Erythrocyte Membrane Fatty Acids and Subsequent Breast Cancer: a Prospective Italian Study

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Background: **The relationship between erythrocyte membrane fatty acids and postmenopausal breast cancer risk was analyzed previously only by retrospective studies, which suggested a protective effect of increased saturation index (SI), i.e., the ratio of membrane stearic to oleic acid. We investigated the relationships in a prospective study of hormones, diet, and prediagnostic breast cancer (the ORDET study) conducted in northern Italy.** ***Methods:*** **A total of 4052 postmenopausal women were followed for an average of 5.5 years; 71 cases of invasive breast cancer were identified. For each case subject, two matched control subjects were chosen randomly from among cohort members. The various fatty acids in erythrocyte membranes were measured as a percentage of total fatty acids. Conditional logistic regression analysis evaluated the association between membrane fatty acid composition and breast cancer risk. The SI, which is influenced by the activity of the enzyme delta 9 desaturase (Δ9-d), was also investigated. All statistical tests were two-sided.** ***Results:*** **Oleic (highest versus lowest tertile of percentage of total fatty acids, odds ratio [OR] = 2.79; 95% confidence interval [CI] = 1.24 to 6.28) and monounsaturated fatty acids (highest to lowest tertile, OR = 5.21; 95% CI = 1.95 to 13.91) were positively associated with breast cancer risk. The SI (highest to lowest tertile, OR = 0.29; 95% CI = 0.13 to 0.64) was inversely associated with breast cancer risk. The analysis suggested an inverse association between total polyunsaturated fatty acids and breast cancer risk, but individual polyunsaturated fatty acids behaved differently. There was no association between saturated fatty acids and breast cancer risk.** ***Conclusions:*** **We have found that monounsaturated fats and SI in erythrocyte membranes are predictors of postmenopausal breast cancer. Both of these variables depend on the activity of the enzyme Δ9-d. The dietary, metabolic, and hormonal factors acting on Δ9-d expression and activity and, therefore, on patterns of fatty acid metabolism, should be further investigated as possible determinants of breast cancer. [J Natl Cancer Inst 2001;93:1088–95]**

That dietary factors contribute to the etiology of human breast cancer has not been established unequivocally *(1);* however, variations in fat consumption and metabolism are suspected to contribute to the marked regional differences in the incidence rates of breast cancer *(2,3)* and to the increases in risk that follow migration from a low-incidence to a high-incidence area *(4–6).*

The fatty acid composition of the erythrocyte membrane, more than a mere biomarker of dietary fat intake, is the integrated result of interactions between dietary fatty acid intake, other dietary factors, and endocrine changes *(7–9),* in part because of the long half-life (120 days) of erythrocytes. The parameter may, therefore, be an appropriate biomarker for investigating not only dietary intake but also the relations of the patterns of fatty acid metabolism to breast cancer risk.

The saturation index (SI) in red blood cell membranes is the ratio of stearic acid (the most common saturated fatty acid [SFA]) to oleic acid (the most common monounsaturated fatty acid [MUFA]). A reduction in SI was observed in patients with breast and other cancers in several *(10,11)* but not in all *(12,13)* case–control studies. Furthermore, in a recent prospec-

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See "Notes" following "References."

tive cohort study carried out in Sweden *(14),* a high SI in plasma phospholipids was observed to be protective against breast cancer.

The polyunsaturated fatty acid (PUFA) content of red blood cell membranes may also by itself be related to breast cancer: A case–control *(12)* and a cohort *(15)* study indicated that breast cancer patients have a lower proportion of n-6 PUFAs in their red blood cell membranes *(12)* and serum phospholipids *(15),* whereas no association was found between levels of serum phospholipid n-3 PUFAs *(15)* and the risk of the disease.

