 through July 2000, 1429 women had breast lumps that were diagnosed histologically at 1 of the 3 main hospitals affiliated with the STIB. Of those women, 432 were confirmed to have breast cancer, 336 of whom completed a food-frequency questionnaire, completed a detailed risk-factor questionnaire, and provided a blood sample. Six of these women were excluded because they had a prior history of breast cancer, and 8 were excluded because their blood sample was not adequate for analyses, yielding a final sample of 322 breast cancer case women for inclusion in the present study.

Control women were randomly selected from women in the BSE trial with no breast biopsy. A single control group was selected for studies of breast cancer and for concurrent studies of benign breast conditions. For each benign and malignant case woman enrolled between September 1995 and August 1997, 20 potential control women of the same age were randomly selected and listed. Potential control women were contacted, starting with the first 2 names on the list, until 2 women of the same age and menstrual status as their matched case subjects were recruited. Three hundred sixty-seven control women were recruited in this manner (64% of the eligible women contacted). Control subjects for case subjects that were enrolled between September 1997 and August 2000 were frequency-matched to the case subjects by 5-y age group and hospital affiliation of their factory at baseline. In-person interviews were completed for 704 (82%) of 862 control women selected in this manner, for a total of 1071 control women. One control woman was excluded because of a calculated daily energy intake >4000 kcal that was considered unreliable, 32 did not provide a blood sample, and for 8 the blood sample provided was not adequate for analyses, yielding a total of 1030 control women for inclusion in the present analyses. In the statistical analyses for the present report, the individual matching on age and menstrual status was not retained, and the breast cancer case women were compared with all interviewed control women from both studies.

Before enrollment, informed consent was obtained from each woman. The institutional review board of the Fred Hutchinson Cancer Research Center and the Station for Prevention and Treatment of Cancer of the STIB approved the study, in accordance with the assurances of the Office for Human Research Protections of the US Department of Health and Human Services.

Specimens were processed within 5 h of the blood draw, and washed RBC aliquots were stored in a −70 °C freezer until being air mailed to Seattle on dry ice. Blood was stored at the Fred Hutchinson Cancer Research Center at −70 °C. A food-frequency questionnaire and risk-factor questionnaire were administered to each woman to collect information on the woman's dietary intake, demographic characteristics, reproductive and gynecologic history, smoking and alcohol habits, medical history, family history of breast cancer, and occupational and recreational physical activity.

Analyses of RBC fatty acids

RBCs (250 μL) were mixed with an equivalent volume of distilled water, and lipids were extracted with 2-propanol and chloroform according to Rose and Oklander (23). Butylated hydroxytoluene (5 mg) per 100 mL 2-propanol was added as an antioxidant. The lipid extract was dissolved in 5 mL acetyl chloride reagent and processed according to Lepage and Roy (24). After transesterification, fatty acid methyl esters were recovered in hexane, dried under nitrogen (40 °C), and re-dissolved in 80 μL hexane for gas chromatography analysis.

Fatty acid methyl esters were injected in a split mode (1:50) and were separated on a gas chromatograph (model 5890B; Hewlett-Packard, Avondale, PA). The gas chromatograph system was equipped with a flame ionization detector, electronic pressure control, Chemstation software (Hewlett-Packard), and automatic sampler (model 7673; Hewlett-Packard). As part of quality control measures, the long-term precision of the RBC fatty acids was monitored with repeat analysis of an in-house RBC quality control pool that was extracted in each batch of 23 study samples. The accuracy of the chromatographic system was monitored with the use of commercial standards (GLC-87, NIH-D, and NIH_F; Nu Chek, Elysian, MN). The CVs of the quality control pool for the major fatty acids ranging >5% were ≤2%; for minor fatty acids ranging between 0.2% and 5% the CVs were ≤9.8%. The case or control status was unknown to the laboratory personnel. Fatty acid composition is reported as a weight percentage of the total RBC fatty acids.

Statistical analyses

The frequency of demographic and reproductive characteristics in the case and control women were compared, and the percentages among the case women were standardized to the age distribution of control women, with the use of indirect adjustment methods (25). Fatty acid composition was first evaluated as continuous variables. Differences in mean intake across the group were evaluated with the use of a Satterwhite *t* test for unequal variances. To model the associations more efficiently we then categorized the fatty acid concentrations into quartiles based on the distribution of fatty acid concentrations among the control women. Conditional logistic regression models were used to calculate odds ratios (ORs) as estimates of the relative risks and their 95% CI for breast cancer associated with each quartile of fatty acids (26). All statistical analyses were performed with the use of the STATISTICAL ANALYSIS

SYSTEM (SAS/PC V.8.2; SAS Institute, Cary, NC), and tests were considered statistically significant at $P < 0.05$. Because case and control women were not recruited and interviewed at an equal rate during the 5 y of data collection, all analyses were conditioned on year of interview (1995–1996, 1997, 1998–1999, and 2000–2001). Further, because of the method of selecting control women to match all women undergoing biopsy, the mean age of the control women is significantly younger than the mean age of the breast cancer case women. To account for this difference in age distribution, all frequencies are standardized to the age distribution of the control women, and ORs for each concentration of RBC fatty acids were adjusted for age by using the 5-y age categories.

