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Adipose Tissue Omega-3 and Omega-6 Fatty Acid Content and Breast Cancer in the EURAMIC Study

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The fatty acid content of adipose tissue in postmenopausal breast cancer cases and controls from five European countries in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer (EURAMIC) breast cancer study (1991–1992) was used to explore the hypothesis that fatty acids of the omega-3 family inhibit breast cancer and that the degree of inhibition depends on background levels of omega-6 polyunsaturates. Considered in isolation, the level of omega-3 or omega-6 fat in adipose tissue displayed little consistent association with breast cancer across study centers. The ratio of long-chain omega-3 fatty acids to total omega-6 fat showed an inverse association with breast cancer in four of five centers. In Malaga, Spain, the odds ratio for the highest tertile relative to the lowest reached 0.32 (95% confidence interval 0.13–0.82). In this center, total omega-6 fatty acid was strongly associated with breast cancer. With all centers pooled, the odds ratio for long-chain omega-3 to total omega-6 reached 0.80 for the second tertile and 0.65 for the third tertile, a downward trend bordering on statistical significance (p for trend = 0.055). While not definitive, these results provide evidence for the hypothesis that the balance between omega-3 and omega-6 fat may play a role in breast cancer. *Am J Epidemiol* 1998;147:342–52.

adipose tissue; breast neoplasms; fatty acids; fatty acids, omega-3

Breast cancer rates vary greatly across industrialized nations. The incidence rate in the United States is nearly five times that of Japan. In the neighboring countries of Spain and France, annual female breast cancer incidences differ by 50 percent (86 and 129 per 100,000, respectively) (1). Despite clear associations of hormonal, reproductive, and genetic factors with breast cancer risk, most of the variation in occurrence across populations does not appear to be attributable to established risk factors (2, 3). In the search for additional etiologic agents, fat consumption has received extensive attention. Cohort studies, however, provide little evidence that total fat consumption independent of its energy contribution strongly influences breast cancer risk in postmenopausal women (4, 5). Research is now shifting to the potential role of particular types of fat in the development of breast cancer.

Polyunsaturated fats are essential nutrients that cannot be synthesized, so they must be obtained from the diet. Their component fatty acids possess carbon chains containing two or more double bonds. Polyunsaturates are divided into two major families, omega-6 and omega-3, with the former predominant in most diets. Both omega-3 and omega-6 polyunsaturates give rise to physiologically active products, including prostaglandins, leukotrienes, and thromboxanes (known collectively as eicosanoids) (6). In addition to producing competing families of eicosanoids and other metabolites, omega-3 fatty acids compete with omega-6 fatty acids for incorporation into cell membranes (7).

Corn and other common seed oils provide rich sources of omega-6 polyunsaturates (primarily as the 18-carbon linoleic acid). Seafood, in contrast, is rich in the longer chain (20 or more carbon) omega-3 fatty acids docosahexanoic acid (DHA) and eicosapen-

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Abbreviations: DHA, docosahexanoic acid; EPA, eicosapentanoic acid; EURAMIC, European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer.

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tanoic acid (EPA). Nonmarine sources often include alpha-linolenic acid, an 18-carbon omega-3 polyunsaturate, but at generally much lower levels than linoleic acid.

Laboratory studies point to an involvement of polyunsaturated fatty acids in tumor growth and metastasis (8, 9). Mammary tumors produce large amounts of omega-6 metabolites (e.g., prostaglandin E2) (10) that may compromise immune function and aid tumor growth. Omega-3-derived metabolites have been shown to block tumor growth. An increase in omega-3 at the expense of omega-6 fat could potentially inhibit production of omega-6 metabolites and increase production of omega-3 metabolites, thereby exerting anticarcinogenic effects. In fact, increasing the ratio of omega-3 to omega-6 fatty acid in mammary tumor cell cultures reduces the production of prostaglandin E2 by those cells (11, 12). Substitution of fish oil for corn oil in the diet also inhibits both spontaneous mammary tumor incidence and growth of transplanted tumors in rodent models (13–15). In several of these models, the magnitude of the increase in the ratio of fish to corn oil appears critical to tumor suppression (16–18). Purified long-chain omega-3 fatty acids produce the same tumor growth inhibition seen with fish oil (19).

A study using population-based controls and tissue taken from a site remote from the tumor was therefore undertaken to investigate the relation between adipose tissue omega-3 fatty acids and breast cancer. The fatty acid composition of adipose tissue samples is used as a biomarker to investigate the relation between exposure to omega-3 fatty acids and postmenopausal breast cancer in European populations that differ greatly in their dietary fat intakes as well as breast cancer risks (1, 20, 21). The primary hypothesis under exploration is that stores of essential fatty acids of the omega-3 family (reflecting exposure through diet) are inversely associated with breast cancer in postmenopausal women and that this association is modulated by stores of omega-6 fatty acids.

