Original Contribution

Association between Serum *trans*-Monounsaturated Fatty Acids and Breast Cancer Risk in the E3N-EPIC Study

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The authors assessed the association between serum phospholipid fatty acids as biomarkers of fatty acid intake and breast cancer risk among women in the E3N Study (1989–2002), the French component of the European Prospective Investigation into Cancer and Nutrition. During an average of 7 years of follow-up, 363 cases of incident invasive breast cancer were documented among 19,934 women who, at baseline (1995–1998), had completed a diet history questionnaire and provided serum samples. Controls were randomly matched to cases by age, menopausal status at blood collection, fasting status at blood collection, date, and collection center. Serum phospholipid fatty acid composition was assessed by gas chromatography. Adjusted odds ratios for risk of breast cancer with increasing levels of fatty acids were calculated using conditional logistic regression. An increased risk of breast cancer was associated with increasing levels of the *trans*-monounsaturated fatty acids palmitoleic acid and elaidic acid (highest quintile vs. lowest: odds ratio = 1.75, 95% confidence interval: 1.08, 2.83; *p*-trend = 0.018). *cis*-Monounsaturated fatty acids were unrelated to breast cancer risk. A high serum level of *trans*-monounsaturated fatty acids, presumably reflecting a high intake of industrially processed foods, is probably one factor contributing to increased risk of invasive breast cancer in women.

breast neoplasms; diet; fatty acids; *trans* fatty acids

Breast cancer incidence is the highest among women's malignancies in Western Europe and North America (1), and incidence is rapidly increasing in Japan (2). Much controversy has surrounded the hypothesis that a high intake of fat increases the risk of breast cancer. Results of intervention trials have suggested a modest beneficial effect of a reduction in total fat intake on breast cancer incidence (3) and recurrence (4). Case-control studies have generally shown a positive association between total fat consumption and the incidence of breast cancer (5), while pooled cohort data have shown no relation (6). Nevertheless, some studies have

suggested that a high intake of *cis*-monounsaturated oleic acid (7–9) or n-3 polyunsaturated fatty acids (PUFAs) (7, 10–13) could reduce breast cancer risk. Conversely, a high intake of *trans*-unsaturated fatty acids (7, 14) and n-6 PUFAs (15, 16) could increase the risk of postmenopausal breast cancer.

Epidemiologic studies are limited by their assessment of nutrition through food frequency questionnaires, a method shown to be prone to measurement error (17). Moreover, the conversion of quantities of food items consumed into their fatty acid content is exceptionally complex, for numerous

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reasons related to the imprecision of qualitative and quantitative estimates of fat in food. The fatty acid composition of a given food can vary according to cooking methods and industry supply, and food composition tables are incomplete.

In contrast, biomarkers of dietary fatty acids offer objective, qualitative measures of bioavailable amounts of these nutrients irrespective of the source and quality of food, particularly for fatty acids that are not endogenously synthesized (18, 19). We investigated the relation between serum phospholipid fatty acid levels as biomarkers of past dietary fatty acid intake and breast cancer risk in a large French cohort study, the E3N Study (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) (20). This cohort study is the French component of the European Prospective Investigation into Cancer and Nutrition (EPIC) (21). We hypothesized a negative association between oleic acid, n-3 PUFAs, and breast cancer risk and a positive association with n-6 PUFAs and *trans*-fatty acids.

MATERIALS AND METHODS

Study cohort

The E3N Study is an ongoing prospective study that was designed to examine associations between cancer risk and dietary, lifestyle, and reproductive factors in women (20). The E3N cohort comprises female members of a national health insurance scheme covering teachers in the French education system and their spouses. Overall, 98,995 female volunteers aged 40–65 years were enrolled between February 1989 and November 1991 after replying to a baseline questionnaire and giving their written informed consent. The study was approved by the French National Commission for Data Protection and Privacy. In the baseline questionnaire and subsequent self-administered questionnaires, participants provided information on anthropometric characteristics, reproductive history, health status, lifetime use of hormonal treatments, family history of breast cancer, and smoking status. Usual diet was assessed through a validated 208-item diet history questionnaire sent out between June 1993 and June 1995 (22). The response rate for the dietary questionnaire was 81 percent of the total cohort at baseline. Responders to the dietary questionnaire constituted the French component of EPIC (21). Following a common protocol, blood samples were collected, aliquotted into plasma, serum, lymphocytes, and erythrocytes, and stored in liquid nitrogen. In France, approximately 25,000 E3N participants volunteered for blood collection between 1995 and 1998 (23). Along with blood samples, information on fasting, smoking, use of medication in the preceding 12 hours, and menopausal status was collected. Body mass index was calculated as measured weight (kg) divided by the square of height (m).

