Int. J. Cancer: **124,** 924–931 (2009)

Dietary intakes of ω-6 and ω-3 polyunsaturated fatty acids and the risk of breast cancer

Anne C.M. Thiébaut[1], Véronique Chajès[2], Mariette Gerber[3], Marie-Christine Boutron-Ruault[1], Virginie Joulin[2], Gilbert Lenoir[2], Franco Berrino[4], Elio Riboli[5], Jacques Bénichou[6] and Françoise Clavel-Chapelon[1]*

[1]*INSERM, ERI-20, Institut Gustave Roussy, Villejuif Cedex, France*
[2]*CNRS-FRE 2939, Institut Gustave Roussy, Villejuif Cedex, France*
[3]*Centre de Recherche en Cancérologie, INSERM-CRLC, Montpellier, France*
[4]*Istituto Nazionale per la Cura e lo Studio dei Tumori, Unità Operativa di Epidemiologia, Milan, Italy*
[5]*Imperial College, Department of Epidemiology & Public Health, London, United Kingdom*
[6]*INSERM, U657, CHU et Faculté de Médecine-Pharmacie de Rouen, Unité de Biostatistique, Rouen, France*

Experimental studies suggest detrimental effects of ω-6 polyunsaturated fatty acids (PUFA), and beneficial effects of ω-3 PUFAs on mammary carcinogenesis, possibly in interaction with antioxidants. However, PUFA food sources are diverse in human diets and few epidemiologic studies have examined whether associations between dietary PUFAs and breast cancer risk vary according to food sources or antioxidant intakes. The relationship between individual PUFA intakes estimated from diet history questionnaires and breast cancer risk was examined among 56,007 French women. During 8 years of follow-up, 1,650 women developed invasive breast cancer. Breast cancer risk was not related to any dietary PUFA overall; however, opposite associations were seen according to food sources, suggesting other potential effects than PUFA *per se*. Breast cancer risk was inversely associated with α-linolenic acid (ALA) intake from fruit and vegetables [highest *vs.* lowest quintile, hazard ratio (HR) 0.74; 95% confidence interval (CI) 0.63, 0.88; *p* trend < 0.0001], and from vegetable oils (HR 0.83; 95% CI 0.71, 0.97; *p* trend 0.017). Conversely, breast cancer risk was positively related to ALA intake from nut mixes (*p* trend 0.004) and processed foods (*p* trend 0.068), as was total ALA intake among women in the highest quintile of dietary vitamin E (*p* trend 0.036). A significant interaction was also found between ω-6 and long-chain ω-3 PUFAs, with breast cancer risk inversely related to long-chain ω-3 PUFAs in women belonging to the highest quintile of ω-6 PUFAs (*p* interaction 0.042). These results emphasize the need to consider food sources, as well as interactions between fatty acids and with antioxidants, when evaluating associations between PUFA intakes and breast cancer risk.

Key words: fatty acids; vitamin E; breast cancer; prospective studies; prevention

Breast cancer is the most frequent malignancy among women in Western Europe, North America[1] and more recently in Japan.[2] In France, about 50,000 incident cases were observed and 11,200 deaths (19% of cancer mortality) were due to breast cancer in 2005.[3] It has been estimated that as many as half of breast cancer deaths could be avoided through dietary modifications,[4] although the available evidence is far from convincing for most dietary factors.[5] The role of fat intake in breast cancer etiology has been investigated for long[6] but still remains controversial.[7] A meta-analysis of epidemiologic studies[8] observed a significant increase in breast cancer risk with high saturated fat intake but no association with monounsaturated and polyunsaturated fatty acid (PUFA) intakes. A recent prospective study[9] reported varying associations of breast cancer with dietary fat depending on its food sources, hence suggesting a possible explanation for the discrepant results across epidemiologic studies. Moreover, in a meta-analysis covering 97 studies in rodents, Fay et al.[10] underlined the need to distinguish between the effects of ω-6 and ω-3 PUFAs, with ω-6 PUFAs showing strong tumor-enhancing effects and ω-3 PUFAs nonsignificant protective effects. Additional experimental studies suggest that high intakes of ω-3 PUFAs could exert inhibitory effects on mammary tumorigenesis through competition with ω-6 PUFAs[11] or formation of oxidation products[12] that may in turn depend on the antioxidant status.[13–15] So far, such interactions have hardly been examined in epidemiologic studies.

We analyzed the relationship of breast cancer risk to PUFA intake, overall and by food sources, in a large prospective cohort of French women with a high diversity of dietary habits across the national territory.[16] The analysis focused on dietary ω-3 PUFA intakes which have been hypothesized to encompass a potential for preventive strategies. Nowadays, a number of dietary fish oil supplements and foods enriched with ω-3 PUFAs are available on the market, although their benefit on cancer prevention has not yet been ascertained.[17] As a secondary analysis, we investigated potential interactions of ω-3 PUFA intakes with intakes of ω-6 PUFAs and vitamin E, a liposoluble antioxidant, in relation to breast cancer risk.

Material and methods

Study population

E3N is a prospective cohort study on cancer risk factors conducted in France among women insured with the "Mutuelle Générale de l'Education Nationale" (MGEN), a national health insurance scheme covering teachers, teacher spouses and employees of the National Education System.[18] Overall, 98,995 women volunteers aged 40-65 years were enrolled in 1989-1991 after replying to a baseline questionnaire and giving their informed consent. The study was approved by the French National Commission for Data Protection and Privacy.

In 1993, a diet history questionnaire[19] was sent to participants who had previously answered both the baseline questionnaire and a second questionnaire on reproductive history and hormonal treatments (n = 95,644). After two reminders for nonrespondents, 77,613 dietary questionnaires were collected between June 1993

Abbreviations: ALA, α-linolenic acid; BMI, body mass index; CI, confidence interval; E3N, Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale; EPA, eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; HR, hazard ratio; INSERM, Institut National de la Santé et de la Recherche Médicale; MGEN, Mutuelle Générale de l'Education Nationale; PUFA, polyunsaturated fatty acids.

Grant sponsors: Foundation de France, French League Against Cancer, Carrefour Foundation, French Ministry of Research, French League Against Cancer, the European Community, 3M Company, "Mutuelle Générale de l'Education Nationale", the French Institute of Health and Medical Research, Gustave Roussy Institute and several General Councils in France.

Present address of Anne C.M. Thiébaut: INSERM, U657, Institut Pasteur, Paris, France.