MATERIALS AND METHODS

Study design and subject recruitment

The European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast (EURAMIC) was conducted in five European countries between 1991 and 1992 to test the relation between long-term exposure to antioxidants and breast cancer (20). This case-control study used adipose tissue antioxidant and fatty acid levels rather than dietary recall or food record data as its measure of exposure. The EURAMIC Study design has been extensively considered elsewhere (20, 22). Centers recruited incident cases of breast cancer from the gynecologic and surgical units of participating hospitals. Recruitment involved incident primary breast cancers (*International Classification of Diseases for Oncology*, code 174) among postmenopausal women aged 50–74 years at diagnosis. Eligible cases had tumors histologically classified as ductal carcinoma, with primary tumors less than 5 cm, axillary lymph nodes stage less than N3, and no clinical indication of distant metastases at discharge.

Controls were randomly selected from population registries in Germany and Switzerland. Other centers (in the Netherlands, Northern Ireland, and Spain), anticipating low general-population response rates, drew controls from patient lists of the cases' general practitioners (22). The sampling of controls used frequency matching for age (5-year intervals). Potential cases or controls who reported that they had a physician-prescribed change in diet other than sodium or total calorie reduction within the previous year; altered use of dietary supplements containing vitamin E, beta-carotene, or selenium; or weight loss exceeding 5 kg were excluded. Other grounds for exclusion included a history of treatment for alcohol or other chemical abuse, institutionalization, or major psychiatric disorder that might compromise ability to give informed consent. The requirements of all relevant local committees on human experimentation were met, and informed consent was obtained from all subjects.

Tissue sampling and analysis

Adipose tissue was aspirated by needle from the subcutaneous buttock directly into vacuum adapters (23). To assure standardized procedures and to assist in acquiring the appropriate skills for sampling, a videotape showing the technique was distributed to all participating centers. After collection and during transit, samples were kept on dry ice or in liquid nitrogen prior to storage at −70°C. Quality control samples were included in the shipments. After saponification and acidification, the free fatty acids were extracted with hexanoyl and methylated. Gas-liquid chromatography (HRGC 5300 Mega Series, Carlo Erba, Milan, Italy) with split injection was conducted in Zeist, Switzerland, using a 30-m long DB-23 column, inside diameter 0.253 mm, phase layer 0.25 μ, and helium as carrier gas in a temperature-programmed run (24, 25).

Response rates and availability of fatty acid data

Detailed response rates for this population are presented in a paper by van't Veer et al. (22). All subjects were requested to donate adipose tissue. The participation rate for eligible cases was 86 percent, with no

center falling below 75 percent. As expected, because of the need for a clinical visit to obtain the adipose tissue sample, the participation rate for controls was lower than that for cases. The control rate averaged 41 percent, largely as a result of the low participation rate in Zurich (22 percent). The average participation rate for persons approached as potential controls in centers other than Zurich was 58 percent and ranged from 45 percent in Berlin to 91 percent in Malaga.

A total of 317 cases and 367 controls provided fat aspirates. As described in a previous publication (22), 26 subjects had adipose tissue samples too small to analyze. The assays for a further 14 small, but analyzable, samples were rejected after the laboratory determined that the total weight of fatty acids estimated chromatographically diverged excessively from the actual measured weight of the sample. In addition, for the current analyses, two subjects were excluded because some of the chromatographic peaks for omega-3 and omega-6 fatty acids could not be separated. This left 291 cases and 351 controls in the current analyses. Excluded cases and controls showed the same major demographic characteristics as did their included counterparts.

Measured omega-3 fatty acids included α-linolenic, EPA, and DHA. Total omega-6 fatty acid reflected the sum of linoleic, γ-linolenic, eicosadienoic, dihomo-γ-linolenic, and arachidonic acids, although the contribution of linoleic acid dwarfed that of all other components. Between-assay coefficients of variation derived from replicate tissue samples ranged from 1.7 percent for linoleic acid to 4.5 percent for alpha-linolenic acid and 12.8 percent for docosahexanoic acid. Detection limit values for α-linolenic acid and docosahexaenoic acid were set to 0.2 and 0.025, respectively.

Statistical analyses

Analyses used simple descriptive statistics followed by logistic regression modeling to compare fatty acid distributions across study centers. Center-specific modeling employed the LOGISTIC procedure in SAS version 6.10 software for unconditional logistic regression (SAS Institute, Inc., Cary, North Carolina). Pooled analyses used the logistic regression with random effects for distinguishable data option in the EGRET software package (Cytel Software Corporation, Cambridge, Massachusetts), with random effects assigned to each tertile of fatty acid within centers.

Factors considered as potential confounders included age, body mass index, current and past smoking, current alcohol drinking, oral contraceptive use, supplemental hormone use, family history of breast cancer, parity, age at first childbirth, age at menarche, socioeconomic status, and recruitment center. Age was included in all models, and adjustment for recruitment center was included in all models where centers were pooled. With a forward selection process (26), additional variables were added if they met a statistical significance criterion of $p = 0.10$ for inclusion in the model containing a fatty acid term. Variables that did not alter the results for any of the fatty acids by at least 10 percent, however, were dropped to provide a more parsimonious model. The covariates selected in this manner included body mass index and reproductive history. Reproductive history was modeled by indicator variables for nulliparity and for age at first childbirth of 25–34 years and more than 34 years (with age less than 25 years serving as the referent).

