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FATTY-ACID COMPOSITION IN SERUM PHOSPHOLIPIDS AND RISK OF BREAST CANCER: AN INCIDENT CASE-CONTROL STUDY IN SWEDEN

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The study of the relationship between dietary intake of fatty acids and the risk of breast cancer has not yielded definite conclusions with respect to causality, possibly because of methodological issues inherent to nutritional epidemiology. To evaluate the hypothesis of possible protection of n-3 polyunsaturated fatty acids (PUFA) against breast cancer in women, we examined the fatty-acid composition of phospholipids in pre-diagnostic sera of 196 women who developed breast cancer, and of 388 controls matched for age at recruitment and duration of follow-up, in a prospective cohort study in Umeå, northern Sweden. Individual fatty acids were measured as a percentage of total fatty acids, using capillary gas chromatography. Conditional logistic-regression models showed no significant association between n-3 PUFA and breast-cancer risk. In contrast, women in the highest quartile of stearic acid had a relative risk of 0.49 (95% confidence interval, 0.22–1.08) compared with women in the lowest quartile (trend $p = 0.047$), suggesting a protective role of stearic acid in breast-cancer risk. Besides stearic acid, women in the highest quartile of the 18:0/18:1 n-9c ratio had a relative risk of 0.50 (95% confidence interval, 0.23–1.10) compared with women in the lowest quartile (trend $p = 0.064$), suggesting a decrease in breast-cancer risk in women with low activity of the enzyme delta 9-desaturase (stearoyl CoA desaturase), which may reflect an underlying metabolic profile characterized by insulin resistance and chronic hyper-insulinemia. *Int. J. Cancer* 83:585–590, 1999.
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Data derived from animal experiments indicate that the tumor-promoting properties of high-fat diets may be more a function of differences in fatty-acid composition than of fat content *per se* or of total caloric intake. In several animal models, high-fat diets rich in n-6 polyunsaturated fatty acids (PUFA) generally stimulated mammary-tumor development and metastasis, whereas diets rich in n-3 PUFA appeared to inhibit tumor growth and metastasis (Fay *et al.,* 1997). However, the promotion phase of chemically induced carcinogenesis has been shown to be significantly suppressed only when equal parts of high-fat diets rich in n-6 and n-3 long-chain PUFA were fed (Fay *et al.,* 1997).

Epidemiological studies in which fat intake has been assessed in individuals by questionnaire methods have generally provided weak or no support for the hypothesis that dietary intake of n-3 PUFA might protect against breast cancer. Although some case-control studies (Ingram *et al.,* 1991; Landa *et al.,* 1994; Franceschi *et al.,* 1995; Braga *et al.,* 1997) showed inverse associations between breast-cancer risk and consumption of fish rich in long-chain n-3 PUFA, these findings were not confirmed by several prospective cohort studies (Vatten *et al.,* 1990; Toniolo *et al.,* 1994). Pooled analyses of multiple case-control (Howe *et al.,* 1990) or cohort studies (Hunter *et al.,* 1996) showed no association between intake of PUFA and the risk of breast cancer. In none of these studies, however, was any distinction made between n-6 and n-3 PUFA. Furthermore, conclusive evidence for a role of individual fatty acids in breast-cancer risk may be precluded by the many methodological limitations in measurements of dietary intake of fatty acids.

Beyond food-questionnaire methodology, objective data points on consumption of fish can be obtained from long-chain n-3 PUFA analysis of serum or plasma phospholipids, which have been shown to accurately reflect recent dietary intake of long-chain fatty acids from fish (Bjerve *et al.,* 1993; Ma *et al.,* 1995). Therefore, measurement of serum phospholipid fatty acids may be appropriate for examining whether the type of PUFA is related to breast-cancer risk.

To test the hypothesis that n-3 PUFA may have a protective role against breast cancer, we conducted a prospective cohort study in Umeå, northern Sweden, in which we compared the fatty-acid composition of serum phospholipids of women who developed breast cancer and that of a sub-set of cohort members who did not. Main findings were an absence of association between breast-cancer risk and phospholipid levels of n-3 PUFA of marine origin. A less expected finding, however, was a negative association of risk with level of stearic acid (18:0) and with the ratio 18:0/18:1n-9c, and a positive association with level of palmitic acid (16:0).