***Correspondence to:** INSERM, ERI-20, Institut Gustave Roussy, Espace Maurice Tubiana, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France. Fax: +33 (0)1 42 11 40 00. E-mail: clavel@igr.fr

Received 6 May 2008; Accepted after revision 29 July 2008
DOI 10.1002/ijc.23980
Published online 9 September 2008 in Wiley InterScience (www.interscience.wiley.com).

and July 1997 (81.1% response rate). Of these, 2,104 were excluded because of miscoding ($n = 2,050$), duplicate answers ($n = 46$) or empty questionnaire ($n = 8$). In addition, we excluded 985 respondents who did not give their consent to follow-up by the health insurer (MGEN) in case of dropout. The remaining 74,524 subjects with available dietary measurements constituted the French component of the European Prospective Investigation into Cancer and Nutrition (EPIC).[20]

Information on educational level, reproductive history, history of benign breast diseases, familial history of breast cancer and hormonal treatments was obtained from the questionnaires preceding the dietary questionnaire and from a follow-up questionnaire accompanying the dietary questionnaire. Anthropometric measurements and smoking history were updated using all available follow-up questionnaires at the time of the analysis. Menopausal status and age at menopause were determined using information on last menstruation, hot flushes, hysterectomy, oophorectomy and use of hormonal treatments recorded in each follow-up questionnaire.

We excluded participants in the top and bottom 1% of the ratio of reported energy intake to basal metabolic rate, computed on the basis of age, height and weight at the time of the dietary survey.[21] Of the remaining 73,034 subjects, 4,500 who had reported cancer diagnosis (except basal cell skin carcinoma and lobular breast carcinoma *in situ*) before responding to the dietary questionnaire and 901 with unavailable follow-up information after this questionnaire were excluded. Finally, to focus on dietary intakes, we excluded 11,626 women who reported use of vitamin E, C or β-carotene supplements in 1995, 2000 or 2002, leaving 56,007 subjects for the analysis.

Dietary assessment

The diet history questionnaire[19] was designed to assess usual dietary intake during the past year and consisted of 2 parts. In the first part, divided into 8 meals or food occasions (from breakfast to after-dinner snacks), subjects were asked about the frequency of consumption (11 possible responses, from less than once per month to every day) of a given list of foods and food groups (*e.g.,* fruit, meat, cheese) and the amounts usually consumed, using either natural units (number of eggs, teaspoons, etc.) or portion sizes as illustrated in a booklet of colored photos (7 possible responses).[22] The second part of the questionnaire inquired the relative frequency of consumption of individual foods within food groups of the first part (*e.g.,* oranges, apples, etc. for fruit). It also included specific questions on the usual types of fat used as a spread on bread, used for cooking, added after cooking and used in salad dressings. Overall, the questionnaire covered the daily consumption of 208 food items, beverages and recipes.

The food composition table was initially derived from the French national database.[23] Moreover, specific analyses were performed to determine the fatty acid content of a number of food items, including sunflower, olive and peanut oils (3 major types of oils consumed in France), margarine, vegetables and processed meat. For the remaining foods, missing information on PUFA content [*i.e.,* linoleic acid, arachidonic acid, α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)] was completed using the food composition table that had been developed for another French study, based on a compilation of published data.[24] Dietary intake of long-chain ω-3 PUFAs was computed as the sum of EPA, DPA and DHA intakes. We also considered the ratio of total ω-6 PUFA intake over total ω-3 PUFA intake. Other nutrients assessed included ethanol, vitamins C and E, and carotenoids expressed as β-carotene equivalents.

The reproducibility and relative validity of the diet history questionnaire was assessed in 1990-1991 using an independent sample of 115 women with an educational level comparable with that of the E3N-EPIC participants.[19] The reference method for dietary assessment was based on the average of 12 24-hr dietary recalls completed monthly on varying days of the week during the year after completion of the diet history questionnaire. Spearman's correlation coefficient between the diet history questionnaire and the average of the 12 recalls was 0.49 for total fat intake, 0.48 for total PUFA intake, 0.46 for ω-6 PUFA intake and 0.35 for ω-3 PUFA intake (varying from 0.30 for EPA to 0.38 for ALA).

Case ascertainment

Cases were ascertained through active follow-up, with questionnaires sent in December 1994, April 1997, June 2000 and July 2002. All follow-up questionnaires asked participants whether any cancer had been diagnosed, requesting the address of their physician for confirmation. Deaths in the cohort were detected from reports by family members or the postal service, and by searching the health insurance company (MGEN) database, which contains updated information on vital status. Information on cause of death was obtained from the National Service on Causes of Deaths (INSERM CepiDC).

A total of 1,864 incident breast cancer cases were identified in the study subjects between their response to the dietary questionnaire and the mailing date of the last follow-up questionnaire in 2002. Of these, 63 were only self-reported cases, the remainder being confirmed by a pathology report (96.6%). These cases were included, as self-reporting proved to be extremely accurate in this cohort. After exclusion of 214 cases of carcinoma *in situ*, 1,650 cases of invasive breast cancer were available for analysis.

Statistical analysis

A total of 448,439 person-years (average follow-up 8.0 years) accumulated from the date of return of the dietary questionnaire to the date of cancer diagnosis for all cancer cases ($n = 3,349$), to the date of the last questionnaire returned for nonrespondents ($n = 3,573$) and deceased subjects ($n = 504$), or to the mailing date of the last follow-up questionnaire in 2002 for replies received after this date ($n = 48,581$). Subjects diagnosed with *in situ* breast cancer ($n = 214$) or cancer of another site ($n = 1,485$) were censored at their date of diagnosis.

The relationship between nutrient intakes and breast cancer risk was investigated using Cox proportional hazards regression models with age as the time scale.[25] The estimated hazard ratio (HR) and the corresponding 95% confidence interval (CI) were computed after stratifying on 5-year interval birth cohorts,[26] and adjusting for the following confounders: alcohol consumption (continuous), smoking history (never, ever), history of breast cancer in mother or sister(s) (never, ever), personal history of benign breast disease (never, ever), age at menarche (<12, 12-13, ≥ 14 years), parity (nulliparous, one or 2 children and age at first birth ≤ 30 years, 3 or more children and age at first birth ≤ 30 years, age at first birth >30 years), physical activity at baseline (in quintiles of metabolic equivalents), menopausal status (pre, post as a time-dependent covariate) and body mass index (BMI, in quintiles defined according to the baseline distribution, as a time-dependent variable). For postmenopausal women, analyses were further adjusted for age at menopause (≤ 45, >45 years) and current use of menopausal hormones (no, yes as a time-dependent variable). We tested for an interaction between menopausal status (as a time-dependent covariate) and PUFA intake. Missing ages at menopause were imputed to 47 years if menopause was artificial, or 51 years if menopause was natural, ages which corresponded to the medians observed among postmenopausal women with available age at menopause who reported artificial or natural menopause, respectively. For each remaining adjustment factor, missing data were imputed to the mode among the subjects with complete data, since the proportion of cases having missing data did not exceed 5%. We verified that HR estimates hardly changed after additional adjustment for educational level; however, educational level was not associated with breast cancer risk in the E3N-EPIC population that is rather homogenous with respect to this variable.