The associations between fatty acids and breast cancer were modeled on the basis of both tertiles of fat and continuous fatty acid variables. Cutpoints are computed separately for each center on the basis of the total population within that center to ensure adequate numbers within each tertile.

RESULTS

Other risk factors

Valid adipose tissue fatty acid data were obtained for 642 women who met the study inclusion criteria. The mean age of both cases and controls was 62 years. Mean body mass index was 27 percent for cases and 26 percent for controls. Details of the relations between established risk factors and breast cancer in the EURAMIC Study have been published recently and will only be summarized here (22). Among the factors considered, body mass index, age at first childbirth, and familial history of breast cancer showed significant positive associations with breast cancer. Smokers, drinkers, and persons with a history of benign breast disease were mildly but not statistically significantly overrepresented in the eligible case population compared with controls.

Omega-3 and omega-6 differences between study centers

As expected, less than a tenth of the polyunsaturates for any center belong to the omega-3 family, within which alpha-linolenic acid predominates (table 1). Median omega-3 values vary substantially across centers. Malaga, Spain, and Zurich, Switzerland, have the lowest median omega-3 and lowest alpha-linolenic acid levels. Medians between centers vary by as much as 38 percent for total omega-3 and 54 percent for alpha-linolenic acid among controls. The highest levels of DHA (C22:6 ω3), the major long-chain omega-3 fatty acid, occur in Malaga, Spain, and Berlin, Germany. Median values in

TABLE 1. Adipose tissue fatty acids in breast cancer cases and controls, by center, postmenopausal European women in the EURAMIC Study, 1991–1992*

Fatty acid(s)	All centers				Zeist, the Netherlands				Coleraine, Northern Ireland			
	Cases (n = 291)		Controls (n = 351)		Cases (n = 70)		Controls (n = 63)		Cases (n = 95)		Controls (n = 99)	
Total ω3† polyunsaturates (as % of total fatty acid)	0.77	0.62–0.93	0.78	0.63–0.94	0.89	0.74–0.98	0.92	0.78–0.99	0.83	0.74–1.02	0.89	0.79–0.98
Total long-chain ω3† (as % of total)	0.18	0.13–0.26	0.20	0.15–0.25	0.17	0.11–0.25	0.23	0.16–0.29	0.15	0.12–0.22	0.18	0.13–0.22
DHA† (as % of total)	0.15	0.10–0.21	0.15	0.12–0.20	0.14	0.09–0.18	0.18	0.11–0.23	0.12	0.09–0.17	0.14	0.10–0.18
ALA† (as % of total)	0.59	0.43–0.71	0.57	0.42–0.73	0.67	0.59–0.78	0.68	0.54–0.76	0.67	0.59–0.78	0.71	0.62–0.78
Total ω6† polyunsaturates (as % of total fatty acid)	13.17	10.8–16.5	12.53	10.8–14.8	14.22	11.6–16.0	14.19	11.8–16.2	11.26	9.6–14.2	11.61	10.0–15.0
Linoleic acid (as % of total fatty acid)	12.35	10.2–15.6	11.67	10.0–14.0	13.41	10.9–15.3	13.37	11.0–15.3	10.52	9.0–13.5	10.91	9.5–14.2
Ratios to ω6: total ω3:total ω6 (×100)	5.69	4.34–7.63	6.01	4.72–8.06	6.42	5.31–7.28	6.42	5.39–7.21	7.44	6.03–9.05	7.44	5.90–9.02
Total long chain ω3:total ω6 (×100)	1.37	0.90–1.93	1.56	1.07–2.12	1.30	0.74–1.78	1.52	0.94–2.17	1.41	0.86–1.92	1.47	0.97–1.94
Total DHA:total ω6 (×100)	1.05	0.76–1.54	1.26	0.84–1.67	0.97	0.60–1.53	1.18	0.70–1.70	1.05	0.67–1.45	1.23	0.79–1.48
Total ALA:total ω6 (×100)	4.46	3.01–6.00	4.23	3.27–5.93	4.99	3.96–5.79	4.61	4.05–5.60	6.00	4.94–7.08	5.89	4.89–7.51
Total monounsaturates (as % of total fatty acid)	55.57	52.9–58.4	56.42	53.1–59.7	55.29	52.7–58.0	53.58	51.1–56.3	55.26	52.8–57.9	54.94	52.9–57.5