MATERIAL AND METHODS

Cohort studies

Data for this study have been collected in 3 ongoing cohort studies in the town of Umeå and its surroundings in northern Sweden. The Västerbotten Intervention Project (VIP) began in 1986, and comprised a total of 24778 men and 27168 women by the end of May 1997. The northern Sweden component of the WHO multinational study for Monitoring of Trends and Cardiovascular Disease study (MONICA) comprises 2507 men and 2540 women recruited in 1986, 1990 and 1994. The Mammary-Screening Project (MSP) started in 1995, and by the end of May 1997 17486 women were involved.

Study population

Originally, 624 women (208 cases and 416 referents) were selected for this study: 2 referents for each case were randomly selected from the corresponding cohort and matched for age, age of blood sample and sampling centre. Owing to lack of blood samples, 40 subjects were excluded from the study. The final study population with blood samples was thus 584 women (196 cases and 388 referents). The VIP contributed 103 cases and 214 referents, the MONICA, 9 cases and 6 referents, and the MSP, 84 cases and 168 referents. The number of cases with time between sample collection and date of diagnosis less than 3 months were 6 in the VIP, none in the MONICA and 72 in the MSP.

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Reproductive-history data

Data concerning reproductive history were not systematically collected at baseline. A questionnaire was therefore sent out retrospectively, in November 1997, to all cases with breast cancer within the 3 cohorts, and to the selected referents, and a 85% response rate was obtained (169 cases and 324 referents) (Table I). The questionnaire covers information on age at menarche (years), number of full-term pregnancies, age at first full-term pregnancy (years), menopausal status, age at menopause (years), use of oral contraception and use of hormone-replacement therapy. Among the subjects, 15 women died (14 cases and one referent) and 8 moved out of the study area, before the closure date of recruitment for cases and referents.

Baseline questionnaire information

In the VIP and MONICA cohorts, information on dietary habits, working conditions and social factors were collected at baseline by questionnaires. For the MSP, questionnaire information was obtained only for reproductive factors. In the VIP and MONICA cohorts, weight was measured with light indoor clothing without shoes, and height was measured by a graded scale fixed to the wall (Table I). In the MSP, height and weight were self-reported.

Blood sampling

In each of the 3 studies, 20 ml of blood was collected at baseline from every subject; 10 ml was collected with heparin and 10 ml with EDTA, as anti-coagulants. The blood was then aliquoted into 10 tubes: 6 tubes with plasma, 2 with buffy coat and 2 with erythrocytes, and stored at −80°C. For the VIP cohort, 95% (94 cases, 204 referents) of the subjects had fasted for at least 4 hr and 58% (52 cases, 129 referents) had fasted for more than 8 hr before giving blood samples. In the MONICA project, 94% (6 cases, 8 referents) had fasted for at least 4 hr. For the MSP, subjects were not required to report in fasting state, and only a very small proportion was actually fasting in this cohort. The study was approved by the ethical committee of Umeå University, and all study participants gave their informed consent for future use of blood samples for research purposes.

Follow-up

Incident cases of breast cancer from baseline up to end of May 1997 were reported through linkage with the regional cancer registry covering the northern region of Sweden, complemented by linkage with the National Cancer Registry covering the whole of Sweden. The Swedish unique personal identification number was used for linkage. Follow-up for vital status (death), or losses to follow-up due to migration from the country, were also determined for the whole population through local and national population registries.

Entry and statistical analysis of data

Data collected from the questionnaire were coded, and edited with SPSS, and descriptive statistics were also obtained with SPSS. Conditional logistic-regression analysis was performed using the SAS system (Breslow and Day, 1980). Odds ratios and 95% confidence intervals (CI) were calculated to estimate the relative risk for quartiles as well as for continuous variables. Tests for trend were performed by using the means within each category in the logistic-regression model. Quartile cut points were determined by distribution of the fatty-acid levels among the referents, and the lowest quartile was used as the reference category. Potential confounding effects of age at menarche, parity, age at first full-term pregnancy, use of hormones and menopausal status were adjusted for in multivariate logistic-regression models. Body-mass index (BMI) and age at menarche were kept as continuous data, whereas parity and age at first full-term pregnancy were categorized according to tertiles. Use of hormone-replacement therapy was classified as use of hormones during or after menopause, ''yes'' or ''no''. When information on age at menopause was uncertain (21 women) or not obtained at all (91 women), the women were categorized as pre-menopausal when younger than 51 years and post-menopausal at 51 years and older at the time of blood collection. Women whose age for menopause occurred the same year as the blood sampling were classified as pre-menopausal (11 women).