Potential confounding by other nondietary factors and instruction arm of the BSE trial was evaluated by conducting univariate analyses and then adding each variable found to be independently associated with breast cancer risk separately into the main model. Family history of breast cancer, age at menarche, age at first full-term pregnancy, age at first live birth, total live births, number of prior benign breast lumps, duration of oral contraceptive use, duration of intrauterine device (IUD) use, number of induced abortions, menopausal status, years of breastfeeding, years since last induced abortion, frequency of BSE practice, education, smoking habit, alcohol use, body mass index (in kg/m^2), and physical activity were evaluated as possible confounders. Variables were considered confounders if they changed the estimated OR of the main independent variable (RBC fatty acid) by ≥10%. Duration of breastfeeding, age at first birth, time since last induced abortion, and duration of IUD use were all maintained as covariates in the final model. To assess the potential for effect modification as a result of menopausal status, we stratified the data by menopausal status as determined at the time of interview. The significance of a trend in risk across RBC fatty acid composition was evaluated by entering quartiles of the RBC fatty acid proportions into the logistic model as different values of a single ordinal variable.

TABLE 1
Selected demographic characteristics of breast cancer cases and control women in Shanghai, China[1]

Characteristic	Cases (n = 330)	Controls (n = 1038)
	n (%)	
Age		
35–39 y	12 (3.6)	13 (1.3)
40–44 y	90 (27.3)	462 (44.5)
45–49 y	71 (21.5)	216 (20.8)
50–59 y	47 (14.2)	121 (11.7)
≥60 y	110 (33.3)	226 (21.8)
Education completed[2]		
Elementary school or less	95 (28.8)	196 (18.9)
Middle school	210 (63.6)	811 (78.1)
College or more	25 (7.6)	30 (2.9)
BMI[2]		
≤20 kg/m^2	51 (16.1)	196 (18.9)
>20 to ≤25 kg/m^2	194 (61.4)	607 (58.5)
>25 kg/m^2	85 (22.7)	235 (22.6)
Intensity of occupational and recreational activity[2]		
Light	74 (23.0)	188 (18.1)
Moderate	244 (73.5)	779 (75.1)
Heavy	12 (4.6)	71 (6.8)
Spouse smoking[2]		
Nonsmoker	124 (47.4)	268 (35.5)
1–9 cigarettes/d	52 (17.5)	114 (11.0)
10–19 cigarettes/d	60 (19.9)	209 (20.1)
≥20 cigarettes/d	94 (15.2)	347 (33.4)
Family history of breast cancer[2,3]		
No	305 (93.3)	1004 (96.7)
Yes	14 (11.9)	17 (1.6)
Unknown	11 (2.6)	17 (1.6)

[1] P values were determined from age-adjusted model stratified by year of interview (1995–1996, 1997, 1998– 1999, and 2000–2001) with conditional logistic regression; included 8 cases and 8 controls with inadequate samples for red blood cell analyses.
[2] Indirect age-adjusted percentages based on age distribution of the controls.
[3] $P < 0.05$.

RESULTS

Dietary, demographic, and reproductive characteristics of the study subjects have been previously reported (27). Briefly, compared with the control women, the case women were significantly older and were more likely to have education beyond high school, a family history of breast cancer, and a nonsmoking spouse (**Table 1**). Case women were also more likely to have been younger at menarche, to have been >30 y at their first live birth, and to have breastfed for a longer duration and were less likely to have used an IUD for >14 mo and ever had a clinical breast examination (**Table 2**). The prevalence of alcohol consumption (11% of case women and 12% of control women who ever drank alcohol) and cigarette smoking (2% of case women and 2.4% of control women who ever smoked) was low among both case and control women.

Fatty acid values are presented in **Table 3** as a percentage of the total fatty acids in the RBCs. Palmitic and stearic acids, the principal saturated fatty acids in the RBCs, were significantly higher in case women than in control women. The percentages of total n−3 polyunsaturated fatty acids (PUFAs), EPA, DHA, total n−6 PUFAs, linoleic acid (LA), arachidonic acid (AA), total PUFAs, total MUFAs, and erucic acid were lower in the case women than in the control women. Also, the SI (n–7) was significantly different between the case and control women.