TABLE I – BASELINE CHARACTERISTICS (MEANS AND PROPORTIONS) BY INTAKES OF α-LINOLENIC ACID AND LONG-CHAIN ω-3 POLYUNSATURATED FATTY ACIDS (PUFA), E3N-EPIC COHORT (n = 56,007), 1993-2002

	Quintile of α-linolenic acid intake (median percent energy)			Quintile of long-chain ω-3 PUFA intake (median percent energy)		
	I (0.32)	III (0.41)	V (0.56)	I (0.08)	III (0.18)	V (0.40)
Age at entry (years)	53.4	52.8	52.5	52.8	52.5	53.6
Education level (%)[1]						
<12 years	12.6	11.3	12.4	13.3	11.8	11.5
12-14 years	48.8	50.9	50.1	48.7	50.9	48.1
15-16 years	17.1	18.1	17.6	17.7	17.6	17.8
17+ years	17.3	15.9	16.0	16.3	15.7	18.2
Nonalcohol energy intake (kcal/d)	2,125	2,097	2,057	2,138	2,134	1,939
Total fat intake (% energy)	32.7	38.8	42.7	36.7	38.4	39.7
Saturated fat intake (% energy)	13.1	15.5	16.2	15.3	15.3	14.7
Ratio ω-6/ω-3 PUFA	11.1	9.6	8.5	11.7	9.8	7.5
Alcohol intake (g/d)	8.4	11.0	14.3	8.9	11.4	13.0
Dietary vitamin E intake (mg/d)	12.9	14.2	15.6	13.4	14.2	15.0
Mother/sister(s) ever diagnosed with breast cancer (%)	7.4	7.1	7.4	7.4	7.4	7.5
History of benign breast disease (%)	27.5	28.3	28.2	27.5	28.1	27.5
Ever smoked (%)	42.1	47.7	53.2	44.6	47.3	52.0
Body mass index (kg/m^2)	22.7	23.2	23.6	22.6	23.1	23.8
Height (cm)	161	161	161	162	161	161
Age at menarche (%)						
<12 years	17.5	20.3	22.8	18.5	20.0	23.7
12-13 years	51.4	51.7	50.7	51.4	51.1	50.7
14+ years	31.1	28.0	26.5	30.2	28.9	25.6
Combined age at first birth and parity (%)						
Nulliparous	16.2	14.9	15.5	15.3	14.3	17.4
First birth before 30, 1-2 children	47.5	49.7	50.7	48.9	49.8	49.8
First birth before 30, 3+ children	27.7	27.5	27.1	27.1	27.9	26.0
First birth at 30+	8.7	7.9	6.7	8.7	7.9	6.8
Age at menopause (%)						
Premenopausal	41.9	43.9	45.1	45.2	46.6	38.4
≤45 years	7.5	7.3	7.4	7.5	6.8	8.1
46-54 years	46.8	45.3	44.4	44.1	43.3	49.6
55+	3.8	3.5	3.2	3.3	3.3	3.9
Current use of menopausal hormones (%)	31.2	33.1	33.0	29.1	31.0	36.9

[1]Percentages do not add up to 100% because of missing values.

We calculated PUFA intake from the 208 food items grouped into food groups such as added fat (vegetable oils, butter, margarine, duck fat), meat (poultry, red and processed meat), marine sources (canned and fresh fish, seafood) and processed foods (food items potentially including partially hydrogenated vegetable oils, *i.e.,* margarine, ready-to-use salad dressing, breakfast cereals, industrial bread and rusk, croissants, biscuits and cakes, chocolate bars and candies, nut mixes, pizza, tarts and sandwiches, French fries, fruit pies and dessert). In all models, PUFA intakes were expressed as percentages of nonalcohol energy (nutrient densities) and adjusted for nonalcohol energy intake.[27] Alternatively, we expressed PUFA intakes as the exponentiated residuals of the linear regression of log-transformed PUFA intake on log-transformed energy intake (residual method).[27] We also evaluated the addition effect of PUFA intake on breast cancer risk (as if subjects were given supplementation in PUFA) by fitting crude PUFA intake along with energy intake from other sources.[28] In both instances, we generally found similar results (data not shown). Nutrient intakes were categorized into quintiles according to the distribution observed in the entire cohort. Tests for trend across quintiles were performed by fitting the median value for each quintile of nutrient intake as a continuous variable and assessing whether its coefficient equaled 0 using the Wald chi-square statistic.

To investigate interactions between ω-3 and ω-6 PUFA and vitamin E intakes, HRs of breast cancer associated with increasing ω-3 PUFA intake were calculated per quintile of ω-6 PUFA (expressed as nutrient density) or vitamin E intakes. As they showed similar results, the second to fourth quintiles of dietary vitamin E intakes were grouped together. We performed tests for interaction by applying the likelihood ratio test to PUFA trend variable (2 degrees of freedom). As there was no clear evidence for an interaction between PUFA and carotenoid intakes (expressed as β-carotene equivalents) with respect to breast cancer, results are not included here. We verified that the proportional hazards assumption was not violated for our main exposure and other fixed covariates by testing interactions between these and functions of age. Moreover, we conducted a lag analysis by excluding the first year of follow-up for all subjects and 168 cases of invasive breast cancer among them. All p values were 2-sided. Statistical analyses were performed using SAS statistical software (release 9.1, 2004; SAS Institute Inc, Cary, NC).

Results

At baseline, total fat intake contributed an average 38.3% of nonalcohol energy intake, including 6.7% from PUFA intake. Linoleic acid was the predominantly consumed PUFA (5.9% energy), followed by ALA (0.43% energy). Among long-chain ω-3 PUFAs (0.22% energy), DHA was the major fatty acid (0.13% energy). The main food sources for linoleic acid intake were added fat (59.3% total intake) including sunflower oil (34.7%), processed foods (24.8%) including nut mixes (7.2%), and meat (10.7%). Arachidonic acid originated mostly from meat (70.9%), followed by marine sources (14.7%) and processed foods (11.8%), whereas long-chain ω-3 PUFAs originated mostly from marine sources (80.3%, including 32.8% from fatty fish), followed by meat (15.8%). In contrast, a variety of foods contributed to ALA intake: processed foods (28.2%) including nut mixes (12.1%), fruit and vegetables (16.4%), cheese (15.2%), butter and margarine (11.4%), meat (11.1%) and vegetable oils (8.7%). Table I shows the characteristics of subjects at inclusion according to quintiles of ALA and long-chain ω-3 PUFA intake (as nutrient density). Increasing quintiles of ALA intake were associated with increasing total and saturated fat intakes as percentages of nonalcohol

TABLE II – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR BREAST CANCER ACCORDING TO QUINTILES OF POLYUNSATURATED FATTY ACID (PUFA) INTAKES, E3N-EPIC COHORT (n = 56,007), 1993-2002