	Zurich, Switzerland				Berlin, Germany				Malaga, Spain			
	Cases (n = 54)		Controls (n = 74)		Cases (n =16)		Controls (n = 47)		Cases (n = 58)		Controls (n =68)	
Total ω3 polyunsaturates (as % of total fatty acid)	0.57	0.53–0.71	0.62	0.57–0.72	1.01	0.93–1.22	0.93	0.81–1.12	0.66	0.57–0.75	0.58	0.52–0.65
Total long-chain ω3 (as % of total)	0.16	0.11–0.20	0.17	0.13–0.22	0.30	0.24–0.45	0.24	0.19–0.30	0.28	0.22–0.38	0.24	0.17–0.29
DHA (as % of total)	0.13	0.08–0.16	0.12	0.10–0.16	0.25	0.18–0.33	0.18	0.15–0.22	0.23	0.18–0.31	0.20	0.14–0.23
ALA (as % of total)	0.45	0.37–0.50	0.48	0.41–0.50	0.69	0.59–0.80	0.69	0.58–0.85	0.38	0.32–0.42	0.35	0.32–0.39
Total ω6 polyunsaturates (as % of total fatty acid)	13.29	11.7–14.8	13.41	12.1–15.5	11.48	10.8–13.1	11.39	10.3–13.1	17.88	13.4–22.1	11.74	10.6–13.7
Linoleic acid (as % of total fatty acid)	12.62	11.0–13.8	12.45	11.4–14.6	10.62	9.8–11.8	10.68	9.5–12.4	16.69	12.4–21.1	10.96	9.6–12.6
Ratios to ω6: total ω3:total ω6 (×100)	4.61	3.57–5.36	4.56	4.12–5.40	9.18	8.45–11.1	8.25	7.34–9.19	3.67	3.06–4.72	5.04	4.09–5.69
Total long-chain ω3:total ω6 (×100)	1.19	0.88–1.58	1.20	0.91–1.57	2.55	1.90–4.03	2.06	1.70–2.45	1.70	1.14–2.03	2.04	1.38–2.50
Total DHA: total ω6 (×100)	0.90	0.64–1.22	0.92	0.69–1.18	1.99	1.50–3.03	1.58	1.31–1.86	1.38	0.93–1.66	1.70	1.16–1.99
Total ALA: total ω6 (×100)	3.31	2.73–3.90	3.41	2.78–3.96	6.06	5.15–6.89	6.37	5.35–7.09	2.10	1.56–2.90	2.96	2.54–3.38
Total monounsaturates (as % of total fatty acid)	54.89	52.1–56.8	55.51	52.0–57.1	57.07	55.2–59.1	57.55	54.8–60.2	58.45	53.7–63.2	66.10	63.5–68.4

* Medians followed by 25th and 75th percentiles.

† ω3 = omega-3 = sum of α-linolenic, eicosapentanoic, and docosahexanoic acids; long-chain ω3 = sum of eicosapentanoic and docosahexanoic acids; DHA = docosahexanoic acid; ALA = α-linolenic acid; ω6 = omega-6 = sum of linoleic, γ-linolenic, eicosadienoic, dihomo-γ-linolenic, and arachidonic acids.

Zurich Switzerland, and Coleraine, Northern Ireland, are about 40 percent lower. Omega-6 polyunsaturates are far more prevalent than are omega-3s. Linoleic acid, a member of the omega-6 family, makes up the vast bulk of the polyunsaturates in all centers. Medians for both linoleic acid and total omega-6 show a 20 percent difference for controls between extreme centers, with the lowest levels in Berlin and Coleraine and the highest in Zeist, the Netherlands.

Omega-3 and omega-6 differences between cases and controls

A comparison of median adipose omega-3 fatty acid percentages in all centers combined reveals little difference between cases and controls. Center-specific comparisons of total omega-3 and its major individual components, α-linolenic and DHA, with disease status show differences of varying magnitude and direction across centers. Logistic regression models were used to evaluate the relation between breast cancer and these fatty acids while controlling for other risk factors.

Models of breast cancer by omega-3 and omega-6 fatty acids

Inverse associations between breast cancer and total adipose tissue omega-3 fatty acid levels appear in three centers (Zeist, Coleraine, and Zurich), with the second and third tertiles displaying odds ratios below

TABLE 2. Odds ratios for increasing tertiles of adipose tissue fatty acids by study center and for all centers pooled,* postmenopausal European women in the EURAMIC Study, 1991–1992