In the multivariate models, the results of which are presented in **Table 4**, a significant direct association was observed between greater percentage of palmitic acid and breast cancer risk but no association with stearic acid. The percentage of total n−3 PUFA was associated with a significantly lower risk of breast cancer. This association appeared to be explained primarily by the effect of EPA. Also a nonsignificant inverse association was observed between the percentage of DHA and risk of breast cancer. No association was observed with the percentage of total n−6 fatty acids or AA and breast cancer risk; however, higher LA concentrations were associated with a reduced risk of breast cancer. Further, among women with a higher percentage of γ-linolenic acid (GLA), there was an increased risk. Also a significant direct association was observed between a greater percentage of palmitoleic acid and breast cancer risk, although the ORs for the third and fourth quartile were similar, suggesting a possible threshold effect. A similar although less dramatic association was found for higher concentrations of vaccenic acid. The SI for palmitic acid

TABLE 2
Selected reproductive characteristics of breast cancer case ($n = 330$) and control women ($n = 1038$) in Shanghai, China[1]

Characteristic	Cases[2]	Controls
	n (%)	
Age at menarche[3]		
≤13 y	61 (20.8)	167 (16.1)
14 y	63 (19.1)	201 (19.4)
15 y	77 (23.3)	204 (19.7)
16 y	60 (18.2)	215 (20.7)
≥17 y	69 (20.9)	250 (24.1)
Live births[3]		
None	18 (4.9)	37 (3.6)
1	176 (65.8)	702 (67.6)
2	55 (16.4)	118 (11.4)
≥3	81 (17.6)	177 (17.1)
Missing	0 (0.0)	4 (0.4)
Age at first live birth		
No live births	20 (5.4)	41 (4.0)
≤24 y	96 (22.6)	262 (25.2)
25–29 y	155 (53.4)	586 (56.5)
≥30 y	59 (26.4)	149 (14.4)
Duration of breastfeeding[3]		
Never	62 (20.7)	223 (21.48)
≤6 mo	62 (20.7)	206 (19.9)
7–12 mo	96 (34.4)	357 (34.4)
13–24 mo	47 (11.5)	110 (10.6)
≥25 mo	63 (19.1)	142 (13.7)
Duration of oral contraceptive use		
Never used	293 (89.5)	949 (91.4)
≤1 y	20 (6.1)	33 (3.2)
≥1 y	17 (5.2)	55 (5.3)
Missing	0 (0.0)	1 (0.1)
Duration of intrauterine device use		
Never used	164 (39.7)	358 (34.5)
≤9 mo	33 (12.7)	113 (10.9)
>9 to ≤14 mo	68 (26.8)	238 (22.9)
>14 mo	52 (15.8)	292 (28.1)
Missing	13 (5.0)	37 (3.6)
Induced abortions		
0	17 (4.4)	35 (3.4)
1	145 (42.1)	420 (40.5)
2	120 (37.0)	421 (40.6)
Missing	48 (16.5)	162 (15.6)
Time since last induced abortion		
No induced abortions	145 (42.1)	420 (40.5)
0–10 y	35 (14.0)	126 (12.1)
11–15 y	46 (17.9)	181 (17.4)
16–20 y	24 (6.9)	119 (11.5)
>20 y	43 (9.2)	145 (14.0)
Missing	37 (9.9)	47 (4.5)
Frequency of clinical breast examination[4]		
Never	115 (34.8)	208 (20.0)
Once or more	80 (25.8)	376 (36.2)
Missing	135 (39.4)	454 (43.7)
Breast lumps evaluated by medical worker		
Missing	312 (93.9)	1006 (96.9)
1	13 (4.4)	22 (2.1)
2	3 (1.0)	7 (0.7)
3	2 (0.6)	3 (0.3)
Menopause		
No	170 (64.6)	675 (65.0)
Yes	160 (35.5)	363 (35.0)

[1] *P* values were determined from an age-adjusted model stratified by year of interview (1995–1996, 1997, 1998– 1999, and 2000–2001) with conditional logistic regression; included 8 cases and 8 controls with inadequate samples for red blood cell analyses.
[2] Indirect age-adjusted percentages based on age distribution of the controls.
[3] $P < 0.01$.
[4] $P < 0.05$.

to palmitoleic acid was inversely associated with breast cancer risk. This inverse association was driven primarily by lower concentrations of palmitoleic acid (SI denominator) with increasing SI quartile rather than changes in palmitic acid (SI numerator).

DISCUSSION

In this case-control study of RBC fatty acids and breast cancer risk, we observed a significant inverse association between total n−3 fatty acids, and more specifically EPA, and risk of breast cancer. GLA, palmitic acid, palmitoleic acid, and also vaccenic acid were all positively associated with disease, whereas SI for palmitic acid to palmitoleic acid was inversely associated with breast cancer risk.