Ascertainment of breast cancer cases

Follow-up questionnaires were sent biennially to ascertain whether participants had been diagnosed with any of various diseases and to update data on medication use, menopausal status, and lifestyle factors. When a diagnosis of

breast cancer was reported, we examined the relevant medical records and obtained medical records from the attending physicians.

For this analysis, we designed a case-control study nested within the E3N-EPIC cohort among women who completed the dietary questionnaire and provided serum samples (*n* = 19,934). Among them, we selected those with available information on age at blood collection, date of collection, center of collection, menopausal status at collection, and fasting status at collection, which left us with a subcohort of 17,540 subjects. During a follow-up period of up to 7 years from blood collection, until July 2002, we identified 384 cases of incident invasive breast cancer. Controls (*n* = 768) were randomly selected among subjects who were free of cancer until the date of diagnosis of their matched case. Two controls per case were matched on age (±2 years), menopausal status (pre- or postmenopausal) at blood collection, fasting status (yes or no) at blood collection, study center (40 centers), and date of blood collection (same year).

Analysis of serum phospholipid fatty acids

Serum samples were divided into batches of nine blinded samples corresponding to samples from three cases and their matched controls in random order. Total lipids were extracted from serum samples (200 μl) with chloroform-methanol 2:1 (v/v) containing antioxidant butylated hydroxytoluene and L-A-phosphatidylcholine-dimyristoyl-d$_{54}$ (Cambridge Isotope Laboratories, Inc., Andover, Massachusetts) as an internal standard. Phospholipids were purified by adsorption chromatography (Supelco, St. Quentin Fallavier, France). Fatty acid methyl esters were formed by transmethylation with Methyl-Prep II (Alltech, Templemars, France). Analyses were carried out on a 6890N gas chromatograph (Agilent, Massy, France). A BPX70 capillary column was used for separation of fatty acid methyl esters (SGE, Courtaboeuf, France). The relative amount of each fatty acid, expressed as a percentage of total fatty acids, was quantified by integrating the area under the peak and dividing the result by the total area. Fatty acids were also expressed as absolute concentrations in serum (μmol/liter) based on the quantity of the methyl deuterated internal standard.

For quality control, we added one aliquot of the same serum sample to each batch of nine samples analyzed over 77 days and assessed between-batch reproducibility. As expressed in percentage of total fatty acids, coefficients of variation for major fatty acids were 6.63 percent for 15:0, 5.36 percent for 17:0, 0.75 percent for 16:0, 1.04 percent for 18:0, 0.67 percent for *cis*-18:1n-9, 8.98 percent for *trans*-monounsaturated fatty acids, 0.74 percent for 18:2n-6, 1.10 percent for 20:4n-6, 10.51 percent for 18:3n-3, 2.54 percent for 20:5n-3, and 1.48 percent for 22:6n-3.

Statistical analysis

Of the 1,152 selected participants, eight serum samples (two cases, six controls) could not be retrieved from the blood repository. Thirty samples were oxidized (eight cases, 22 controls). We further excluded 10 cases for which we obtained later histologic reports indicating that they had in

situ noninvasive breast cancer and one control who did not answer any questionnaire after her blood collection. As a consequence, some triplets of one case and the two corresponding controls were incomplete; we excluded those in which only the case ($n = 1$) or only the two controls ($n = 19$ triplets or 38 controls) remained. This left 363 cases and 702 matched controls. Moreover, the definition of menopausal status was revised after controls were selected; when we excluded controls who did not match their reference case's menopausal status at blood collection and/or cases for whom neither control was of the same menopausal status, the study population was restricted to 349 cases and 645 matched controls. Results for the larger population (363 cases, 702 controls) were similar to those for the restricted population, so we present results for the larger group. Baseline characteristics of cases and controls were compared using univariate logistic regression stratified by triplet.

Using values for the 40 individual fatty acids, we calculated the serum content of the following seven groups of fatty acids: saturated fatty acids, total monounsaturated fatty acids (MUFAs), *trans*-MUFAs (palmitoleic acid + elaidic acid), long-chain n-6 PUFAs, total n-6 PUFAs, long-chain n-3 PUFAs, and total n-3 PUFAs. We calculated the ratios of 18:2n-6 (linoleic acid) to 18:3n-3 (α-linolenic acid), long-chain n-6 PUFAs to long-chain n-3 PUFAs, and total n-6 PUFAs to total n-3 PUFAs. We also determined the saturation index (SI) as the ratio of stearic acid to oleic acid (SI_{n-9}) and the ratio of palmitic acid to palmitoleic acid (SI_{n-7}). The SI is an indicator of activity of the rate-limiting enzyme delta-9 desaturase, which transforms palmitic acid and stearic acid into the MUFAs palmitoleic acid and oleic acid, respectively. For analysis, serum phospholipid fatty acids and SIs were divided into quintiles based on the distribution among controls.