PUFA	Quintile	Median	Cases	Person-years	HR[1]	95%CI	p trend[2]
Total ω-6 PUFA (% energy)	I	3.41	337	89,107	1.00		
	II	4.63	341	89,739	1.02	0.87, 1.18	
	III	5.63	329	90,177	0.97	0.84, 1.13	
	IV	6.82	330	89,964	0.98	0.84, 1.14	
	V	8.97	313	89,453	0.93	0.80, 1.09	0.30
Linoleic acid (% energy)	I	3.33	338	89,114	1.00		
	II	4.54	340	89,711	1.01	0.87, 1.18	
	III	5.53	324	90,219	0.96	0.82, 1.11	
	IV	6.72	339	89,219	1.01	0.86, 1.17	
	V	8.86	309	89,437	0.92	0.79, 1.07	0.28
Arachidonic acid (% energy)	I	0.05	326	88,897	1.00		
	II	0.07	346	89,865	1.04	0.89, 1.21	
	III	0.09	328	89,825	0.98	0.84, 1.15	
	IV	0.11	310	90,254	0.92	0.79, 1.08	
	V	0.14	340	89,598	0.99	0.85, 1.16	0.54
Total ω-3 PUFA (% energy)	I	0.44	324	90,687	1.00		
	II	0.54	330	90,490	0.99	0.85, 1.16	
	III	0.62	344	89,738	1.03	0.89, 1.20	
	IV	0.71	318	89,389	0.94	0.80, 1.10	
	V	0.90	334	88,136	0.99	0.84, 1.15	0.69
α-linolenic acid (% energy)	I	0.32	328	89,605	1.00		
	II	0.37	321	89,940	0.96	0.83, 1.12	
	III	0.41	342	89,880	1.01	0.87, 1.18	
	IV	0.46	301	89,768	0.89	0.76, 1.04	
	V	0.56	358	89,246	1.05	0.90, 1.23	0.62
Long-chain ω-3 PUFA (% energy)	I	0.08	332	90,892	1.00		
	II	0.13	345	90,353	1.04	0.89, 1.21	
	III	0.18	323	90,200	0.96	0.82, 1.12	
	IV	0.25	329	89,220	0.96	0.82, 1.12	
	V	0.40	321	87,774	0.94	0.80, 1.10	0.25
Ratio ω-6/ω-3 PUFA	I	5.48	335	88,401	1.00		
	II	7.33	352	89,463	1.06	0.91, 1.23	
	III	8.95	343	89,823	1.04	0.89, 1.21	
	IV	10.91	305	90,363	0.93	0.80, 1.09	
	V	14.76	315	90,389	0.97	0.83, 1.14	0.32

[1]Cox proportional hazards models adjusted for age (time metric), nonalcohol energy and ethanol intakes, smoking history, history of benign breast disease, history of breast cancer in first-degree relatives, age at menarche, parity, body mass index, menopausal status, age at menopause and use of menopausal hormone treatment.–[2]Two-sided test using median nutrient intake in each quintile as a continuous variable.

energy, increasing alcohol consumption, a greater likelihood of smoking and increasing BMI. The same held for long-chain ω-3 PUFAs, except for the absence of a trend with saturated fat intake.

Overall, breast cancer risk was not related to ω-6 (total, linoleic acid, arachidonic acid) or ω-3 (total, ALA, long-chain) PUFA intake, or the ratio of ω-6 to ω-3 PUFAs (Table II). Interaction tests with menopausal status or hormone treatment use among postmenopausal women were not statistically significant. When we stratified on total ω-6 PUFA intake, we found a decreased risk of breast cancer associated with high long-chain ω-3 PUFA intake confined to the highest quintile of ω-6 PUFA intakes (highest *vs.* lowest quintile of long-chain ω-3 PUFA, HR 0.62; 95%CI 0.44, 0.86; p trend 0.021; p interaction 0.042). Breast cancer risk was not related to long-chain ω-3 PUFA intake in any but the highest quintile of dietary ω-6 PUFAs, and remained unrelated to total ω-3 or ALA intake regardless of ω-6 PUFA consumption.

Analyzing the relationship between breast cancer risk and individual PUFA intake from their main food sources (Table III), we found decreased breast cancer risk associated with high linoleic acid or ALA intake from vegetable oils, as well as with high ALA intake from fruit and vegetables. Conversely, an increase in breast cancer risk was associated with high linoleic acid or ALA intake from processed foods, including nut mixes. Breast cancer risk was related to neither ALA intake from other food sources (cheese, butter, margarine or meat) nor longer chain ω-6 (arachidonic acid) and ω-3 PUFA intake from any source. No test for interaction with menopausal status was statistically significant.

Analyses stratified by vitamin E intake are presented in Table IV; results are not shown for intermediate quintiles of dietary vitamin E where null associations were observed. A significant trend of increasing breast cancer risk with increasing ALA intake was found in women in the top quintile of dietary vitamin E (p 0.036); however, the test for interaction was not statistically significant. When we considered ALA intake from various food sources (data not shown), the positive associations with processed foods and nut mixes and the inverse associations with vegetable oils, fruit and vegetables were independent of dietary vitamin E intake. There was no association with longer chain PUFAs, neither arachidonic acid nor ω-3 PUFAs, regardless of dietary vitamin E intake.

The statistically significant associations of breast cancer risk with linoleic acid and ALA intakes from vegetable oils, ALA intake from fruit and vegetables, and linoleic acid and ALA intakes from nut mixes remained when we excluded the first year of follow-up (data not shown). The inverse association between long-chain ω-3 PUFA intake and breast cancer risk among women in the highest quintile of ω-6 PUFA intake also remained statistically significant (p trend 0.018); however, the test for interaction became borderline significant (p 0.061).