Fatty acid and tertile	Center											
	Zeist, the Netherlands		Coleraine, Northern Ireland		Zurich, Switzerland		Berlin, Germany		Malaga, Spain		All centers pooled	
	OR†	95% CI†	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Total ω3† fat (DHA†, EPA†, and ALA†)												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.85	0.36–2.04	0.35	0.17–0.72	0.37	0.15–0.93	3.04	0.47–19.78	1.57	0.64–3.87	0.81	0.42–1.58
3	0.81	0.33–1.96	0.64	0.31–1.33	0.41	0.17–1.00	2.50	0.38–16.44	4.56	1.74–11.93	1.07	0.56–2.05
p for trend‡		0.63		0.22		0.05		0.39		0.01		0.85
Total long-chain ω3 fat (DHA, EPA)												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.49	0.20–1.19	0.51	0.25–1.05	0.80	0.32–2.01	0.99	0.17–5.66	1.10	0.43–2.81	0.70	0.38–1.28
3	0.30	0.11–0.77	0.64	0.31–1.36	0.53	0.21–1.31	2.21	0.41–11.88	3.45	1.41–8.46	0.93	0.50–1.70
p for trend		0.01		0.23		0.17		0.33		0.01		0.81
DHA												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.93	0.37–2.29	0.46	0.22–0.94	0.66	0.27–1.59	0.70	0.11–4.35	0.98	0.40–2.43	0.74	0.40–1.39
3	0.35	0.14–0.89	0.57	0.28–1.17	1.03	0.42–2.56	2.64	0.47–14.75	3.84	1.49–9.87	1.09	0.58–2.07
p for trend		0.03		0.10		0.99		0.20		0.01		0.81
ALA												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	1.65	0.68–4.00	0.50	0.24–1.04	0.75	0.31–1.83	4.01	0.69–23.18	0.40	0.16–0.99	0.78	0.45–1.34
3	1.29	0.53–3.12	0.66	0.31–1.39	0.54	0.22–1.30	0.88	0.15–5.18	1.92	0.76–4.85	0.92	0.53–1.60
p for trend		0.52		0.27		0.17		0.97		0.27		0.78
Total ω6† fat (LA†, AA†, DGLA†)												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.59	0.24–1.45	0.78	0.38–1.59	0.91	0.38–2.20	0.95	0.20–4.56	3.25	1.16–9.14	1.10	0.54–2.27
3	0.84	0.35–2.06	0.81	0.39–1.67	0.64	0.26–1.57	1.10	0.20–6.17	17.11	5.58–52.47	1.42	0.69–2.94
p for trend		0.70		0.57		0.33		0.92		0.01		0.35
Ratio of total ω3 to ω6 fat												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.46	0.19–1.13	1.05	0.51–2.15	0.43	0.17–1.06	1.55	0.28–8.67	0.43	0.17–1.07	0.67	0.37–1.19
3	0.82	0.34–1.98	0.85	0.41–1.78	0.65	0.27–1.57	2.46	0.49–12.51	0.14	0.05–0.38	0.70	0.38–1.19
p for trend		0.63		0.67		0.32		0.27		0.01		0.19
Ratio of long-chain ω3 to total ω6												
1	1.00		1.00		1.00		1.00		1.00		1.00	
2	0.68	0.28–1.67	0.83	0.40–1.71	0.70	0.29–1.69	0.52	0.09–3.07	1.02	0.41–2.51	0.80	0.51–1.27
3	0.48	0.19–1.21	0.78	0.38–1.60	0.55	0.22–1.35	1.35	0.25–7.26	0.32	0.13–0.82	0.65	0.41–1.03
p for trend		0.12		0.50		0.19		0.67		0.02		0.055

Ratio of DHA to total ω6														
1	1.00		1.00		1.00		1.00		1.00		1.00			
2	0.82	0.34–2.00	0.87	0.42–1.79	0.86	0.35–2.12	0.75	0.12–4.85	1.54	0.63–3.73	0.98	0.53–1.75		
3	0.52	0.21–1.29	0.66	0.33–1.36	1.04	0.43–2.50	2.84	0.51–15.87	0.31	0.12–0.80	0.77	0.42–1.42		
p for trend	0.16		0.26		0.93		0.19		0.02		0.42			
Ratio of ALA to total ω6														
1	1.00		1.00		1.00		1.00		1.00		1.00			
2	1.16	0.48–2.79	1.42	0.69–2.91	0.83	0.39–2.22	1.54	0.31–7.72	0.16	0.06–0.43	0.83	0.45–1.52		
3	1.25	0.51–3.03	1.00	0.48–2.12	0.67	0.28–1.65	1.62	0.32–8.21	0.10	0.04–0.28	0.66	0.36–1.21		
p for trend	0.63		0.97		0.40		0.56		0.01		0.17			

* Center-specific odds ratios are followed by 95% confidence intervals. Values are the result of unconditional logistic regression models adjusted for age (years), body mass index, nulliparity, family history of breast cancer, and age exceeding 35 years at first childbirth. Models for all centers pooled include study center as a random effects term in addition to the covariates included in the center-specific analyses. Tertile cutpoints are based on the total population within each center.

† OR, odds ratio; CI, confidence interval; ω3 = omega-3 = sum of α-linolenic, eicosapentanoic, and docosahexanoic acid; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; ALA, α-linolenic acid; ω6 = omega-6 = sum of linoleic, γ-linolenic, eicosapentanoic, dihomo-γ-linoleic, and arachidonic acids; LA, linoleic acid; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid.

‡ Test for trend based on 3-level ordinal variable assigning values of 1, 2, and 3 to fatty acid values falling within the first, second, and third tertiles within each center.

one. A positive association is seen in Berlin and Malaga, reaching significance in the latter center (table 2). Analyses restricted to only long-chain omega-3 fatty acid reveal a pattern similar to that for total omega-3. Alpha-linolenic acid shows an overall association with disease that is inverse in only two centers and positive in the rest.