There are few epidemiologic studies of either RBC or serum fatty acid concentrations and breast cancer risk. One cohort study reported a significant reduction in risk with increasing DHA concentrations (13), but another found no significant associations for either DHA or EPA (28). Of 6 case-control studies that used fatty acid concentrations of either RBCs or adipose tissue, 3 found no significant association among any individual n−3 fatty acid and breast cancer (12, 28, 29). Maillard et al (30) found that α-linolenic acid (ALA) and DHA concentrations in adipose tissue, as well as a high ratio of ALA to LA, were associated with decreased risk of breast cancer. Klein et al (31) also reported that ALA concentrations in adipose tissue were associated with a reduced risk of breast cancer. Although our results do not support an association with ALA specifically, we do report an inverse association with total n−3 fatty acids. Contrary to these results, a nested case-control study by Rissanen et al (32), which used RBC fatty acid concentrations, found that total n−6 fatty acids and LA were related to a decrease in risk of breast cancer. Those same investigators reported no association between n−3 fatty acids and breast cancer risk. In 2 recent review articles it is stated that case-control studies and cohort studies do not yet adequately support a clear association between n−3 fatty acids and breast cancer risk (33, 34). However, the reviewed studies included different and primarily questionnaire-based assessment measures and were conducted in populations that do not have a wide variation in intake of EPA and DHA. It is possible that some of the inconsistencies may be explained by variation (or lack of variation) in the background diet or in intake of n−3 fatty acids in the target population.

To our knowledge, the positive association we report between GLA and risk of breast cancer has not been previously documented. In vitro and animal work suggests that GLA has tumor-reducing effects. Menendez et al (35) found that GLA increased cytotoxicity in MDA-MB-231 and MCF-7 breast cancer cells. In another study Menendez et al (36) found that GLA reduced the growth rate of MDA-MB-231 cells. Those investigators also report that GLA increased the cytotoxicity of paclitaxel (an anticancer drug). It is unclear why our findings in a human population do not support the published effects of GLA in vitro and in animal studies.

We are aware of only 2 cohort studies and 1 case-control study that have published an association between biomarker concentrations of palmitoleic acid and breast cancer risk. Consistent with our findings, Pala et al (13) report a positive

TABLE 3
Percentage of fatty acids in red blood cell membranes of breast cancer cases and control women in Shanghai, China ($n = 1352$)[1]

	Cases ($n = 322$)	Controls ($n = 1030$)	P^2
Palmitic acid (16:0)	19.51 ± 1.30 (16.72–25.57)	18.73 ± 1.04 (13.02–26.67)	< 0.0001
Stearic acid (18:0)	14.33 ± 1.04 (9.73–17.13)	13.93 ± 0.95 (9.60–18.00)	< 0.0001
Total n–3 fatty acids[3]	7.43 ± 1.29 (2.56–11.0)	7.69 ± 1.07 (2.09–12.27)	0.001
EPA (20:5n−3)	0.53 ± 0.16 (0.12–1.30)	0.60 ± 0.22 (0.13–2.11)	< 0.0001
DHA (22:6n−3)	4.78 ± 1.03 (1.44–7.65)	4.92 ± 0.85 (1.24–7.78)	0.02
DPA (22:5n−3)	1.84 ± .36 (0.50–2.73)	1.86 ± 0.34 (0.53–3.63)	0.22
ALA (18:3n−3)	0.25 ± 0.16 (0.09–1.62)	0.27 ± 0.13 (0.08–1.35)	0.10
Total n–6 fatty acids[4]	27.57 ± 2.64 (13.59–37.54)	28.13 ± 2.42 (15.74–41.15)	0.0004
LA (18:2n−6)	11.67 ± 2.84 (6.98–27.17)	12.17 ± 2.84 (6.57–29.96)	0.006
GLA (18:3n−6)	0.09 ± 0.06 (0.01–0.54)	0.08 ± 0.05 (0.02–0.69)	0.08
AA (20:4n−6)	11.82 ± 1.58 (4.19–14.94)	12.08 ± 1.19 (5.47–14.98)	0.006
Total n–3:total n–6 fatty acids[5]	0.27 ± 0.05 (0.13–0.43)	0.28 ± 0.05 (0.10–0.55)	0.13
Total PUFAs[6]	35.10 ± 2.98 (18.67–43.30)	35.93 ± 2.25 (17.96–45.45)	< 0.0001
Total MUFA[7]	19.18 ± 2.27 (15.23–30.55)	20.55 ± 2.78 (14.05–30.97)	< 0.0001
Oleic acid (18:1n−9)	10.38 ± 1.22 (8.05–20.15)	10.50 ± 1.06 (7.20–17.05)	0.12
Erucic acid (22:1n−9)	0.21 ± .29 (0.03–3.21)	0.35 ± 0.37 (0.02–3.64)	< 0.0001
Palmitoleic acid (16:1n−7)	0.26 ± 0.18 (0.02–1.77)	0.20 ± 0.12 (0.07–1.07)	< 0.0001
Vaccenic acid (18:1n−7)	0.96 ± 0.13 (0.71–1.55)	0.93 ± 0.13 (0.01–1.44)	0.02
Saturation index			
(16:0/16:1n−7)	96.4 ± 64.60 (14.18–1036.12)	112.46 ± 44.38 (20.47–264.08)	< 0.0001
(18:0/18:1n−9)	1.40 ± 0.19 (0.52–1.87)	1.34 ± 0.18 (0.56–2.11)	< 0.0001