Discussion

This prospective cohort study showed no evidence of an association between estimated ω-6 or ω-3 PUFA intakes and breast cancer risk, in agreement with former meta-analyses of prospective studies for linoleic acid[29] and total ω-3 PUFA[17] intakes. However, our study provided some indication that dietary PUFA may play opposite roles in breast cancer risk depending on their food sources and that ω-6 and ω-3 PUFA intakes may interact in their association with breast cancer. Whether dietary vitamin E further

TABLE III – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR BREAST CANCER ACCORDING TO QUINTILES OF POLYUNSATURATED FATTY ACID (PUFA) INTAKE DIVIDED INTO SELECTED FOOD SOURCES, E3N-EPIC COHORT (n = 56,007), 1993-2002

Food source	% PUFA intake	Quintile	Median (% energy)	Cases	Person-years	HR[1]	95%CI	p trend[2]
Vegetable oils	50.0% linoleic acid	I	0.71	354	88,764	1.00		
		II	1.65	330	89,851	0.93	0.80, 1.08	
		III	2.56	349	90,164	0.99	0.86, 1.15	
		IV	3.67	313	90,001	0.89	0.76, 1.03	
		V	5.84	304	89,660	0.86	0.74, 1.00	0.042
	8.7% α-linolenic acid	I	0.007	343	90,632	1.00		
		II	0.013	332	90,204	0.95	0.82, 1.11	
		III	0.022	337	89,591	1.00	0.81, 1.10	
		IV	0.035	341	89,019	0.89	0.82, 1.11	
		V	0.070	297	88,994	0.87	0.71, 0.97	0.017
Fruit and vegetables	16.4% α-linolenic acid	I	0.037	360	89,758	1.00		
		II	0.055	347	90,108	0.94	0.81, 1.09	
		III	0.069	343	89,605	0.92	0.79, 1.06	
		IV	0.085	306	89,795	0.80	0.68, 0.93	
		V	0.115	294	89,173	0.74	0.63, 0.88	<0.001
Processed foods[3]	24.8% linoleic acid	I	0.46	298	89,559	1.00		
		II	0.85	336	90,013	1.14	0.97, 1.33	
		III	1.21	328	89,821	1.11	0.95, 1.30	
		IV	1.67	343	90,049	1.17	1.00, 1.37	
		V	2.69	345	88,997	1.18	1.01, 1.38	0.080
	28.2% α-linolenic acid	I	0.033	305	89,086	1.00		
		II	0.067	321	89,978	1.05	0.90, 1.23	
		III	0.099	339	90,191	1.11	0.95, 1.30	
		IV	0.139	333	90,063	1.10	0.94, 1.28	
		V	0.226	352	89,122	1.16	0.99, 1.35	0.068
Of which nut mixes[4]	7.2% linoleic acid	I	0.00	373	105,643	1.00		
		II	0.10	282	85,961	0.92	0.79, 1.08	
		III	0.36	307	85,973	1.01	0.87, 1.18	
		IV	0.48	332	86,036	1.09	0.94, 1.27	
		V	1.04	356	84,827	1.17	1.01, 1.37	0.004
	12.1% α-linolenic acid	I	0.000	373	105,643	1.00		
		II	0.012	280	85,973	0.91	0.78, 1.07	
		III	0.044	309	85,945	1.02	0.87, 1.19	
		IV	0.059	334	86,021	1.10	0.94, 1.28	
		V	0.128	354	84,857	1.17	1.01, 1.36	0.004

[1]Cox proportional hazards models adjusted for age (time metric), nonalcohol energy and ethanol intakes, smoking history, history of benign breast disease, history of breast cancer in first-degree relatives, age at menarche, parity, body mass index, menopausal status, age at menopause and use of menopausal hormone treatment.–[2]Two-sided test using median nutrient intake in each quintile as a continuous variable.–[3]Food items potentially including partially hydrogenated vegetable oils, *i.e.*, margarine, ready-to-use salad dressing, breakfast cereals, industrial bread and rusk, croissants, biscuits and cakes, chocolate bars and candies, nut mixes, pizza, tarts and sandwiches, French fries, fruit pies and dessert.–[4]Single item in E3N-EPIC diet history questionnaire; nutrient content estimated assuming a mix of different types of nuts, including 31% walnut, 26% peanut, 16% pistachio, 9% almond, 5% dried exotic fruit, 5% hazelnut, 5% cashew nut, 2% seeds and 1% coconut. The reference category corresponds to 23.7% non-consumers; the upper categories were based on quartiles among consumers.

affects dietary PUFA and breast cancer association was not clearly demonstrated from our study.

The epidemiologic evidence regarding ALA and breast cancer relationship is conflicting. Only one case-control study in Uruguay[30] found a positive association between ALA intake and breast cancer risk. In contrast, the Netherlands Cohort Study,[31] a case-control study in Italy[32] and a case-control study using adipose breast tissue[33] showed decreased breast cancer risks associated with high ALA levels. Other case-control studies reported no association of breast cancer risk with dietary ALA intakes,[34] or plasma[35,36] and erythrocyte membrane[37] levels. The discrepancy across studies seems unlikely to be explained by different levels of consumption: for instance, median ALA intake in the E3N-EPIC cohort was 0.9 g/d, compared with 1.0 g/d in the Uruguayan controls.[30]

Instead, another possible explanation for the lack of consistency among studies may be the different dietary sources of ALA. In the Uruguayan study,[30] meat was its major dietary source, whereas oils and raw vegetables were the main contributors in the Italian study finding an inverse association.[32] The diversity of food sources that we observed for ALA intake in our study is consistent with another French study.[24] In line with previous studies, we found decreased breast cancer risk associated with high consumption of ALA from fruit and vegetables, and vegetable oils, and increased breast cancer risk associated with high consumption of nut mixes and processed meat. Because nut mixes were proposed as a single item eaten with aperitifs, we cannot exclude residual confounding by alcohol consumption; we note, however, that another French study reported a positive association between breast cancer risk and ALA intake originating mainly from nuts.[38] Moreover, the stronger association that we observed between ALA intake and breast cancer risk in the highest quintile of dietary vitamin E intake favors a direct association with nuts, a major dietary source for both these nutrients. Altogether, these results suggest that ALA intake may not have a direct biological effect on breast cancer occurrence but may reflect different food patterns involving other nutrients that could affect breast cancer risk. Thus, the inverse association of breast cancer risk with ALA intake from vegetables and vegetable oils, as with linoleic acid, may indeed result from other compounds, such as folate or lignan that have been related to a reduced risk in this cohort.[39,40]

Alternatively, whether all *cis vs. cis*/*trans*-ALA isomers originating from different food sources may exert different effects on carcinogenesis remains to be determined. It is of interest that linoleic acid intake also showed opposite associations with breast cancer depending on whether it came from (unaltered) vegetable oils or from processed foods. Partially hydrogenated vegetable oils used in the preparation of margarines and a number of industrial foods are a source of *trans* fatty acids including isomers of ALA and linoleic acid[41,42] that have been associated with an increased

TABLE IV – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR BREAST CANCER ACCORDING TO QUINTILES OF POLYUNSATURATED FATTY ACID (PUFA) INTAKE STRATIFIED ON QUINTILES OF DIETARY VITAMIN E INTAKE, E3N-EPIC COHORT (n = 56,007), 1993-2002