Odds ratios and 95 percent confidence intervals were estimated using conditional logistic regression matched on the triplets constituted by a case and her two controls. We selected a parsimonious multivariate model by including variables which were statistically significantly associated with breast cancer risk (at the 5 percent level) and which changed the risk estimates for at least one of the major fatty acids by 10 percent or more. The final parsimonious model was adjusted for years of education (in four categories: <12, 12–14, 15–16, or ≥17 years), body mass index at blood collection (as a continuous variable), adult height (as a continuous variable), menopausal hormone use before or at blood collection (ever vs. never), alcohol consumption at the time the dietary questionnaire was completed (as a continuous variable), age at first birth and parity combined (nulliparous, first birth before age 30 years and one or two children, first birth before age 30 years and three or more children, first birth at age 30 years or older), family history of breast cancer in first-degree relatives (yes vs. no), and personal history of benign breast disease (yes vs. no). Tests for linear trend across quintiles of phospholipid fatty acids were performed using the median level in each quintile. SAS statistical software (version 9.1; SAS Institute, Inc., Cary, North Carolina) was used for all analyses. All statistical tests were two-sided; *p* values of less than 0.05 were considered statistically significant.

TABLE 1. Baseline characteristics of control and case subjects in the E3N-EPIC Study, France, 1995–1998

Characteristic	Controls ($n = 702$)	Cases ($n = 363$)	*p* value*
Mean age (years) at blood collection	56.8 (6.3)†	56.8 (6.3)	0.31
Mean body mass index‡ at blood collection	24.1 (3.6)	23.8 (3.7)	0.14
Mean adult height (cm)	160.4 (5.6)	161.1 (6.0)	0.05
Age (years) at menarche (%)			
<12	19.1	19.1	0.97
12–13	53.1	52.7	
≥14	27.7	28.2	
Combined age at first birth and parity (%)			
Nulliparous	12.8	16.0	0.03
First birth before age 30 years, 1–2 children	46.7	46.3	
First birth before age 30 years, ≥3 children	30.6	24.0	
First birth after age 30 years	9.8	13.8	
Age (years) at menopause (%)			
Premenopausal	24.6	23.1	0.43
≤45	7.6	8.3	
46–54	61.1	59.8	
>54	6.7	8.8	
Ever use of menopausal hormones (%)	57.8	63.6	0.02
Years of education (%)			
<12	11.4	11.1	0.05
12–14	46.6	48.3	
15–16	22.9	16.5	
≥17	19.1	24.2	
History of benign breast disease (%)	31.8	41.1	0.002
Familial history of breast cancer (%)	14.4	21.2	0.007
Smoking status at blood collection (%)			
Nonsmoker (never or former smoker)	93.5	92.3	
Smoker	6.6	7.7	0.46

* Baseline characteristics of cases and controls were compared using Student's *t* test for continuous variables and the chi-squared test for categorical variables.

† Numbers in parentheses, standard deviation.

‡ Weight (kg)/height (m)^2.

RESULTS

Body mass index, age at menarche, age at menopause, and smoking status did not differ between cases and controls (table 1). However, greater proportions of taller women, women with higher education, nulliparous women, women who first

TABLE 2.   Mean serum concentrations of phospholipid fatty acids at baseline among control and case subjects in the E3N–EPIC Study, France, 1995–1998