Analysis of fatty acids in serum phospholipids

Total lipids were extracted from serum samples (300 µl) with 6 ml of chloroform-methanol 2:1 (v:v) (Folch *et al.*, 1957) containing 100 µl of anti-oxidant butylated hydroxytoluene (BHT, 1 mg/ml in methanol) and 100 µl of di-12:0 PC (0.05 mM/l in ethanol). The extract was washed with 1.5 ml NaCl and the mixture was allowed to separate into 2 phases by standing. The separated chloroform layer was transferred to a tube and evaporated to dryness under nitrogen. The lipid extract was dissolved in 200 µl of chloroform-methanol 2:1 and directly used for the column procedure.

Phospholipids were purified by adsorption chromatography on silica tubes. The column was treated with 2 ml chloroform-methanol 2:1. Then the lipid sample was applied to the column and the tube was washed with 100 µl of this same system solvent added to the column. Neutral lipids were eluted with 5 ml of chloroform and phospholipids with 8 ml of methanol. Methanol fractions containing phospholipids were collected in screw-cap tubes with a Teflon seal. Fractions obtained from the column procedure were evaporated to dryness. Dichloromethane (80 µl) was added to dissolve the lipids, followed by 25 µl Methyl-Prep II to convert the fatty acids to their methyl esters. The mixture was incubated for 10 min at room temperature. Fatty-acid methyl esters (FAME) were extracted twice into hexane, which was evaporated to about 50 µl and dissolved in 1 ml iso-octane.

TABLE I – CHARACTERISTICS OF STUDY POPULATION

Variables	Cases (n 196)				References (n 388)			
	Median	Mean	Missing[1]	25th and 75th Percentiles	Median	Mean	Missing[1]	25th and 75th Percentiles
Age (years)	55	55	0	(50–60)	55	55	0	(50–60)
Reproductive variables[2]								
Age at menarche (years)	13	13	0	(12–14)	13	13	6	(12–14)
Parity	2	2	0	(2–3)	2	2	12	(2–3)
Age at first full-term pregnancy	24	24	2	(21–27)	24	24	12	(21–26)
Lactation (months)	8	10	4	(5–14)	9	11	23	(4–16)
Age at menopause	50	50	8	(47–52)	50	50	13	(48–53)
Anthropometry[3]								
Weight (kg)	66	67	15	(60–74)	66	68	37	(60–74)
Height (cm)	1.64	1.63	14	(1.60–1.67)	1.63	1.63	28	(1.59–1.67)
BMI (kg/m^2)	24	25	17	(23–27)	25	26	39	(23–28)

[1]True missing among those who answered questionnaires.–[2]169 cases, 324 referents (covered by retrospective reproductive questionnaire).–[3]179 cases, 345 referents (covered by base-line anthropometric questionnaire).

FAME composition was determined by capillary gas chromatography: 0.5 µl was injected through an on-column injector at 65°C into a gas chromatograph with the aid of an automatic injector (Hewlett Packard, Palo Alto, CA) operated at column and detector temperatures of 220°C and 250°C respectively. A 30-m × 0.32-mm I.D. fused silica capillary column with 0.25-µm film thickness was used for the separation of FAME (Supelco, Bellefonte, PA). Helium was used as carrier gas at a flow rate of 1 ml/min, with N2 as make-up gas for the flame ionization detector. The temperature was programmed to rise from 65 to 135°C at a rate of 5°C/min, from 135 to 200°C at a rate of 2°C/min, and from 200 to 220°C at a rate of 3°C/min, then kept constant for 10 min. Identification of FAME was obtained by comparison with the relative retention times of pure standard mixtures (Sigma, St. Louis, MO). The relative amount of each fatty acid (percent of total area) was quantified by integrating the peak and dividing the results by the total area for all fatty acids.

Within-day coefficients of variation (CV) were based on the analysis of 10 serum samples, all extracted and analyzed during the same day. CV ranged from 0.45% for large peaks to 3.4% for the smallest peaks. The between-day CV were based on the analysis of one independent serum control on separate days (up to 58 days). CV (n = 58) for major fatty acids were 2.34 for 16:0, 1.77 for 18:0, 1.36 for 18:1 n-9, 1.01 for 18:2 n-6, 3.60 for 20:4 n-6, 3.98 for 18:3 n-3, 3.62 for 20:5 n-3 and 3.17 for 22:6 n-3.