PUFA	Quintile	Median	Cases	Person-years	HR[1]	95%CI	p trend[2]	p interaction
Lowest quintile of dietary vitamin E intake (median 8.5 mg/d)								
α-linolenic acid (% energy)	I	0.32	108	27,217	1.00			
	II	0.37	84	19,994	1.06	0.79, 1.41		
	III	0.41	68	17,147	0.99	0.73, 1.35		
	IV	0.46	57	14,134	0.98	0.70, 1.36		
	V	0.56	43	10,405	0.99	0.68, 1.42	0.83	
Long-chain ω-3 PUFA (% energy)	I	0.08	103	23,925	1.00			
	II	0.13	63	17,973	0.80	0.58, 1.09		
	III	0.18	64	16,706	0.84	0.61, 1.15		
	IV	0.25	71	15,756	0.96	0.71, 1.31		
	V	0.40	59	14,535	0.86	0.62, 1.20	0.67	
Arachidonic acid (% energy)	I	0.05	92	21,132	1.00			
	II	0.07	74	18,058	0.94	0.69, 1.29		
	III	0.09	55	16,408	0.76	0.54, 1.06		
	IV	0.11	56	15,821	0.79	0.56, 1.10		
	V	0.14	83	17,477	1.03	0.75, 1.41	0.93	
Highest quintile of dietary vitamin E intake (median 21.1 mg/d)								
α-linolenic acid (% energy)	I	0.32	46	13,831	1.00			
	II	0.37	49	16,185	0.93	0.62, 1.39		
	III	0.41	65	17,416	1.14	0.78, 1.67		
	IV	0.46	56	19,199	0.89	0.60, 1.32		
	V	0.56	104	23,274	1.36	0.95, 1.94	0.036	0.32
Long-chain ω-3 PUFA (% energy)	I	0.08	59	15,190	1.00			
	II	0.13	59	17,256	0.87	0.61, 1.25		
	III	0.18	63	17,655	0.91	0.64, 1.30		
	IV	0.25	69	18,506	0.95	0.67, 1.34		
	V	0.40	70	21,299	0.80	0.56, 1.13	0.29	0.72
Arachidonic acid (% energy)	I	0.05	65	16,242	1.00			
	II	0.07	71	17,257	1.03	0.74, 1.45		
	III	0.09	49	17,589	0.71	0.49, 1.03		
	IV	0.11	63	19,027	0.85	0.60, 1.21		
	V	0.14	72	19,790	0.90	0.64, 1.28	0.42	0.66

[1]Cox proportional hazards models adjusted for age (time metric), nonalcohol energy and ethanol intakes, smoking history, history of benign breast disease, history of breast cancer in first-degree relatives, age at menarche, parity, body mass index, menopausal status, age at menopause and use of menopausal hormone treatment.–[2]Two-sided test using median nutrient intake in each quintile as a continuous variable.

risk of breast cancer in biomarker studies,[43,44] including the present cohort.[45]

Overall, we found no association between long-chain ω-3 PUFA intake and breast cancer risk, in agreement with most epidemiologic cohort studies.[17,46–48] As exceptions, inverse associations have been reported in Chinese and Japanese women having intakes up to 40 times greater than Western ones.[49–51] We note that, like in other Western populations,[24,52] meat consumption sensibly contributed to long-chain ω-3 PUFA intake in this cohort, in addition to marine sources. Although null associations persisted regardless of food sources, we did observe a statistically significant interaction with ω-6 PUFAs, suggesting that women with lowest long-chain ω-3 PUFA intake but highest ω-6 PUFA intake (therefore very low long-chain ω-3 to ω-6 PUFA ratio) could benefit from increasing their long-chain ω-3 PUFA intake. Similarly, some epidemiologic studies have shown decreased breast cancer risks associated with high ratios of (long-chain) ω-3 to ω-6 PUFA intake, based on dietary questionnaires[53,54] or biomarkers.[33,55,56] In the same line, a Chinese prospective study[49] found a direct association between ω-6 PUFA intakes and breast cancer risk confined to women having the lowest intakes of long-chain ω-3 PUFAs. Such observation is consistent with the competition between ω-6 and ω-3 PUFAs for eicosanoid production as an underlying mechanism.[11,57]

We did not find any evidence of an interaction between long-chain ω-6 or ω-3 PUFA and dietary vitamin E with respect to breast cancer risk. Long-chain PUFA contribute to the production of peroxides[57] which have been hypothesized to play a role in breast cancer etiology.[58] Although peroxides have a proven genotoxic and cytotoxic action on normal cells, they might favor apoptosis in tumor cells.[59] Experimental evidence indicates that antioxidants, notably the lipid-soluble vitamin E, may interact with the effect of long-chain PUFAs on tumor growth by preventing the formation of peroxides.[12,13,60] So far, a limited number of epidemiologic studies have been published, and their results were divergent: whereas 2 prospective studies suggested an inverse association of breast cancer risk with combined high intakes of vitamin E and PUFA,[61,62] a case-control study reported a decreased risk of breast cancer associated with high arachidonic acid intakes among women with low vitamin E intakes, but an increased risk among women with both high arachidonic acid and vitamin E intakes.[34]

Our prospective analysis of the relationship between dietary PUFA intake and breast cancer risk had several strengths and limitations. The strengths of our study included the minimal loss to follow-up (only 6.4% nonrespondents), the high degree of reliability of the dietary questionnaire comparable to other studies,[19] its detailed list of foods including 14 types of fish, allowing the distinction between leaner and fattier fishes and an accurate estimation of long-chain ω-3 PUFA intake,[57] high variability of dietary patterns across France[16] and of ALA food sources[24] and a sufficient sample size to allow stratified analyses. In contrast, our study was limited by a single dietary measurement, and the inability to assess other liposoluble antioxidants (*e.g.,* carotenoids) or to distinguish between vitamin E compounds (*e.g.,* α-tocopherol). Changes in dietary habits or food composition may have occurred before the onset of breast cancer, further enhancing measurement error inherent to the use of dietary questionnaire.[63,64] Moreover, we could not assess consumption of ω-3 supplements during the study period although it was likely to be marginal.[24] How resulting misclassification between categories of fatty acid or vitamin E consumption[65] may affect estimated associations between PUFA intakes and breast cancer risk is unclear. At least, misclassification is likely to be nondifferential with respect to breast cancer due to the prospective design. Finally, we cannot exclude results due to chance alone owing to the high number of tests performed.

In conclusion, findings from this large French cohort indicate that the association of breast cancer risk with ω-6 and ω-3 PUFA intake may differ according to food sources, either reflecting different food patterns or geometrical isomers. The study also provided further support to the hypothesis of an interaction between ω-6 and ω-3 PUFAs with respect to breast cancer risk. Altogether, these results emphasize the need to distinguish among food sources of PUFAs, *e.g.,* fish, vegetables, vegetable oils *vs.* processed foods and to consider interactions between ω-6 and ω-3 PUFAs when evaluating potential beneficial effects of high ω-3 PUFA intakes for breast cancer prevention. The hypothesis of an effect modification by antioxidant intakes deserves further consideration.