Fatty acid	Controls ($n = 702$)		Cases ($n = 363$)	
	% of total fatty acids	Level (μmol/liter)	% of total fatty acids	Level (μmol/liter)
15:0, pentadecanoic acid	0.20 (0.05)*	9.07 (2.62)	0.20 (0.05)	8.91 (2.64)
17:0, heptadecanoic acid	0.44 (0.07)	18.42 (5.72)	0.44 (0.07)	17.96 (5.01)
16:0, palmitic acid	25.03 (1.7)	963.00 (261.26)	25.1 (1.58)	951.51 (246.92)
18:0, stearic acid	14.38 (1.23)	556.03 (166.81)	14.27 (1.1)	542.61 (145.94)
Total saturates†	40.78 (1.51)	1,586.10 (427.08)	40.72 (1.34)	1,559.79 (392.83)
16:1, palmitoleic acid‡	0.66 (0.19)	28.35 (11.74)	0.67 (0.19)	28.31 (11.26)
cis-16:1n-7, *cis*-palmitoleic acid	0.50 (0.16)	20.75 (9.32)	0.50 (0.16)	20.52 (8.96)
trans-16:1n-7, *trans*-palmitoleic acid	0.16 (0.06)	7.62 (3.29)	0.17 (0.06)	7.79 (3.27)
18:1, oleic acid§	11.25 (1.46)	437.06 (129.99)	11.25 (1.41)	430.70 (122.83)
cis-18:1n-9, oleic acid	9.43 (1.43)	364.95 (114.60)	9.39 (1.35)	357.79 (106.36)
cis-18:1n-7	1.61 (0.24)	62.88 (17.84)	1.64 (0.24)	63.48 (18.16)
trans-18:1n-9, elaidic acid	0.21 (0.13)	9.22 (5.65)	0.22 (0.14)	9.43 (5.87)
trans-MUFAs,¶ 16:1n-7 + 18:1n-9	0.37 (0.17)	16.79 (7.61)	0.39 (0.17)	17.22 (7.99)
Total MUFAs#	12.29 (1.58)	487.98 (144.06)	12.31 (1.53)	481.34 (136.10)
18:2n-6, linoleic acid**	21.34 (3.05)	820.76 (248.12)	21.41 (2.93)	814.07 (236.97)
cis-18:2n-6, *cis*-linoleic acid	21.30 (3.05)	816.68 (245.17)	21.33 (2.94)	809.37 (236.04)
trans-18:2n-6, *trans*-linoleic acid	0.07 (0.14)	3.97 (10.24)	0.07 (0.13)	3.80 (5.24)
18:3n-6, γ-linolenic acid	0.30 (0.21)	12.76 (9.91)	0.30 (0.21)	12.98 (12.03)
20:4n-6, arachidonic acid	11.9 (1.84)	458.50 (142.73)	11.95 (1.92)	453.31 (136.03)
Long-chain n-6 PUFAs¶	16.70 (2.25)	651.88 (202.71)	16.69 (2.25)	641.96 (189.11)
Total n-6 PUFAs††	37.89 (3.35)	1,466.80 (417.56)	37.98 (2.99)	1,451.54 (390.76)
18:3n-3, α-linolenic acid	0.19 (0.18)	9.28 (13.00)	0.19 (0.15)	8.56 (7.05)
20:5n-3, eicosapentaenoic acid	1.50 (0.91)	59.39 (41.57)	1.48 (0.86)	57.64 (36.66)
22:6n-3, docosahexaenoic acid	5.86 (1.41)	226.08 (81.65)	5.91 (1.36)	225.01 (75.86)
Long-chain n-3 PUFAs	8.65 (2.20)	337.50 (126.98)	8.67 (2.08)	333.66 (116.00)
Total n-3 PUFAs‡‡	8.84 (2.21)	346.71 (132.78)	8.86 (2.10)	342.14 (118.59)
Ratios				
18:2n-6/18:3n-3	139.85 (60.25)	110.40 (39.09)	143.92 (61.64)	113.89 (40.35)
Long-chain n-6 PUFAs/n-3 PUFAs	2.06 (0.6)	2.05 (0.59)	2.05 (0.59)	2.04 (0.57)
Total n-6 PUFAs/n-3 PUFAs	4.58 (1.3)	4.51 (1.26)	4.58 (1.34)	4.51 (1.29)
18:0/18:1n-9	1.56 (0.29)	1.56 (0.29)	1.55 (0.27)	1.55 (0.27)
16:0/16:1n-7	40.59 (11.89)	36.21 (9.35)	40.03 (10.16)	35.74 (8.03)

* Numbers in parentheses, standard deviation.
† Including 8:0, 10:0, 11:0, 13:0, 20:0, 21:0, 22:0, 23:0, and 24:0.
‡ Including *cis*- and *trans*-16:1n-7.
§ Including *cis*- and *trans*-18:1n-9 and 18:1n-7.
¶ MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
# Including 17:1, 20:1n-9, 22:1n-9, and 24:1n-9.
** Including *cis*- and *trans*-18:2n-6.
†† Including 20:2n-6, 20:3n-6, 22:2n-6, 22:4n-6, and 22:5n-6.
‡‡ Including 20:3n-3 and 22:5n-3.

gave birth after age 30 years, postmenopausal women using menopausal hormones, and women with a personal history of benign breast disease or a familial history of breast cancer were found among cases than among controls (table 1).