Total omega-6 fatty acid shows a strong positive association with breast cancer in one center (Malaga; at a p for trend of 0.01), while demonstrating neutral or mildly inverse associations elsewhere. Results for linoleic acid were virtually identical to those for total omega-6 (data not shown). Pooling all centers (table 2, last column) yields little indication of an inverse relation with breast cancer for total or long-chain omega-3 and weak evidence of a positive association for omega-6 fatty acid.

Ratio of omega-3 to omega-6 fatty acid and breast cancer

Analyses based on the ratio of omega-3 to omega-6 fatty acids were carried out to evaluate the importance of the balance between these fats (table 2). The ratio of total omega-3 to omega-6 fatty acid yields an odds ratio below one for the upper tertile in four of five centers, reaching statistical significance in Malaga (odds ratio for the second and third tertiles relative to the lowest, 0.43 and 0.14; p for trend = 0.01). For long-chain omega-3, the ratio to omega-6 shows an inverse association in the same four centers, with evidence of dose-response in most centers and a statistically significant downward trend in Malaga. The relation for the ratio of alpha-linolenic acid to omega-6 is inconsistent across centers. A strong inverse association is seen in Malaga. In pooled analyses, the effect estimates for total omega-3, long-chain omega-3, and alpha-linolenic acid are all greater when considered in relation to omega-6 fat rather than by themselves. The association for long-chain omega-3 is the strongest statistically, with evidence of a dose-response pattern and a downward trend that approached nominal statistical significance (odds ratios = 0.80 and 0.65 for the second and third tertiles, respectively; p for trend = 0.05).

The results of models based on continuous fatty acid terms (not presented) were generally similar to those seen for the models based on tertiles.

DISCUSSION

This study found no significant inverse association between the simple percentage of total omega-3 fatty acids in adipose tissue and incident breast cancer in the general study population. Total omega-6 polyunsatu-

rate shows weak indications of an association with breast cancer when all centers are pooled. The association is largely driven by a single center (Malaga, Spain). Total and long-chain omega-3 polyunsaturates are also positively associated with breast cancer in this center. The ratio of omega-3 (and, in particular, long-chain omega-3) to omega-6 fat yielded more consistent results. An inverse association for the long-chain omega-3 to omega-6 ratio appeared in four of the five centers, with evidence of a downward trend across tertiles. With all centers pooled, this trend approached statistical significance.

A protective role of omega-3 fatty acids in breast cancer could stem from several hypothesized mechanisms (figure 1). The best known mechanism through which omega-3 fatty acids exert physiologic effects and hence could affect carcinogenesis is a modulation of eicosanoid metabolism. The predominant polyunsaturate in the typical Western diet is linoleic acid, a member of the omega-6 family. Linoleic acid can be converted to arachidonic acid, which in turn serves as the parent compound for eicosanoids that can powerfully affect cell function. Tumor cells typically produce large amounts of arachidonate-derived eicosanoids such as prostaglandin E2, which may have immunosuppressive properties (27). Increasing levels of omega-3 fatty acids inhibit the delta-5 and delta-6 desaturase pathways involved in the formation of arachidonic acid from linoleic acid. Long-chain omega-3 fatty acids compete directly with arachidonic acid for incorporation into cell membranes. They also compete for the enzyme pathways that give rise to eicosanoids, and through these pathways, EPA yields a family of metabolites with effects that are often different from those associated with arachidonate (28). Omega-3 fatty acids could thus inhibit tumor development and/or growth by both competitive inhibition of arachidonic acid metabolism and the effects of their own metabolites.

Other proposed anticarcinogenic mechanisms for omega-3 fatty acids include effects in three general areas: reduction of estradiol hydroxylase and circulating estrogen levels noted with high dietary fish oil intakes could block tumor promotion (28, 29), modification of hepatic phase I and phase II detoxification systems affecting carcinogen detoxification and/or activation (30), and lipid peroxidation products of these highly unsaturated fats producing direct cytotoxic effects on tumor cells (8). Additional potential roles of omega-3 fats in carcinogenesis include the suppression of fatty acid synthase activity and assorted effects on gene expression (31, 32).

Most of the relevant evidence regarding possible anticarcinogenic effects of omega-3 fatty acids in human populations comes from studies addressing fish consumption. The preponderance of ecologic studies supports an inverse relation of fish consumption with breast cancer (33, 34). While case-control and cohort studies are less consistent (35–37), many recent studies report a statistically significant negative association between estimated consumption of fish or other seafood and postmenopausal disease (38–42).