[1] All values are $\bar{x}$ ± SD; range in parentheses (all such values). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ALA, α-linolenic acid; LA, linoleic acid; GLA, γ-linolenic acid; AA, arachidonic acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.
[2] *t* Test for unequal variances.
[3] 18:3n−3 + 20:3n−3 + 20:5n−3 + 22:5n−3 + 22:6n−3.
[4] 18:2n−6 + 18:3n−6 + 20:2n−6 + 20:3n−6 + 20:4n−6 + 22:2n−6 + 22:4n−6.
[5] (n−3 PUFAs)/(n−6 PUFAs).
[6] n−3 PUFAs + n−6 PUFAs.
[7] 14:1 + 16:1n−7 + 16:1n−9 + 17:1n−9 + 18:1n−5 + 18:1n−8 + 18:1n−9 + 22:1n−9 + 24:1n−9.

association between RBC fatty acid concentration of palmitoleic acid and risk of breast cancer (OR: 2.32; 95% CI: 1.03, 5.20). Chajes et al (12) reported no association between palmitoleic acid and breast cancer risk but, similar to our findings and those of Pala et al (13), report a nonsignificant inverse association between SI, as determined by the ratio of stearic acid to oleic acid, and breast cancer risk. Contrary to our findings, Simonsen et al (37, 38) report a significant reduction in risk among women in the highest compared with the lowest quartiles of serum palmitoleic acid. Thus, although findings for palmitoleic acid alone have not been consistent, the inverse association between the SI and breast cancer risk suggests that the ratio of the most prevalent saturated fatty acids to MUFAs in the erythrocyte membrane may be of more relevance than the concentration of either individual fatty acid. Palmitic acid is the primary end product of FAS-dependent de novo free fatty acid synthesis, and palmitoleic acid is primarily produced through desaturation of palmitic acid by δ-9 desaturase. Hence, these 2 fatty acids and the ratio of the 2 are more reflective of lipid metabolism than is dietary intake. Because the inverse association appears to be primarily due to lower concentrations of palmitoleic acid, this association may suggest a role for reduced δ-9 desaturase activity in breast cancer prevention.

RBC fatty acids reflect recent dietary intake (approximately the past 3 wk) and thus are not appropriate measures for identifying associations resulting from intake earlier in life. In addition, change in dietary intake, absorption, or metabolism because of the presence of breast cancer could alter RBC fatty acid concentrations in women with this disease. However, this is an unlikely explanation for our findings; such effects would be most likely to occur in the later stages of disease. We found no differences in our results by disease stage at diagnosis (data not shown).

Bias could also have been introduced into our results if the women who agreed to participate in the study had a different diet from those who chose not to participate. However, as both case and control women chose not to participate in roughly similar proportions, it is unlikely that this explains our results.

Another limitation of this study is that some control women had their blood drawn later than did the case women. During the period of this study, China underwent economic reform, increasing market availability of some food products, including meats. If this shift resulted in increases in meat intake over the study period, it could bias our results to show evidence of a protective effect of the saturated and n−6 fatty acids found in meat products. However, our findings do not provide such evidence. Also, we corrected for the effect of this possible source of bias by stratifying by year of interview (1995–1996, 1997, 1998–1999, and 2000–2001) in the conditional logistic regression models.

In summary, our results provide support for a protective effect of total n−3 PUFAs and more specifically EPA for breast cancer. Further, our results suggest the importance of considering the ratio of fatty acids, because there was a significant direct association among both palmitic acid and

TABLE 4
Concentrations of red blood cell (RBC) fatty acids (FAs) among women in Shanghai, China (n = 1352), and risk of breast cancer[1]