Palmitic acid was the prevailing saturated fatty acid in serum phospholipids, while oleic acid was the major MUFA (table 2). Among PUFAs, linoleic acid was the most common fatty acid, and it constituted approximately one fifth of

TABLE 3. Odd ratios for breast cancer according to quintile of serum phospholipid fatty acids (% of total fatty acids) in the E3N-EPIC Study (363 cases, 702 controls), France, 1995–1998

	Quintile of serum fatty acids									p-trend
	1 (referent)	2		3		4		5		
	(OR* = 1)	OR†	95% CI*	OR†	95% CI	OR†	95% CI	OR†	95% CI	
Saturated fatty acids										
15:0, pentadecanoic acid	1	1.08	0.69, 1.70	1.09	0.72, 1.63	0.78	0.48, 1.27	0.85	0.54, 1.32	0.27
17:0, heptadecanoic acid	1	0.81	0.52, 1.26	0.81	0.53, 1.25	0.60	0.38, 0.94	0.72	0.45, 1.16	0.10
16:0, palmitic acid	1	0.93	0.59, 1.45	1.43	0.91, 2.23	1.26	0.80, 1.98	1.12	0.70, 1.77	0.42
18:0, stearic acid	1	1.10	0.73, 1.65	0.82	0.53, 1.26	1.02	0.68, 1.54	0.73	0.46, 1.13	0.18
Total saturates‡	1	0.88	0.56, 1.36	0.78	0.49, 1.24	1.20	0.76, 1.90	0.80	0.48, 1.35	0.87
MUFAs*										
16:1, palmitoleic acid§	1	0.85	0.55, 1.30	1.18	0.78, 1.80	1.23	0.79, 1.92	1.39	0.89, 2.18	0.06
18:1, oleic acid¶	1	1.01	0.66, 1.55	1.17	0.76, 1.81	1.01	0.66, 1.55	1.24	0.81, 1.91	0.36
Total MUFAs#	1	0.94	0.61, 1.43	1.41	0.93, 2.15	1.00	0.65, 1.53	1.24	0.81, 1.90	0.37
n-6 PUFAs*										
18:2n-6, linoleic acid**	1	1.33	0.87, 2.02	1.53	0.99, 2.36	1.11	0.72, 1.71	1.11	0.71, 1.74	0.97
18:3n-6, γ-linolenic acid	1	0.99	0.64, 1.53	0.74	0.46, 1.18	0.83	0.50, 1.39	0.93	0.54, 1.58	0.63
20:4n-6, arachidonic acid	1	0.92	0.60, 1.40	1.18	0.78, 1.78	0.99	0.66, 1.51	0.94	0.62, 1.44	0.90
Long-chain n-6 PUFAs	1	1.25	0.82, 1.89	0.98	0.64, 1.49	0.92	0.59, 1.42	1.07	0.69, 1.66	0.86
Total n-6 PUFAs††	1	0.97	0.63, 1.49	1.34	0.88, 2.04	0.94	0.61, 1.45	0.97	0.61, 1.53	0.99
n-3 PUFAs										
18:3n-3, α-linolenic acid	1	1.13	0.75, 1.68	1.16	0.76, 1.76	0.81	0.54, 1.23	0.84	0.54, 1.32	0.17
20:5n-3, eicosapentaenoic acid	1	0.77	0.51, 1.17	1.00	0.65, 1.54	0.85	0.55, 1.30	0.98	0.64, 1.52	0.75
22:6n-3, docosahexaenoic acid	1	1.18	0.77, 1.81	1.12	0.72, 1.72	1.40	0.90, 2.18	1.15	0.73, 1.80	0.44
Long-chain n-3 PUFAs	1	1.49	0.97, 2.28	1.03	0.66, 1.62	1.40	0.91, 2.17	1.35	0.86, 2.13	0.31
Total n-3 PUFAs‡‡	1	1.37	0.90, 2.10	0.98	0.62, 1.53	1.35	0.88, 2.07	1.32	0.84, 2.08	0.28
Ratios										
18:2n-6/18:3n-3	1	0.94	0.62, 1.43	1.06	0.69, 1.63	1.18	0.78, 1.78	1.08	0.70, 1.66	0.50
Long-chain n-6 PUFAs/n-3 PUFAs	1	0.86	0.58, 1.29	0.78	0.51, 1.20	0.72	0.47, 1.11	0.90	0.58, 1.40	0.54
Total n-6 PUFAs/n-3 PUFAs	1	0.95	0.63, 1.44	0.86	0.56, 1.33	1.03	0.67, 1.56	0.76	0.48, 1.20	0.35
18:0/18:1n-9	1	1.14	0.76, 1.69	0.91	0.59, 1.40	0.90	0.59, 1.38	0.91	0.59, 1.41	0.40
16:0/16:1n-7	1	0.88	0.58, 1.35	0.88	0.57, 1.36	0.63	0.40, 0.99	0.66	0.41, 1.05	0.03

* OR, odds ratio; CI, confidence interval; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

† Adjusted for body mass index, alcohol consumption, height, menopausal hormone use, educational level, parity, family history of breast cancer, and history of benign breast disease.