Acknowledgements

Dr. Anne Thiébaut is grateful to "Fondation de France" in the French League Against Cancer for their financial support. Dr. Véronique Chajès is the recipient of a grant from Carrefour Foundation. The authors also wish to thank Ms. Rafika Chaït and Mr. Yann Follain (E3N-EPIC) for collecting and entering the pathology report data, Ms. Maryvonne Niravong (E3N-EPIC), Dr. Jayne Ireland (CIQUAL), Dr. Serge Hercberg and Dr. Pierre Astorg (SU.VI.MAX) for their help in drawing up the food composition table, Dr. Nicole Combe (ITERG) for performing the fatty acid analysis of selected foods, Mr. Baptiste Prestel (E3N-EPIC) for updating the analysis dataset, Dr. Marina Touillaud (E3N-EPIC) for her help with the revisions, and Mr. Garth Evans for his assistance with the English. The study of individual polyunsaturated fatty acid intakes and breast cancer risk was part of a collaborative project (Nutrialis) funded by the French Ministry of Research. The E3N study is being carried out with the financial support of the French League Against Cancer, the European Community, 3M Company, "Mutuelle Générale de l'Education Nationale", the French Institute of Health and Medical Research (INSERM), Gustave Roussy Institute and several General Councils in France. The authors are indebted to women participants and practitioners for their active collaboration to the E3N study.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide, version 1.0. Lyon, France: IARC Press, 2001.
2. Minami Y, Tsubono Y, Nishino Y, Ohuchi N, Shibuya D, Hisamichi S. The increase of female breast cancer incidence in Japan: emergence of birth cohort effect. Int J Cancer 2004;108:901–6.
3. Molinié F, Colonna M. Evolution de l'incidence et de la mortalité par cancer en France de 1980 à 2005: estimations à partir des données des registres du réseau FRANCIM et du CepiDC [Time trends in incidence and mortality by cancer in France from 1980 to 2005: estimations from FRANCIM network registry and CepiDC data]. FRANCIM; Hospices Civils de Lyon; Institut de Veille Sanitaire; CepiDC, 2008. http://www.invs.sante.fr/surveillance/cancers/estimations_cancers/default.htm (accessed on June 19, 2008).
4. Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995;103:165–70.
5. World Cancer Research Fund, American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 2007, 269 p.
6. Tannenbaum A. The genesis and growth of tumors. III. Effects of a high-fat diet. Cancer Res 1942;2:468–75.
7. Prentice RL, Caan B, Chlebowski RT, Patterson R, Kuller LH, Ockene JK, Margolis KL, Limacher MC, Manson JE, Parker LM, Paskett E, Phillips L, et al. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006;295:629–42.
8. Boyd NF, Stone J, Vogt KN, Connelly BS, Martin LJ, Minkin S. Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. Br J Cancer 2003;89:1672–85.
9. Wirfält E, Mattisson I, Gullberg B, Olsson H, Berglund G. Fat from different foods show diverging relations with breast cancer risk in postmenopausal women. Nutr Cancer 2005;53:135–43.
10. Fay MP, Freedman LS, Clifford CK, Midthune DN. Effect of different types and amounts of fat on the development of mammary tumors in rodents: a review. Cancer Res 1997;57:3979–88.
11. Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 1999;83:217–44.
12. Welsch CW. Review of the effects of dietary fat on experimental mammary gland tumorigenesis: role of lipid peroxidation. Free Radic Biol Med 1995;18:757–73.
13. Gonzalez MJ, Schemmel RA, Dugan L, Jr, Gray JI, Welsch CW. Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. Lipids 1993;28:827–32.
14. Cognault S, Jourdan ML, Germain E, Pitavy R, Morel E, Durand G, Bougnoux P, Lhuillery C. Effect of an α-linolenic acid-rich diet on rat mammary tumor growth depends on the dietary oxidative status. Nutr Cancer 2000;36:33–41.
15. Bougnoux P, Maillard V, Chajès V. Omega-6/omega-3 polyunsaturated fatty acids ratio and breast cancer. World Rev Nutr Diet 2005;94:158–65.
16. Kesse E, Boutron-Ruault MC, Clavel-Chapelon F. Regional dietary habits of French women born between 1925 and 1950. Eur J Nutr 2005;44:285–92.
17. MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, Lim YW, Traina SB, Hilton L, Garland R, Morton SC. Effects of omega-3 fatty acids on cancer risk: a systematic review. JAMA 2006;295:403–15.
18. Clavel-Chapelon F. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer: results from a large cohort of French women. Br J Cancer 2002;86:723–7.
19. van Liere MJ, Lucas F, Clavel F, Slimani N, Villeminot S. Relative validity and reproducibility of a French dietary history questionnaire. Int J Epidemiol 1997;26(Suppl 1):S128–36.
20. Riboli E. The European Prospective Investigation into Cancer and Nutrition (EPIC): plans and progress. J Nutr 2001;131:170S–5.
21. Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, Veglia F, Bueno-de-Mesquita HB, Ocke MC, Brustad M, Braaten T, Jose TM et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5:1329–45.
22. Lucas F, Niravong M, Villeminot S, Kaaks R, Clavel-Chapelon F. Estimation of food portion size using photographs: validity, strengths, weaknesses and recommendations. J Hum Nutr Diet 1995;8:65–74.
23. Favier JC, Ireland-Ripert J, Toque C, Feinberg M. Répertoire Général des Aliments. Table de composition (Composition tables), 2nd edn. Paris, France: Lavoisier, 1999. 897 p.
24. Astorg P, Arnault N, Czernichow S, Noisette N, Galan P, Hercberg S. Dietary intakes and food sources of n-6 and n-3 PUFA in French adult men and women. Lipids 2004;39:527–35.
25. Thiébaut ACM, Bénichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. Stat Med 2004;23:3803–20.
26. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol 1997;145:72–80.
27. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
28. Kipnis V, Freedman LS, Brown CC, Hartman A, Schatzkin A, Wacholder S. Interpretation of energy adjustment models for nutritional epidemiology. Am J Epidemiol 1993;137:1376–80.
29. Zock PL, Katan MB. Linoleic acid intake and cancer risk: a review and meta-analysis. Am J Clin Nutr 1998;68:142–53.
30. De Stefani E, Deneo-Pellegrini H, Mendilaharsu M, Ronco A. Essential fatty acids and breast cancer: a case-control study in Uruguay. Int J Cancer 1998;76:491–4.
31. Voorrips LE, Brants HAM, Kardinaal AFM, Hiddink GJ, van den Brandt PA, Goldbohm RA. Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. Am J Clin Nutr 2002;76:873–82.
32. Franceschi S, Favero A, Decarli A, Negri E, La Vecchia C, Ferraroni M, Russo A, Salvini S, Amadori D, Conti E, Montella M, Giacosa A. Intake of macronutrients and risk of breast cancer. Lancet 1996;347:1351–6.
33. Maillard V, Bougnoux P, Ferrari P, Jourdan ML, Pinault M, Lavillonnière F, Body G, Le Floch O, Chajès V. n-3 and n-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int J Cancer 2002;98:78–83.
34. Nkondjock A, Shatenstein B, Ghadirian P. A case-control study of breast cancer and dietary intake of individual fatty acids and antioxidants in Montreal, Canada. Breast 2003;12:128–35.