	Quartiles of RBC FA concentrations					
	1	2	3	4	*P* for trend	Continuous
Palmitic acid (16:0)						
No. of cases/controls[2]	32/261	43/257	81/256	166/256		
RBC FA cutoff	≤18.16	>18.16 to ≥18.70	>18.70 to ≥19.27	>19.27		
OR (95% CI) (% by wt of total)	1.00	0.98 (0.51, 1.89)	1.29 (0.70, 2.37)	2.20 (1.22, 3.96)	0.0003	1.45 (1.24, 1.71)
OR (95% CI)[3]	1.00	1.20 (0.58, 2.50)	1.34 (0.68, 2.64)	2.18 (1.14, 4.15)	0.004	1.40 (1.16, 1.69)
Stearic acid (18:0)						
No. of cases/controls[2]	42/244	48/257	100/274	132/255		
RBC FA cutoff	≤13.34	>13.34 to ≥14.03	>14.03 to ≥14.61	>14.61		
OR (95% CI) (% by wt of total)	1.00	1.12 (0.61, 2.08)	0.84 (0.48, 1.47)	0.97 (0.56, 1.67)	0.73	0.95 (0.80, 1.14)
OR (95% CI)[3]	1.00	1.57 (0.79, 3.13)	1.06 (0.56, 2.01)	1.26 (0.68, 2.36)	0.83	1.03 (0.84, 1.27)
Total n–3 FAs[4]						
No. of cases/controls[2]	103/258	69/255	85/261	65/256		
RBC FA cutoff	≤7.05	>7.05 to ≥7.64	>7.64 to ≥8.36	>8.36		
OR (95% CI) (% by wt of total)	1.00	0.67 (0.41, 1.08)	0.65 (0.41, 1.03)	0.49 (0.31, 0.80)	0.005	0.99 (0.98, 1.00)
OR (95% CI)[3]	1.00	0.78 (0.46, 1.31)	0.81 (0.48, 1.36)	0.55 (0.32, 0.94)	0.04	0.99 (0.98, 1.00)
EPA (20:5n–3)						
No. of cases/controls[2]	107/253	97/261	72/248	46/268		
RBC FA cutoff	≤0.46	>0.46 to ≥0.56	>0.56 to ≥0.69	>0.69		
OR (95% CI) (% by wt of total)	1.00	1.19 (0.77, 1.87)	0.72 (0.46, 1.15)	0.46 (0.28, 0.75)	0.0006	0.97 (0.83, 1.12)
OR (95% CI)[3]	1.00	1.25 (0.77, 2.04)	0.82 (0.49, 1.37)	0.45 (0.26, 0.77)	0.003	0.92 (0.78, 1.09)
DHA (22:6n–3)						
No. of cases/controls[2]	102/259	63/257	84/257	73/257		
RBC FA cutoff	≤4.40	>4.40 to ≥4.90	>4.90 to ≥5.46	>5.46		
OR (95% CI) (% by wt of total)	1.00	0.61 (0.38, 1.00)	0.71 (0.45, 1.13)	0.55 (0.34, 0.88)	0.03	0.98 (0.96, 1.00)
OR (95% CI)[3]	1.00	0.70 (0.40, 1.20)	0.72 (0.43, 1.21)	0.61 (0.36, 1.04)	0.09	0.98 (0.96, 1.00)
DPA (22:5n–3)						
No. of cases/controls[2]	85/258	76/264	92/253	69/255		
RBC FA cutoff	≤1.62	>1.62 to ≥1.85	>1.85 to ≥2.09	>2.09		
OR (95% CI) (% by wt of total)	1.00	0.59 (0.34, 0.97)	0.79 (0.49, 1.30)	0.49 (0.29, 0.82)	0.03	0.57 (0.34, 0.94)
OR (95% CI)[3]	1.00	0.52 (0.29, 0.90)	0.69 (0.40, 1.20)	0.59 (0.33, 1.04)	0.18	0.65 (0.36, 1.16)
ALA (18:3n–3)						
No. of cases/controls[2]	108/237	110/304	43/231	61/258		
RBC FA cutoff	≤0.18	>0.18 to ≥0.23	>0.23 to ≥0.32	>0.32		
OR (95% CI) (% by wt of total)	1.00	0.79 (0.53, 1.19)	0.69 (0.40, 1.19)	1.21 (0.71, 2.06)	0.80	1.22 (0.79, 1.89)
OR (95% CI)[3]	1.00	0.80 (0.50, 1.25)	0.63 (0.35, 1.13)	0.99 (0.54, 1.82)	0.59	1.04 (0.63, 1.72)
Total n–6 FA[5]						
No. of cases/controls[2]	95/254	92/257	77/261	58/258		
RBC FA cutoff	≤26.61	>26.61 to ≥27.76	>27.76 to ≥29.48	>29.48		
OR (95% CI) (% by wt of total)	1.00	0.77 (0.49, 1.19)	0.76 (0.48, 1.20)	1.03 (0.61, 1.74)	0.84	1.00 (0.999, 1.001)
OR (95% CI)[3]	1.00	0.70 (0.43, 1.14)	0.74 (0.44, 1.23)	0.92 (0.51, 1.65)	0.64	1.00 (.998, 1.001)
LA (18:2n–6)						
No. of cases/controls[2]	111/259	91/257	59/255	61/259		
RBC FA cutoff	≤10.19	>10.19 to ≥11.40	>11.40 to ≥13.63	>13.63		
OR (95% CI) (% by wt total)	1.00	0.66 (0.43, 1.03)	0.46 (0.29, 0.75)	0.88 (0.52, 1.48)	0.15	0.79 (0.34, 1.85)
OR (95% CI)[3]	1.00	0.64 (0.39, 1.04)	0.38 (0.22, 0.64)	0.67 (0.37, 1.21)	0.02	0.48 (0.18, 1.29)
GLA (18:3n–6)						
No. of cases/controls[2]	67/246	94/328	64/172	97/284		
RBC FA cutoff	≤0.05	>0.05 to ≥0.07	>0.07 to ≥0.09	>0.09		
OR (95% CI) (% by wt of total)	1.00	1.08 (0.70, 1.69)	1.87 (1.09, 3.20)	2.27 (1.37, 3.78)	0.0003	1.89 (1.33, 2.70)
OR (95% CI)[3]	1.00	0.98 (0.60, 1.62)	1.72 (0.92, 3.17)	2.05 (1.16, 3.63)	0.003	1.94 (1.31, 2.87)
AA (20:4n–6)						
No. of cases/controls[2]	99/261	67/252	88/258	68/259		
RBC FA cutoff	≤11.43	>11.43 to ≥12.17	>12.17 to ≥12.92	>12.92		
OR (95% CI) (% by wt of total)	1.00	0.66 (0.41, 1.06)	1.05 (0.66, 1.68)	0.74 (0.46, 1.20)	0.57	1.12 (0.97, 1.30)
OR (95% CI)[3]	1.00	0.65 (0.38, 1.11)	1.25 (0.74, 2.12)	0.87 (0.51, 1.50)	0.73	1.16 (0.98, 1.38)
Total n–3:total n–6[6]						
No. of cases/controls[2]	85/261	71/216	102/299	64/254		
RBC FA cutoff	≤0.24	>0.24 to ≥0.27	>0.27 to ≥0.31	>0.31		
OR (95% CI) (% by wt of total)	1.00	0.99 (0.59, 1.64)	0.84 (0.53, 1.33)	0.59 (0.36, 0.97)	0.03	0.02 (0.001, 0.41)
OR (95% CI)[3]	1.00	0.95 (0.54, 1.67)	0.93 (0.56, 1.55)	0.66 (0.38, 1.15)	0.16	0.04 (0.001, 1.35)