‡ Including 8:0, 10:0, 11:0, 13:0, 20:0, 21:0, 22:0, 23:0, and 24:0.

§ Including cis- and trans-16:1 n-7.

¶ Including cis- and trans-18:1 n-9 and 18:1 n-7.

Including 17:1, 20:1n-9, 22:1n-9, and 24:1n-9.

** Including cis- and trans-18:2 n-6.

†† Including 20:2n-6, 20:3 n-6, 22:2n-6, 22:4 n-6, and 22:5n-6.

‡‡ Including 20:3n-3 and 22:5n-3.

the total (table 2). Our chromatographic method allowed us to distinguish the trans-MUFAs palmitoleic acid and elaidic acid from cis-palmitoleic acid and oleic acid without any overlapping. Other trans-MUFAs, such as vaccenic acid (trans-18:1 n-7), were eluted with oleic acid. Trans-linoleic acid was clearly separated from cis-linoleic acid.

Table 3 shows odds ratios for breast cancer by quintile of serum phopholipid fatty acids, expressed as a percentage of total fatty acids. No statistically significant association with breast cancer risk was found for individual or total saturated fatty acids, individual or total MUFAs, linoleic acid, long-chain or total n-6 PUFAs, α-linolenic acid, or long-chain or total n-3 PUFAs. The SI_{n-7}—that is, the ratio between palmitic acid and palmitoleic acid (last line of table 3)—was inversely associated with breast cancer risk (for the highest quintile compared with the lowest, odds ratio (OR) = 0.66,

TABLE 4.   Odds ratios for breast cancer according to quintile of serum phospholipid *cis*- and *trans*-fatty acids (% of total fatty acids) in the E3N-EPIC Study (363 cases, 702 controls), France, 1995–1998

	1 (referent) (OR* = 1)	2		3		4		5		*p*-trend
		OR†	95% CI*	OR†	95% CI	OR†	95% CI	OR†	95% CI	
cis- and *trans*-MUFAs*										
cis-16:1n-7, *cis*-palmitoleic acid	1	0.82	0.54, 1.26	1.22	0.80, 1.85	1.00	0.64, 1.56	1.06	0.67, 1.68	0.61
cis-18:1n-9, oleic acid	1	0.87	0.56, 1.36	1.46	0.95, 2.26	0.74	0.48, 1.15	1.18	0.77, 1.81	0.65
trans-16:1n-7, trans-palmitoleic acid	1	0.88	0.58, 1.33	1.17	0.74, 1.83	1.49	0.93, 2.40	2.24	1.30, 3.86	0.002
trans-18:1n-9, elaidic acid	1	1.14	0.73, 1.78	1.02	0.63, 1.64	1.04	0.64, 1.69	1.45	0.90, 2.33	0.12
trans-MUFAs, 16:1n-7 + 18:1n-9	1	1.23	0.79, 1.93	1.12	0.71, 1.78	1.54	0.97, 2.45	1.75	1.08, 2.83	0.018
cis- and *trans*-linoleic acid										
cis-18:2n-6, *cis*-linoleic acid	1	1.26	0.83, 1.93	1.51	0.98, 2.33	1.11	0.72, 1.71	1.05	0.67, 1.64	0.89
trans-18:2n-6, trans-linoleic acid	1	1.02	0.58, 1.77	1.07	0.63, 1.84	1.13	0.66, 1.94	1.55	0.91, 2.63	0.10

* OR, odds ratio; CI, confidence interval; MUFAs, monounsaturated fatty acids.

† Adjusted for body mass index, alcohol consumption, height, menopausal hormone use, educational level, parity, family history of breast cancer, and history of benign breast disease.

95 percent confidence interval (CI): 0.41, 1.05; *p* for trend = 0.031). The SI_{n-9} was also inversely associated (but not statistically significantly) with breast cancer risk. Similar results were found with quintiles of serum fatty acids expressed as μmol/liter (data not shown).