35. Chajès V, Hultén K, Van Kappel AL, Winkvist A, Kaaks R, Hallmans G, Lenner P, Riboli E. Fatty-acid composition in serum phospholipids and risk of breast cancer: an incident case-control study in Sweden. Int J Cancer 1999;83:585–90.
36. Saadatian-Elahi M, Toniolo P, Ferrari P, Goudable J, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E. Serum fatty acids and risk of breast cancer in a nested case-control study of the New York University Women's Health Study. Cancer Epidemiol Biomarkers Prev 2002;11:1353–60.
37. Pala V, Krogh V, Muti P, Chajès V, Riboli E, Micheli A, Saadatian M, Sieri S, Berrino F. Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. J Natl Cancer Inst 2001;93:1088–95.
38. Gerber M, Thiébaut A, Astorg P, Clavel-Chapelon F, Combe N. Dietary fat, fatty acid composition and risk of cancer. Eur J Lipid Sci Technol 2005;107:540–59.
39. Lajous M, Romieu I, Sabia S, Boutron-Ruault MC, Clavel-Chapelon F. Folate, vitamin B12 and postmenopausal breast cancer in a prospective study of French women. Cancer Causes Control 2006;17: 1209–13.
40. Touillaud MS, Thiébaut ACM, Fournier A, Niravong M, Boutron-Ruault MC, Clavel-Chapelon F. Dietary lignan intake and postmenopausal breast cancer risk by estrogen and progesterone receptor status. J Natl Cancer Inst 2007;99:475–86.
41. Wolff RL, Combe NA, Destaillats F, Boue C, Precht D, Molkentin J, Entressangles B. Follow-up of the Δ4 to Δ16 *trans*-18:1 isomer profile and content in French processed foods containing partially hydrogenated vegetable oils during the period 1995-1999. Analytical and nutritional implications. Lipids 2000;35:815–25.
42. Wolff RL. Are *trans* isomers of α-linolenic acid of nutritional significance for certain populations? Lipids 2002;37:1147–8.
43. Kohlmeier L, Simonsen N, van't Veer P, Strain JJ, Martin-Moreno JM, Margolin B, Huttunen JK, Navajas JF-C, Martin BC, Thamm M, Kardinaal AFM, Kok FJ. Adipose tissue *trans* fatty acids and breast cancer in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. Cancer Epidemiol Biomarkers Prev 1997;6:705–10.
44. Bougnoux P, Giraudeau B, Couet C. Diet, cancer, and the lipidome. Cancer Epidemiol Biomarkers Prev 2006;15:416–21.
45. Chajès V, Thiébaut ACM, Rotival M, Gauthier E, Maillard V, Boutron-Ruault MC, Joulin V, Lenoir GM, Clavel-Chapelon F. Association between serum *trans*-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC study. Am J Epidemiol 2008;167:1312–20
46. Missmer SA, Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, van den Brandt PA, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, et al. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. Int J Epidemiol 2002;31:78–85.
47. Terry PD, Terry JB, Rohan TE. Long-chain (n-3) fatty acid intake and risk of cancers of the breast and the prostate: recent epidemiological studies, biological mechanisms, and directions for future research. J Nutr 2004;134:3412S–20S.
48. Engeset D, Alsaker E, Lund E, Welch A, Khaw KT, Clavel-Chapelon F, Thiébaut A, Chajès V, Key TJ, Allen NE, Amiano P, Dorronsoro M, et al. Fish consumption and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 2006;119:175–82.
49. Gago-Dominguez M, Yuan JM, Sun CL, Lee HP, Yu MC. Opposing effects of dietary n-3 and n-6 fatty acids on mammary carcinogenesis: the Singapore Chinese Health Study. Br J Cancer 2003;89:1686–92.
50. Hirose K, Takezaki T, Hamajima N, Miura S, Tajima K. Dietary factors protective against breast cancer in Japanese premenopausal and postmenopausal women. Int J Cancer 2003;107:276–82.
51. Wakai K, Tamakoshi K, Date C, Fukui M, Suzuki S, Lin Y, Niwa Y, Nishio K, Yatsuya H, Kondo T, Tokudome S, Yamamoto A, et al. Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. Cancer Sci 2005;96:590–9.
52. Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition 2006;22:47–53.
53. Goodstine SL, Zheng T, Holford TR, Ward BA, Carter D, Owens PH, Mayne ST. Dietary (n-3)/(n-6) fatty acid ratio: possible relationship to premenopausal but not postmenopausal breast cancer risk in U.S. women. J Nutr 2003;133:1409–14.
54. Wirfält E, Mattisson I, Gullberg B, Johansson U, Olsson H, Berglund G. Postmenopausal breast cancer is associated with high intakes of ω6 fatty acids (Sweden). Cancer Causes Control 2002;13:883–93.
55. Simonsen N, van't Veer P, Strain JJ, Martin-Moreno JM, Huttunen JK, Navajas JF, Martin BC, Thamm M, Kardinaal AF, Kok FJ, Kohlmeier L. Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. Am J Epidemiol 1998;147: 342–52.
56. Bagga D, Anders KH, Wang HJ, Glaspy JA. Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer. Nutr Cancer 2002;42:180–5.
57. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004;79:935–45.
58. Gago-Dominguez M, Castelao JE, Pike MC, Sevanian A, Haile RW. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2829–39.
59. Halliwell B. The antioxidant paradox. Lancet 2000;355:1179–80.
60. Dutta A, Dutta SK. Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review. J Am Coll Nutr 2003;22:258–68.
61. Verhoeven DTH, Assen N, Goldbohm RA, Dorant E, van't Veer P, Sturmans F, Hermus RJJ, van den Brandt PA. Vitamins C and E, retinol, beta-carotene and dietary fibre in relation to breast cancer risk: a prospective cohort study. Br J Cancer 1997;75:149–55.
62. Michels KB, Holmberg L, Bergkvist L, Ljung H, Bruce A, Wolk A. Dietary antioxidant vitamins, retinol, and breast cancer incidence in a cohort of Swedish women. Int J Cancer 2001;91:563–7.
63. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, Bingham S, Schoeller DA, Schatzkin A, Carroll RJ. Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol 2003;158:14–21.
64. Bingham SA, Luben R, Welch A, Wareham N, Khaw KT, Day N. Are imprecise methods obscuring a relation between fat and breast cancer? Lancet 2003;362:212–4.
65. Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 2003;133(Suppl 3):933S–40.