RESULTS

Characteristics of patients

Table I shows the characteristics of the 196 cases and their matched controls. Clinical characteristics were not different between cases and referents.

Fatty-acid composition of serum phospholipids

The mean values of fatty acids in serum phospholipids are given in Table II. The major fatty acids in serum phospholipids were palmitic acid (16:0), linoleic acid (18:2 n-6), stearic acid (18:0), oleic acid (18:1 n-9c), arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3). These fatty acids constituted more than 85% of the total area. Peaks accounting for less than 1% of the total area were detected and quantified. For each individual fatty acid, we observed wide variability among subjects. Mean values of fatty acids expressed as percent of total area were not different between case and control subjects.

Relative risks (odds ratio) of breast cancer by serum phospholipid fatty-acid levels

Fatty acids were considered at individual level for major fatty acids, as well as total saturates, total mono-unsaturates and total PUFA of each series.

No significant associations were found between risk of breast cancer and individual n-3 PUFA of marine origin (20:5 n-3, 22:6 n-3) or total n-3 PUFA (Table III).

Among n-6 PUFA, neither linoleic acid nor long-chain n-6 PUFA levels were associated with breast-cancer risk. Furthermore, no relationship was found between risk and level of individual mono-unsaturated fatty acids, or total mono-unsaturates.

Among individual saturated fatty acids, levels of palmitic acid (16:0) and stearic acid (18:0) were linked to breast-cancer risk. When adjusting for several risk factors (age at menarche, age at first full-term pregnancy, number of children, use of hormone-replacement therapy, height and weight), women in the highest quartile of 16:0 had a relative risk of 2.09 (95% CI, 0.95–4.63) compared with women in the lowest quartile (trend $p = 0.043$). In contrast, women in the highest quartile of 18:0 had a relative risk of 0.49 (95% CI, 0.22–1.08) compared with women in the lowest quartile (trend $p = 0.047$). The ratio of stearic acid to oleic acid (18:1 n-9c) was also associated with the risk of breast cancer: women in the highest quartile had a relative risk of 0.50 (95% CI, 0.23–1.10) compared with women in the lowest quartile (trend $p = 0.064$).

Calculations were done also for sub-groups according to menopausal status (pre- and post-menopausal women). In each subgroup, the same tendency remained for the effect of high palmitic-acid level as a risk and for a protective effect of stearic acid, but was somewhat inconsistent (data not shown).

TABLE II – FATTY-ACID COMPOSITION OF SERUM PHOSPHOLIPIDS IN CASES AND CONTROLS

Fatty acids	Controls (n 390)		Cases (n 196)	
	Mean (%)	Range	Mean (%)	Range
Saturates				
14:0	0.37	0.11–0.98	0.38	0.16–0.93
16:0	26.68	23.67–32.13	26.90	21.84–31.42
18:0	13.94	10.16–18.89	13.74	9.79–21.27
Total[1]	41.62	37.56–47.84	41.67	38.60–51.62
Mono-unsaturates				
16:1 n-7	0.85	0.20–2.30	0.85	0.40–2.40
18:1 n-9t	0.29	0.04–0.98	0.31	0.04–1.31
18:1 n-9c	10.77	7.44–15.32	10.91	7.83–21.35
18:1 n-7c	1.59	0.18–3.25	1.61	0.92–2.89
Total[2]	13.76	9.72–19.34	13.93	10.07–26.12
n-6 PUFA				
18:2 n-6c	22.45	11.48–31.13	22.60	14.69–30.30
18:3 n-6c	0.08	0.0–0.29	0.08	0.0–0.40
20:2 n-6c	0.37	0.18–0.87	0.36	0.17–0.72
20:3 n-6c	3.24	1.77–5.61	3.27	1.70–5.63
20:4 n-6c	9.51	5.55–14.30	9.40	5.68–13.18
22:4 n-6c	0.28	0.03–1.43	0.28	0.02–0.64
22:5 n-6c	0.18	0.10–0.42	0.19	0.10–0.39
Total n-6	35.94	24.23–42.02	36.0	25.21–43.29
n-3 PUFA				
18:3 n-3c	0.30	0.11–0.71	0.31	0.13–0.76
20:5 n-3c	1.74	0.47–12.12	1.62	0.47–6.73
22:5 n-3c	1.16	0.64–1.83	1.14	0.58–1.84
22:6 n-3c	5.38	2.33–9.85	5.29	2.35–9.69
Total n-3	8.55	4.44–22.43	8.36	4.51–16.36

[1]Included: 14:0, 15:0, 17:0, and 20:0.–[2]Included: 20:1 n-9c.