Three previous studies have investigated the association between breast cancer and omega-3 fatty acids by using adipose tissue omega-3 content as an indicator of long-term exposure, with mixed results (42–44). In the United States, London et al. (43) reported a nearly identical median percentage of long-chain omega-3 fatty acids (primarily DHA, with a small EPA component) in gluteal adipose tissue from postmenopausal cases and controls with nonproliferative breast disease. All quintiles of EPA yielded odds ratios below one, but the downward trend was not statistically significant. Petrek et al. (44) examined abdominal and mammary adipose tissue in New York women with breast cancer and in controls undergoing breast biopsy and reported that neither tissue displayed significant case-control differences with regard to omega-3 fatty acids. In both of the US studies, the use of women with breast disease as controls could be problematic if omega-3 fatty acids protect against nonproliferative breast disease, although such activity is not established. In Finland, Zhu et al. (42) found lower levels of the long-chain, marine-origin fatty acid EPA in triglycerides of mammary adipose tissue samples taken from postmenopausal cases than in tissue taken from women with benign breast disease. In contrast, mammary phospholipids of cases had significantly lower DHA and nonsignificantly lower EPA. None of these studies found an association between omega-6 fatty acid and breast cancer, and none specifically addressed the balance between omega-3 and omega-6 fat.

Anticarcinogenicity through competitive inhibition of omega-6 fatty acid metabolism implies that the absolute amount of omega-3 fatty acid taken in by an individual should be less important than the balance between omega-3 and omega-6 intake. If intake of omega-6 is high, a larger amount of omega-3 is needed to offset that intake. Most of the postulated mechanisms of action proposed for omega-3 fatty acids also emphasize the role of the long-chain fatty acids associated with marine oils. This is compatible with the finding that an inverse association with breast cancer is observed most consistently for analyses based on long-chain rather than total or medium-chain omega-3 fatty acids and is stronger for the ratio of omega-3 to

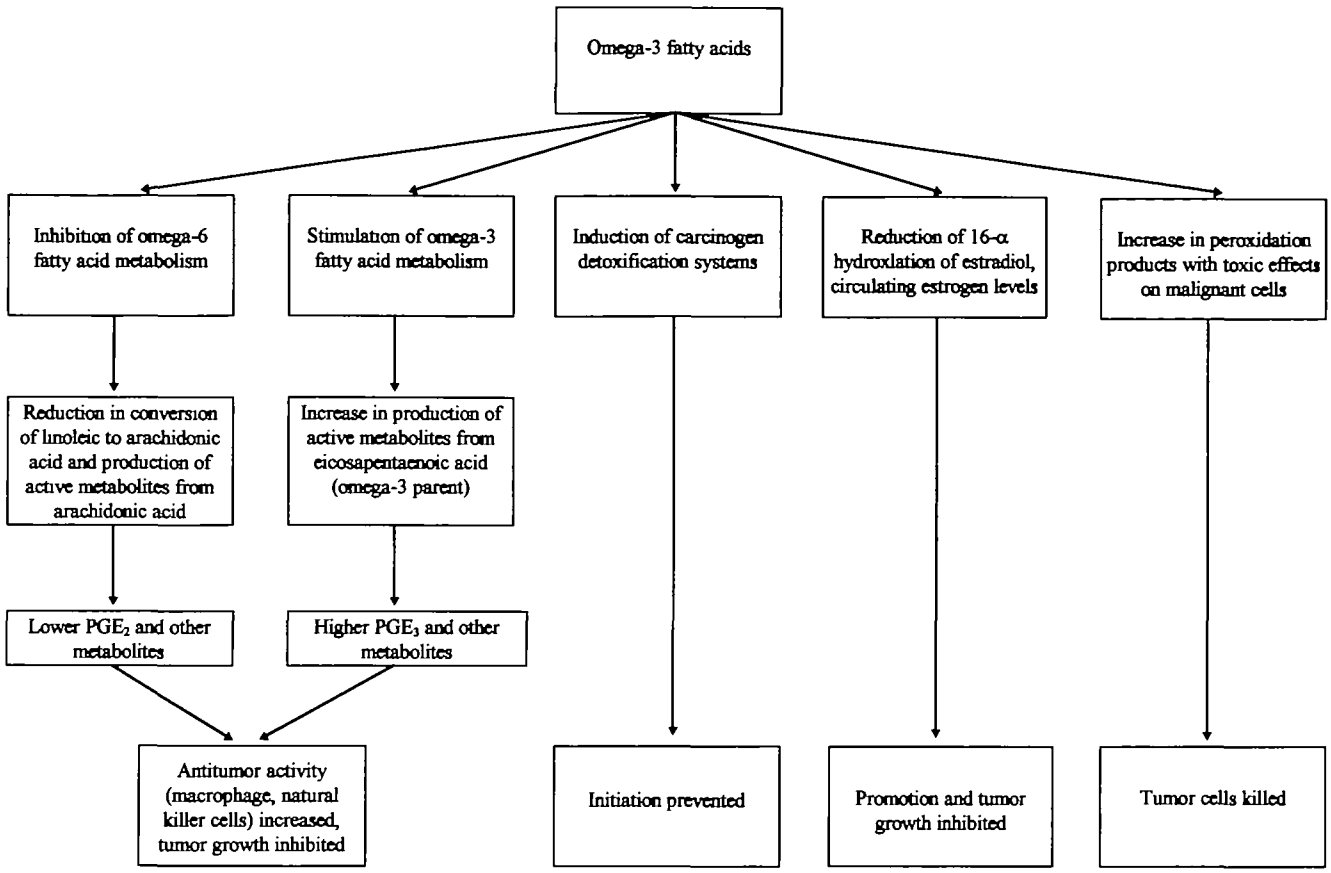

FIGURE 1. Hypothesized mechanisms for anticarcinogenic effects of omega-3 fatty acids, the EURAMIC Study, 1991–1992.

omega-6 than for the simple percentage of omega-3 fatty acids in adipose tissue.