The intriguing question of whether the fatty acid composition of erythrocyte membranes reflects the fatty acid composition of other tissues (in particular, breast tissue) remains largely unexplored. To our knowledge, only two cross-sectional studies *(16,17)* have addressed that issue. The first study *(16)* supported the hypothesis that the fatty acid composition of membranes differed from tissue to tissue, whereas the second study *(17)* found a statistically significant positive correlation between fatty acid composition in erythrocyte and muscle cell membranes. The results from the latter study suggest that the composition of human erythrocyte membrane fatty acid may reflect fatty acid composition in other body tissues. In addition, the fatty acid composition in breast tissue was associated with mammary tumor growth in several studies in animals *(18–20)* and in studies of human cell lines *(20).*

Our study analyzes the association between breast cancer risk and the prediagnostic fatty acid composition of erythrocyte membranes in terms of individual fatty acids and fatty acid classes. The study was conducted on the ORDET cohort of women described below, consisting of volunteers recruited to assess the association between hormone levels, diet, and the development of breast cancer.

Subjects and Methods

Study Design

During the period from 1987 to 1992, 4052 healthy postmenopausal women, aged 42–69 years, who resided in Varese Province, northern Italy, were enrolled in the ORDET study, a prospective study of hormones, diet, and breast cancer risk *(21).* All of the women were volunteers who were recruited from the general population via radio, television, and newspaper advertising and from among women attending breast cancer prevention units.

Women who had a history of cancer, bilateral ovariectomy, or chronic or acute liver disease or women who had received hormone therapy in the 3 months before recruitment were not eligible. Signed informed consent was obtained from all participants. The study was approved by the Ethical Review Board of the Istituto Nazionale per la Cura e lo Studio dei Tumori, Milan, Italy. Trained nurses collected data on menstrual and reproductive histories, education, occupational history, socioeconomic status, family history of breast cancer, and other potential risk factors for breast cancer according to standardized procedures. Anthropometric measurements were made with the women in light clothes and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist-to-hip ratio (WHR), used as an indicator of abdominal adiposity, was calculated by dividing the waist circumference (measured at the narrowest part of the trunk as observed from the front) by the hip circumference (measured at the level of pubic symphysis). Postmenopausal status was defined as the absence of menstrual bleeding for at least 12 months.

The reproducibility of the information gathered was assessed in a study conducted on 233 cohort members 1 year after recruitment *(22).* All of the variables showed good reproducibility. (Pearson's correlation coefficients were always >.86.)

Case Ascertainment

In June 1995, after an average of 5.5 years of follow-up, the ORDET file was linked to the local cancer registry (Lombardy Cancer Registry) files to identify breast cancer cases. The Lombardy Cancer Registry is a high-quality registry with only 1% of breast cancer patients registered by death certificate only and 90% of cases histologically verified. The ORDET file was also linked to the regional residents' file to check the vital status of cohort members. Twenty-three of the 4052 postmenopausal participants were excluded because they had been diagnosed with breast cancer before enrollment, three women were lost to follow-up, and four were excluded for a diagnosis of breast cancer *in situ.* Therefore, 4022 women were available for study. Among these women, the cancer registry identified 71 cases of invasive breast cancer at the time of file linking.

Control Subjects

For each breast cancer case subject, two matched control subjects were chosen randomly from among cohort members without breast cancer who were alive at the time of diagnosis of the matched case subject. Control subjects were matched to case subjects for age (±5 years), menopausal status at recruitment, daylight-saving period at blood sampling, recruitment center, and recruitment date (±89 days).

Blood Sampling and Storage

Antecubital venous blood (40 mL) was drawn between 8:00 AM and 9.30 AM from fasting subjects on the day of recruitment. The exact time of sampling was recorded. Aliquots of serum, plasma, buffy coat, and red blood cell membranes were stored separately at −80 °C. The ORDET procedure for the processing and cryopreservation of biologic samples was described previously *(21).*

Membrane Separation

Membranes were prepared with the use of the washed cell technique *(23).* The erythrocyte fraction obtained from blood centrifugation was resuspended in 5 mL of buffer (NaCl, 140 mmol/L [pH 7.4]) and centrifuged (10 minutes at 2500*g* at 5 °C). The erythrocytes were lysed by the addition of 39 mL of low-molarity solution (Tris–HCl, 5 m*M* [pH 7.4]). The membrane suspension was shaken gently to ensure complete hemolysis and centrifuged (10 minutes at 22 000*g* at 5 °C) to separate the membranes from cytoplasm, proteases, and other cell components. The pellet was resuspended in 5 m*M* Tris–HCl to a volume of 40 mL and centrifuged three more times (40 minutes at 22 000*g* at 5 °C) with resuspension in 5 m*M* Tris–HCl (pH 7.4) to a final volume of 40 mL. After homogenization, the membrane suspension, containing about 1 mg of membrane protein/1 mL of suspension, was divided into four 1-mL aliquots and conserved at −80 °C.