DISCUSSION

In this study, we examined whether n-3 PUFA of marine origin may protect against the risk of breast cancer. For this purpose, n-3 PUFA (20:5 n-3, 22:6 n-3) levels of serum phospholipids were used as a biomarker of dietary intake of n-3 PUFA, to investigate the relation between exposure to n-3 fatty acids and breast cancer. We compared the fatty-acid composition of phospholipids in prediagnostic sera of 196 women who developed breast cancer and of 388 controls matched for age at recruitment and duration of follow-up, in a prospective cohort study in Umeå, northern Sweden. Contrary to our starting hypothesis, no significant association between n-3 PUFA and breast-cancer risk was found. In contrast, we found that individual saturated fatty acids were associated with risk. Women in the highest quartile for palmitic acid (16:0) had an increased risk compared with women in the lowest quartile, with an OR of 2.09, while the OR for breast cancer among women in the highest quartile of stearic acid (18:0) compared with the lowest quartile was 0.49, suggesting a protective role of stearic acid. Moreover, women in the highest quartile of the 18:0/18:1 n-9c ratio had a relative risk of 0.50 compared with women in the lowest quartile, suggesting a decrease in breast-cancer risk in women with low activity of the enzyme delta 9-desaturase (stearoyl CoA desaturase).

Several experimental findings on stearic acid or on the ratio 18:0/18:1n-9c and breast cancer fit well with our observation. Decreased level of stearic acid in red-cell membranes, as well as

TABLE III – ESTIMATED RELATIVE RISK (ODDS RATIO, CRUDE AND ADJUSTED*) OF BREAST CANCER AND 95% CIs BY PHOSPHOLIPID FATTY-ACID LEVELS OF SERUM SAMPLES OBTAINED FROM THE WHOLE POPULATION (n 584)