The strong positive association between breast cancer and total omega-6 polyunsaturates noted in the Spanish study center is consistent with laboratory evidence supporting an association between linoleic acid exposure and mammary malignancy. The positive association with omega-3 polyunsaturates noted in this center is, however, at odds with the laboratory evidence. Spanish populations consume relatively large amounts of olive oil (45, 46), which appears protective in many southern European population studies (35, 38, 45–47), although not all (48, 49). Increased consumption of the monounsaturate oleic acid as a result of a diet rich in olive oil could drive down the proportions of polyunsaturates in fat stores. If an olive oil-rich diet is protective and is strongly inversely associated with omega-3 stores, this could produce the positive association between omega-3 fat stores and breast cancer. A similar olive oil effect could have shaped the omega-6 results. The ratio of omega-3 to omega-6 fat still exhibits an inverse relation with disease in this center, despite both polyunsaturated fat families showing significant positive associations with breast cancer.

Interpretation of the findings of this study depends upon the viability of adipose tissue as a measure of exposure. Adipose tissue is thought to best reflect dietary exposures for the essential fatty acids, which cannot be endogenously synthesized (with the polyunsaturates linoleic, α-linolenic, and long-chain omega-3 fatty acids being of prime importance). For these fatty acids–the focus of the current analyses–adipose tissue represents a stable, long-term reservoir that integrates exposure levels over time. Studies indicate a typical 2-year half-life for polyunsaturated fatty acids in adipose tissue (50–52). Intake estimated from dietary records and adipose tissue polyunsaturated fat shows significant correlations, even without correction for attenuation due to measurement error (52, 53). Two major validation studies conducted among European populations assessed the agreement between dietary and adipose-based polyunsaturate measures after adjustment for measurement error (54, 55). In a comparison of estimates based on fat biopsies and 14-day dietary records among a Danish population, adjusted Pearson correlation coefficients reached 0.57 for linoleic acid and 0.80 for DHA (54). A Dutch study comparing multiple 24-hour recalls over the course of a year with adipose tissue reported an adjusted coef-

ficient of 0.77 for linoleic acid; it did not examine correlations for omega-3 fatty acids (55). Direct measurement of concentrations in storage tissue avoids potential bias associated with dietary recall techniques and is particularly important for substances such as the omega-3 fatty acids, whose concentrations in specific foods vary greatly by locality and time (56).

Major differences in rate of fat accretion or recent weight loss could compromise the utility of adipose tissue-based exposure measures. To avoid these problems, we excluded women with recent weight loss exceeding 5 kg and corrected for body mass index differences. Other precautions against bias included the use of standardized adipose sampling techniques and storage conditions and the maintenance of blinding during laboratory analyses.

The use of extensive exclusion criteria to guard against factors potentially distorting the results of tissue-based measures of exposure theoretically limits the generalizability of this study's results. It is possible that the relation between polyunsaturated fats and breast cancer differs fundamentally for more aggressive or metastatic disease, for example, although the literature provides no clear reason to suspect this. Taken together, the exclusions would tend to increase the similarity of the case and control populations and to reduce the spectrum of disease seen among cases. The most likely effect would thus be a bias toward no association.

The lower response rate for controls presents another potential source of bias. If control selection favored individuals with "healthier" habits also associated with higher omega-3 or omega-3 to omega-6 ratios, the observed results might have arisen through selection bias. However, the two strongest inverse associations observed between the ratio of long-chain omega-3 to omega-6 fat and breast cancer occurred in the centers with the highest control response rates (Malaga and Zeist), suggesting that any selection bias that might have occurred probably acted to weaken the observed results. Controls were drawn from general practitioners' listings in three centers and population registries in the others (Berlin and Zurich). While this is a potential limitation if differing control sources influenced the results for specific centers, no systematic difference in results with the method of control selection was observed.

In summary, although percentages of omega-3 fatty acid in adipose fat did not differ significantly between cases and controls in this study, the ratio of omega-3 to omega-6 fatty acids demonstrated a mild inverse association with breast cancer in four of five study centers. The most consistent evidence of a trend across increasing tertiles was seen for the long-chain omega-3 fat typical of seafood. The strongest associations observed in any single center, however, were the positive associations with total omega-3 and total omega-6 fatty acid seen in the Spanish study center. This pattern was not observed elsewhere and may, at least in part, derive from high olive oil intakes in that population. The results of this study, while not definitive, underscore the need for further study of the balance between fatty acids in the diet and tissue stores as potential determinants of breast cancer risk.

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