(*Continued*)

TABLE 4 (*Continued*)

	Quartiles of RBC FA concentrations					
	1	2	3	4	*P* for trend	Continuous
Total PUFAs[7]						
No. of cases/controls[2]	108/256	88/259	66/258	60/257		
RBC FA cutoff	≤34.72	>34.72 to ≥35.78	>35.78 to ≥37.04	>37.04		
OR (95% CI) (% by wt of total)	1.00	0.66 (0.42, 1.03)	0.48 (0.30, 0.76)	0.84 (0.50, 1.41)	0.13	0.94 (0.87, 1.00)
OR (95% CI)[3]	1.00	0.75 (0.46, 1.22)	0.45 (0.26, 0.75)	0.82 (0.46, 1.46)	0.1	0.92 (0.85, 1.00)
Total MUFAs[8]						
No. of cases/controls[2]	149/257	95/258	47/256	31/259		
RBC FA cutoff	≤18.42	>18.42 to ≥20.07	>20.07 to ≥22.34	>22.34		
OR (95% CI) (% by wt of total)	1.00	1.08 (0.73, 1.62)	1.10 (0.66, 1.81)	1.26 (0.68, 2.33)	0.47	1.04 (0.96, 1.12)
OR (95% CI)[3]	1.00	1.09 (0.70, 1.70)	1.06 (0.60, 1.89)	1.37 (0.69, 2.73)	0.44	1.05 (0.97, 1.15)
Oleic acid (18:1n–9)						
No. of cases/controls[2]	99/255	86/261	67/258	70/256		
RBC FA cutoff	≤9.81	>9.81 to ≥10.38	>10.38 to ≥11.02	>11.02		
OR (95% CI) (% by wt of total)	1.00	1.05 (0.68, 1.64)	0.90 (0.57, 1.44)	1.32 (0.80, 2.17)	0.47	1.90 (0.33, 10.80)
OR (95% CI)[3]	1.00	1.08 (0.67, 1.75)	0.93 (0.56, 1.56)	1.28 (0.72, 2.27)	0.6	1.19 (0.15, 9.27)
Erucic acid (22:1n–9)						
No. of cases/controls[2]	143/268	117/253	35/254	27/255		
RBC FA cutoff	≤0.13	>0.13 to ≥0.22	>0.22 to ≥0.44	>0.44		
OR (95% CI) (% by wt of total)	1.00	1.27 (0.86, 1.88)	0.69 (0.41, 1.15)	0.67 (0.37, 1.22)	0.11	0.87 (0.67, 1.13)
OR (95% CI)[3]	1.00	1.49 (0.97, 2.31)	0.89 (0.50, 1.59)	0.77 (0.39, 1.52)	0.53	0.97 (0.73, 1.31)
Palmitoleic acid (16:1n–7)						
No. of cases/controls[2]	30/241	61/293	107/234	124/262		
RBC FA cutoff	≤0.13	>0.13 to ≥0.17	>0.17 to ≥0.24	>0.24		
OR (95% CI) (% by wt of total)	1.00	1.95 (1.10, 3.45)	5.02 (2.86, 8.79)	5.72 (3.23, 10.13)	<0.0001	3.60 (2.41, 5.38)
OR (95% CI)[3]	1.00	1.63 (0.87, 3.07)	4.80 (2.60, 8.88)	4.83 (2.58, 9.06)	<0.0001	3.16 (2.02, 4.93)
Vaccenic acid (18:1n–7)						
No. of cases/controls[2]	71/250	75/283	87/246	89/251		
RBC FA cutoff	≤0.85	>0.85 to ≥0.93	>0.93 to ≥1.01	>1.01		
OR (95% CI) (% by wt of total)	1.00	1.09 (0.68, 1.76)	1.52 (0.94, 2.44)	1.82 (1.12, 2.96)	0.006	3.56 (1.77, 7.17)
OR (95% CI)[3]	1.00	1.33 (0.78, 2.26)	1.93 (1.13, 3.27)	2.21 (1.25, 3.88)	0.002	3.71 (1.64, 8.39)
Saturation index (n–7) (16:0/16:1 n–7)						
No. of cases/controls[2]	114/258	118/257	57/257	33/258		
RBC FA cutoff	≤77.8	>77.8 to ≥112.3	>112.3 to ≥141.5	>141.5		
OR (95% CI) (% by wt of total)	1.00	0.99 (0.63, 1.56)	0.38 (0.23, 0.64)	0.17 (0.10, 0.30)	<0.0001	0.99 (0.98, 0.99)
OR (95% CI)[3]	1.00	1.16 (0.70, 1.91)	0.45 (0.25, 0.80)	0.19 (0.10, 0.36)	<0.0001	0.99 (0.99, 1.00)
Saturation index (n–9) (18:0/18:1 n–9)						
No. of cases/controls[2]	53/249	64/280	82/251	123/250		
RBC FA cutoff	≤1.23	>1.23 to ≥1.36	>1.36 to ≥1.47	>1.47		
OR (95% CI) (% by wt of total)	1.00	0.67 (0.38, 1.18)	0.79 (0.46, 1.36)	0.76 (0.45, 1.29)	0.6	0.76 (0.30, 1.95)
OR (95% CI)[3]	1.00	0.97 (0.51, 1.84)	1.02 (0.54, 1.91)	0.98 (0.53, 1.80)	0.99	1.02 (0.35, 3.02)