Fatty acids	OR (95% CI)				*p* for trend
	1st quartile (low)	2nd quartile	3rd quartile	4th quartile	
Saturates					
14:0	1.00	1.16 (0.71–1.90)	1.04 (0.62–1.77)	1.02 (0.57–1.82)	0.995
		1.37 (0.70–2.70)*	1.24 (0.59–2.62)	1.21 (0.56–1.02)	0.679
16:0	1.00	0.60 (0.35–1.03)	1.20 (0.73–1.98)	1.26 (0.74–2.11)	0.138
		0.88 (0.43–1.79)*	1.60 (0.77–3.30)	2.09 (0.95–4.63)	0.043
18:0	1.00	0.59 (0.36–0.98)	0.48 (0.28–0.82)	0.59 (0.34–1.01)	0.040
		0.52 (0.25–1.07)*	0.34 (0.16–0.75)	0.49 (0.22–1.08)	0.047
Total	1.00	1.10 (0.66–1.84)	1.38 (0.81–2.31)	1.02 (0.56–1.85)	0.770
		0.97 (0.45–2.09)*	0.95 (0.43–2.10)	1.15 (0.46–2.85)	0.760
Mono-unsaturates					
16:1 n-7	1.00	0.76 (0.46–1.25)	0.82 (0.49–1.36)	0.73 (0.41–1.32)	0.344
		0.74 (0.38–1.47)*	0.77 (0.37–1.58)	0.69 (0.30–1.56)	0.384
18:1 n-9t	1.00	0.75 (0.43–1.29)	1.03 (0.58–1.84)	0.99 (0.50–1.96)	0.868
		0.64 (0.31–1.32)*	0.91 (0.41–2.03)	0.55 (0.20–1.51)	0.339
18:1 n-9c	1.00	1.29 (0.76–2.18)	1.29 (0.75–2.20)	1.48 (0.84–2.61)	0.200
		1.49 (0.73–3.05)*	0.82 (0.39–1.71)	2.25 (0.98–1.02)	0.205
18:1 n-7c	1.00	1.13 (0.67–1.91)	1.49 (0.86–2.58)	1.38 (0.76–2.52)	0.250
		0.98 (0.49–1.98)*	1.36 (0.65–2.84)	0.89 (0.36–2.20)	0.986
Total	1.00	1.09 (0.65–1.85)	1.01 (0.59–1.73)	1.38 (0.79–2.41)	0.295
		0.87 (0.81–3.92)*	0.65 (0.33–1.29)	1.78 (0.81–3.92)	0.371
n-6 PUFAs					
18:2 n-6	1.00	0.92 (0.56–1.52)	1.01 (0.61–1.67)	1.16 (0.70–1.93)	0.579
		1.22 (0.60–2.46)*	1.25 (0.62–2.51)	1.41 (0.67–2.94)	0.367
18:3 n-6	1.00	0.95 (0.58–1.55)	0.78 (0.46–1.33)	0.69 (0.40–1.19)	0.158
		0.95 (0.48–1.91)*	0.77 (0.35–1.66)	0.51 (0.23–1.14)	0.111
20:4 n-6	1.00	0.73 (0.45–1.18)	0.72 (0.44–1.20)	0.84 (0.55–1.37)	0.443
		0.49 (0.24–0.99)*	0.48 (0.22–1.04)	0.51 (0.24–1.09)	0.091
Total	1.00	1.06 (0.63–1.78)	1.42 (0.84–2.37)	0.88 (0.50–1.55)	0.959
		1.27 (0.60–2.68)*	1.38 (0.66–2.88)	0.91 (0.40–2.06)	0.939
n-3 PUFAs					
18:3 n-3	1.00	1.35 (0.81–2.26)	1.12 (0.66–1.91)	1.44 (0.83–2.50)	0.300
		1.31 (0.64–2.70)*	1.42 (0.68–2.95)	1.36 (0.63–2.96)	0.424
20:5 n-3	1.00	0.65 (0.40–1.05)	0.64 (0.39–1.05)	0.66 (0.41–1.09)	0.114
		0.52 (0.26–1.04)*	0.66 (0.34–1.29)	0.51 (0.25–1.03)	0.081
22:6 n-3	1.00	1.23 (0.77–1.97)	1.02 (0.61–1.71)	0.94 (0.56–1.58)	0.663
		1.50 (0.75–3.00)*	0.86 (0.42–1.77)	0.92 (0.42–2.02)	0.405
Total	1.00	0.87 (0.53–1.43)	1.00 (0.61–1.63)	0.77 (0.46–1.28)	0.398
		0.73 (0.36–1.51)*	0.92 (0.46–1.85)	0.58 (0.27–1.28)	0.281
Ratios					
20:5n-3/20:4n-6	1.00	1.20 (0.74–1.93)	0.97 (0.58–1.63)	0.91 (0.54–1.53)	0.528
		1.46 (0.75–2.83)*	1.52 (0.72–3.24)	0.88 (0.42–1.86)	0.591
18:2n-6/20:4n-6	1.00	1.04 (0.64–1.69)	1.26 (0.77–2.05)	1.21 (0.73–2.01)	0.396
		1.53 (0.76–3.07)*	1.22 (0.66–2.42)	1.95 (0.90–4.24)	0.135
18:0/18:1n-9c	1.00	0.74 (0.45–1.20)	0.76 (0.46–1.26)	0.55 (0.32–0.95)	0.040
		0.63 (0.31–1.29)*	0.48 (0.23–1.02)	0.50 (0.23–1.10)	0.064

*Adjusted for age at menarche, age at first full-term pregnancy, number of children, use of hormone-replacement therapy, height and weight.

increased level of oleic acid, have been reported for patients with cancers at different sites (Wood *et al.*, 1985; Kelly *et al.*, 1990; Persad *et al.*, 1990; Pandey *et al.*, 1995). In addition, a prospective study of survival of breast-cancer patients showed a strong negative association between risk of metastases and stearic-acid level in membrane phosphatidylcholine in the primary tumor (Bougnoux *et al.*, 1992).

The inverse association of breast-cancer risk with stearic acid and with the ratio 18:0/18:1 n-9c is not known. A possible interpretation is that stearic acid is directly involved in the inhibition of tumor development. Stearic acid had already been found to inhibit *in vitro* proliferation of various human cancer cell lines (Fermor *et al.*, 1992), including mammary tumor cells, and additional observations suggest that stearic acid may inhibit epidermal-growth-factor(EGF)-induced breast-cancer cell growth (Wickramasinghe *et al.*, 1996). Furthermore, parenteral administration of stearic acid in a chemically induced mammary-tumor model in rats delayed mammary-tumor development (Habib *et al.*, 1987). However, the direct effects of dietary stearic acid on breast cancer warrant further experimental studies.