Table 4 shows odds ratios for breast cancer by quintile of serum phopholipid *trans*- and *cis*-fatty acids, expressed as a percentage of total fatty acids. For total *trans*-MUFAs, the odds ratio for the highest quintile compared with the lowest was 1.75 (95 percent CI: 1.08, 2.83; *p* for trend = 0.018). We found an increased risk of breast cancer with increasing levels of *trans*-palmitoleic acid (OR = 2.24, 95 percent CI: 1.30, 3.86; *p* for trend = 0.0016) and a trend with elaidic acid (OR = 1.45, 95 percent CI: 0.90, 2.33; *p* for trend = 0.12). Similar results were found when *trans*-MUFAs were expressed in μmol/liter (OR = 1.45, 95 percent CI: 0.85, 2.41; *p* for trend = 0.04). We also observed a trend toward increased risk of breast cancer associated with increasing levels of *trans*-linoleic acid (OR = 1.55, 95 percent CI: 0.91, 2.63; *p* for trend = 0.10). No significant association was found with either *cis*-MUFAs, palmitoleic or oleic acids, or *cis*-linoleic acid.

The number of premenopausal breast cancer cases was too small (84 out of 363 cases) for separate examination of the association between serum phospholipid fatty acid levels and breast cancer risk. The results for postmenopausal breast cancer cases only were similar to those reported for the whole population (data not shown).

DISCUSSION

Based on this large analysis from the E3N-EPIC cohort, we found evidence that women with high serum levels of phospholipid *trans*-palmitoleic and elaidic acids had a risk of breast cancer that was increased 50 percent to twofold in comparison with women with low serum levels. As other cohort studies have found previously (24, 25), we observed an inverse association between the saturation index and the risk of breast cancer, suggesting that a high ratio of saturated fat to monounsaturated fat may be associated with a reduced risk of breast cancer.

Trans-fatty acids are unsaturated fatty acids with at least one double bond in the *trans*- configuration. Because humans do not synthesize *trans*-fatty acids, levels of these fatty acid isomers in serum depend on their availability in the diet. *Trans*-fatty acids occur naturally in fat from ruminant animal meat, milk, and dairy fat and unnaturally in industrially hardened vegetable oils (26). The prevailing isomer present in partially hydrogenated fats is elaidic acid, whereas the prevailing isomer in milk fat is vaccenic acid (27). Dietary exposure to partially hydrogenated vegetable oils occurs through consumption of margarine and such industrially processed foods as cakes, rolls, candies, cookies, chocolate, mayonnaise, potato chips, French fries, and fast foods (28, 29).

Concern about the adverse effects of *trans*-fatty acids from partially hydrogenated fats on cardiovascular disease risk has increased since the early 1990s (30–34). Limited data are available on the potential effect of *trans*-fatty acids on cancer risk. One of the major difficulties relates to the imprecision in estimating *trans*-fatty acid intake using dietary questionnaires and available nutrient databases. In addition to the limitations inherent in dietary assessment methods, nutrient databases are rather incomplete and of questionable accuracy with respect to the *trans*-fatty acid composition of foods. In particular, an average value may not adequately describe the *trans*-fatty acid content of a generic food item; indeed, there is evidence of wide variation in the *trans*-fatty acid content of individual foods within a single category (35). Thus, assessment of *trans*-fatty acid intakes by means of dietary questionnaires is likely to be hampered by substantial measurement error, which could mask relatively modest associations with cancer as compared with cardiovascular disease.

In this context, biomarkers may provide more reliable data on *trans*-MUFA levels in free-living populations. The fatty acid profile of serum, plasma, or erythrocyte membrane phospholipids has been described as reflecting medium-term (weeks to months) intakes of some fatty acids (18, 19). In this population of E3N-EPIC women, despite a longer time lag between blood sampling and dietary assessments (4 years on average), serum levels of individual *trans*-palmitoleic and elaidic acids were significantly correlated with the consumption of manufactured foods (i.e., chocolate bars, candies, biscuits, cakes, and sandwich breads) (for *trans*-palmitoleic acid, $r = 0.091$, $p = 0.003$; for elaidic acid, $r = 0.165$, $p < 0.0001$) but were uncorrelated with dairy fat consumption (for *trans*-palmitoleic acid, $r = -0.02$, $p = 0.47$; for elaidic acid, $r = 0.041$, $p = 0.18$) or meat intake (for *trans*-palmitoleic acid, $r = -0.0034$, $p = 0.91$; for elaidic acid, $r = -0.028$, $p = 0.37$) (our unpublished data). Moreover, our findings are consistent with an analysis of the different isomers of *trans*-MUFAs in processed foods containing partially hydrogenated vegetable oils commercialized in France in 1995–1996 and 1999, showing that elaidic acid was the major isomer present in industrially produced sandwiches, crackers, pizza, cakes, and pastries (27). In that study, formation of *trans*-palmitoleic acid has also been described to occur during the partial hydrogenation of vegetable oils, with a content in manufactured foods approximately 100 times lower than that of elaidic acid (27). Moreover, *trans*-palmitoleic acid has been shown to be present in beef tallow (36), so consumption of *trans*-palmitoleic acid may occur through exposure to foods containing beef tallow. Therefore, the correlation we observed is likely to reflect a contribution of manufactured foods to serum *trans*-palmitoleic and elaidic acids at the time of data collection (1995–1998).