[1] All analyses were stratified by year of interview (1995–1996, 1997, 1998–1999, and 2000–2001) with conditional logistic regression. OR, odds ratio; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ALA, α-linolenic acid; LA, linoleic acid; GLA, γ-linolenic acid; AA, arachidonic acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

[2] Number of subjects in age-adjusted model; 130 subjects were dropped from multivariate model because of missing covariates (48 cases and 82 control women).

[3] Adjusted for age, duration of breastfeeding, age at first birth, time since last induced abortion, and duration of intrauterine device use.

[4] 18:3n–3 + 20:3n–3 + 20:5n–3 + 22:5n–3 + 22:6n–3.

[5] 18:2n–6 + 18:3n–6 + 20:2n–6 + 20:3n–6 + 20:4n–6 + 22:2n–6 + 22:4n–6.

[6] (n–3 PUFAs)/(n–6 PUFAs).

[7] n–3 PUFAs + n–6 PUFAs.

[8] 14:1 + 16:1n–7 + 16:1n–9 + 17:1n–9 + 18:1n–5 + 18:1n–8 + 18:1n–9 + 22:1n–9 + 24:1n–9.

palmitoleic acid and breast cancer risk when analyzed independently, yet when considered as a SI (palmitic acid to palmitoleic acid), there was a significant inverse association with risk. These findings suggest that evaluating the ratio of fatty acids may be an important approach to understanding the impact of variations in lipid metabolism on breast cancer risk.

Finally, to our knowledge we are the first to report positive associations between RBC concentrations of GLA and vaccenic acid and risk of breast cancer. These findings, although intriguing, should be viewed with caution until they are reproduced by others. Overall, our study findings strengthen the argument for an increased intake of n−3 PUFAs to prevent

breast cancer. They also support the assertion that fatty acid analyses must move beyond a simple analysis of individual fatty acids to account for both direct effects of the fatty acids and to reflect alterations in lipid metabolism pathways.

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JS was involved in data collection, protocol development, analyses, and manuscript preparation. IBK was responsible for all fatty acid analyses and manuscript review. RM was involved in initial data analyses and manuscript preparation. JWL was involved in manuscript review and comment. DLG was the primary physician contact in Shanghai and was responsible for oversight of data collection and implementation of study procedures. RMR was responsible for oversight of all statistical analyses, data cleaning, and monitoring. DBT was principal investigator for the Breast Self Exam trial, the additional protocols that funded the current study, and was involved in protocol development and manuscript review. None of authors had a conflict of interest.

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