If we assume that a low stearic-acid level in plasma lipids or cellular membranes was indeed a direct cause of breast cancer in our cohort, the next question is which factors might have caused the decrease in stearic-acid level. Although dietary fatty acids are known to influence serum phospholipid fatty-acid levels, no association, or only weak associations, have been reported between levels of saturated fatty acids in plasma phospholipids and dietary intake levels (Ma *et al.*, 1995). In fact, except for the essential fatty acids, linoleic acid and alpha-linolenic acid, which must be obtained from diet, all other fatty acids in serum lipids may come either from diet or from *de novo* endogenous synthesis. Furthermore, all fatty acids can undergo modifications via desaturation, elongation, retroconversion and/or oxidation. Most stearic acid comes either directly from diet or from elongation of palmitic acid, which is also synthesized *de novo* in considerable amounts. In this regard, the contribution of dietary stearic acid to its level in serum

phospholipid is difficult to evaluate. We have no information on estimated dietary intake of stearic acid in our population. Therefore, the possibility that a low level of stearic acid reflects reduced dietary intake of stearic acid warrants further studies.

Lowered stearate in blood cells of patients with malignancies was proposed as being the consequence of increased delta-9 desaturation to oleic acid (Wood *et al.,* 1985), but the reason for an increase in the enzyme activity is not known. The delta-9 desaturase enzyme or stearoyl-CoA desaturase enzyme is encoded by the SCD-gene family. Various fatty acids have been shown to regulate, *in vitro* and *in vivo*, expression of the SCD-1 gene in liver (Ntambi, 1995). Administration of sterculic acid, an uncommon fatty acid from plants which inhibits delta-9 desaturase, caused a decrease in the ratio of oleic acid to stearic acid in peripheral red cells, serum and liver of rats bearing NMU-induced mammary tumors, and inhibited tumor growth (Khoo *et al.,* 1991). These results suggest that delta 9-desaturase activity may be directly involved in cancer development; but it is also possible that the inhibition of enzyme activity is merely a correlated phenomenon, without any mechanistic relation to tumor development.

Besides fatty acids, insulin is a well-documented regulator of stearoyl-CoA desaturase activity (Ntambi, 1995). Direct evidence for a regulatory effect of insulin comes from studies showing insulin-stimulation of SDC-1-gene expression in liver of diabetic mice (Waters and Ntambi, 1996), in mammary glands of normal mice (Kaput *et al.,* 1994), in cultured hepatocytes (Legrand *et al.,* 1994) and adipocytes (Weiner *et al.,* 1991). These various observations suggest that decreased serum phospholipid levels of stearic acid, increased levels of oleic acid and decreased 18:0/18:1n-9 ratio, associated with increased risk of breast cancer in our cohort, might reflect an underlying metabolic profile characterized by chronic hyper-insulinemia.

Contrary to our starting hypothesis, we found no significant association of breast-cancer risk either with total n-3 polyunsaturated fatty acids or with long-chain n-3 fatty acids of marine origin. The relatively wide ranges of variation in both EPA (0.47–12.12%, controls and cases combined) and DHA (2.33–9.85%) appear to rule out the possibility that inter-individual differences in n-3 PUFA intake were too small to allow a decrease in risk to appear. Our results are similar to those of another prospective cohort study, in Norway, which also reported no association of breast-cancer risk with n-3 PUFA levels in serum phospholipids (Vatten *et al.,* 1993). The use of adipose-tissue fatty-acid composition as a biomarker of dietary fatty acids showed an inverse association between the ratio of long-chain n-3 PUFA to n-6 PUFA and breast-cancer risk, in an ecological study conducted in 5 European countries, reinforcing the hypothesis that the degree of inhibition of fatty acids of the n-3 on breast cancer may depend on levels of n-6 PUFA (Simonsen *et al.,* 1998). However, in our population, we found no significant association between the ratio 20:5 n-3/20:4 n-6 or the ratio n-3 PUFA/n-6 PUFA and breast-cancer risk (data not shown).

In conclusion, this study showed no association between n-3 PUFA in serum phospholipids and risk of breast cancer, and thus does not support the hypothesis that n-3 PUFA may be protective. In contrast, we found a decreased risk of breast cancer among women in the highest quartile of stearic acid, as compared with women in the lowest quartile, suggesting a protective effect of stearic acid. Further epidemiological and experimental data are needed to precisely identify the role of stearic acid in breast cancer.

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