In agreement with our finding of a direct relation between serum *trans*-MUFAs and breast cancer, an ecologic study (37) and a multicenter case-control study (38) previously showed that breast cancer incidence was positively associated with levels of *trans*-fatty acids in gluteal adipose tissue. Similarly, in another case-control study based on breast adipose tissue, an increased risk of breast cancer was associated with high levels of *trans*-palmitoleic acid and elaidic acid, while a decreased risk was associated with high levels of corresponding *cis*- isomers (39). In contrast, other biomarker-based case-control (40, 41) or cohort (24, 25, 42) studies showed no evidence for a positive association between elaidic acid and breast cancer risk. This discrepancy may have resulted, in part, from the methods used for fatty acid analyses, which generally did not permit complete resolution of major *trans*-MUFAs (43). Our study had the advantage of combining wide dietary intake ranges and regional variability in the consumption of margarine, butter, and oil among French women (44) with the use of chromatography permitting complete separation of *trans*-palmitoleic and elaidic acids resulting from industrial processes from *trans*- and *cis*-MUFAs obtained from alternative food sources.

Although our study was limited by the inability to resolve vaccenic acid, the prevailing isomer in ruminant fat, we did not find an association between a high level of 15:0 in serum

phospholipids, validated as a biomarker of dairy fat intake in our population study (our unpublished data), and increased risk of breast cancer. These data might suggest that the source of *trans*-fatty acids—natural foods versus processed foods—is a determinant of breast cancer risk.

We did not find any association between n-6 PUFAs and breast cancer risk as reported in most epidemiologic studies (15, 16). Regarding putative protective fatty acids, we did not find any inverse association between n-3 long-chain PUFAs originating from fatty fish consumption and breast cancer risk. This finding is in agreement with most studies conducted in Western countries (10, 45). In contrast, results from studies conducted in Asian populations have shown evidence of a protective effect of fish (11) and n-3 long-chain PUFAs against breast cancer risk (12, 13). This discrepancy might be the consequence of higher dietary intakes of n-3 long-chain PUFAs in Asian populations as compared with Western populations. Specifically, in a prospective study conducted in Japan, the mean dietary intake of long-chain n-3 PUFAs in the highest quartile, associated with a halved risk, was estimated to reach 0.61 percent of energy (12). Similar high intakes were reported in a case-control study carried out in Japan, where the highest mean intake of long-chain n-3 PUFAs, associated with a halved risk, was estimated at more than 0.55 percent of energy (13). A substantially lower dietary intake of long-chain n-3 PUFAs was found in our French population, where the highest intake reached only 0.23 percent of energy (data not shown). In this context, clear inverse associations may not have been observed in our population because n-3 PUFA intake was below the threshold for a protective effect against breast cancer risk.

Although our findings share some consistency with those of previous studies, we cannot exclude the possibility of chance findings owing to the large number of tests performed. As in other observational studies, we cannot rule out the possibility that the associations we observed resulted from confounding bias, although we adjusted for known risk factors for breast cancer. In addition, cases and controls in this study came from a selected population of highly educated women who were willing to participate in both the dietary survey and blood collection. While this population was not representative of the general population, it is not clear how selection could have seriously affected the associations estimated in this study and other studies.

Our findings show that *trans*-fatty acids presumably originating from industrial processes may increase the risk of invasive breast cancer. However, we might ask whether they are still relevant, because a decline in *trans*-fatty acid intake from margarines has occurred since 1996 in Europe (46). In Denmark, although the mean population intake of *trans*-fatty acids from hydrogenated fats fell to around 1 g per day in 2005, daily intakes exceeding 5 g can still be easily reached by eating manufactured and fast-food products (47). Thus, our findings remain of topical interest, since the reduction in *trans*-fatty acids in margarines is likely to have been counterbalanced by increased consumption of readily available foods containing *trans*-fatty acids (48). For a comprehensive evaluation of the public health effect of *trans*-fatty acids, an assessment of *trans*-fatty acid levels in

populations with diverse dietary practices is essential. Such a collaborative study will be undertaken in the large prospective EPIC cohort.

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