Analysis of Fatty Acids

The fatty acid composition of the erythrocyte membrane phospholipids of each case subject and her two matched control subjects was analyzed on the same day (in the same analysis batch). An independent sample of pooled membrane erythrocytes was included in each batch as control. The coded membrane preparations were sent in a random sequence from the Epidemiology Unit of the Istituto Nazionale per la Cura e lo Studio dei Tumori to the nutrition laboratory of the International Agency for Research on Cancer (IARC), Lyon, France, for analysis. The laboratory was blinded to links between samples and subjects.

Total lipids were extracted from erythrocyte membranes with the use of a modification of the Bligh and Dyer method *(24).* An aliquot of membrane (400 μL) was shaken vigorously for 20 seconds with 2 mL of methanol containing 100 μL of butylated hydroxytoluene solution (1 mg/mL ethanol) as antioxidant and 100 μL of di-12 : 0 phosphatidylcholine as internal standard (0.05 m*M*/L in ethanol). Chloroform (1 mL) was added while shaking the mixture for an additional 20 seconds. Chloroform (2 mL) was then added, and the extract was washed with 1 mL of 0.9% NaCl. After separation into two phases, the chloroform layer was transferred to a tube and evaporated to dryness under nitrogen. The residue containing the phospholipids was dissolved in 200 μL of chloroform–methanol (2 : 1) and purified by adsorption chromatography on silica tubes. Methyl-Prep II (Alltech, Templemars, France) was used to convert the phospholipids to fatty acid methyl esters.

The procedures for phospholipid purification and direct methyl esterification have been reported elsewhere *(14).* The fatty acid methyl esters were analyzed by gas chromatography on a fused-silica capillary column, with the use of an on-column injector and flame ionization detector, under operating conditions previously described *(14).* Fatty acids were identified with the use of commercial standards of known retention times (Sigma Chemical Co., St. Louis, MO) and were quantified as the percentage of the total area under the curve and as moles of fatty acid per liter. Interassay coefficients of variation were calculated on independent control analyses conducted on separate days. The coefficients of

variation were less than 6% for the major peaks and 10% for the smallest peaks. One control subject was excluded from the analysis because her blood sample was missing.

18 : 3,n-6c (gamma-linolenic) and 20 : 0 (arachidic) fatty acids were not detected in the membranes of one control sample; 18 : 3,n-3c (α-linolenic) was not detected in the membranes of one case and one control sample; 20 : 1,n-9c was not detected in the membranes of two case and two control samples; these and the six control samples matched to the excluded breast cancer case subjects were excluded only from the analyses involving these residues. For each the 22 fatty acids detected, we calculated the proportion of the total in each case and control subject.

Fatty Acid Naming and Grouping

We use established common names to refer to fatty acid chains. However, not all fatty acid chains in erythrocyte membranes have common names. It is, therefore, convenient to use a standard systematic notation to specify fatty acid chain length, number of double bonds, and position and configuration of the double bond nearest the methyl end of the chain. The specification begins with two numbers separated by a colon; these numbers refer to the number of carbons in the chain and the number of double bonds, respectively; the next number, preceded by "n," indicates the distance of first double bond, in C atoms, from the methyl end (n-end) of the chain; "c" or "t" follow indicating a *cis* or *trans* configuration, respectively, of the double bond nearest the methyl end. The tables use both systematic names and, when they exist, common names.

We calculated the membrane content (percent) of each of the 22 fatty acids detected and of the following five groups, with the use of total fatty acids as the denominator.

SFAs—14 : 0 (myristic), 15 : 0, 16 : 0 (palmitic), 17 : 0, 18 : 0 (stearic), and 20 : 0 (arachidic).

MUFAs—16 : 1,n-7c (palmitoleic), 18 : 1,n-7c (vaccenic), 18 : 1,n-9c (oleic), 18 : 1,n-9t (elaidic), and 20 : 1,n-9c.

n-6 PUFAs—18 : 2,n-6c (linoleic), 20 : 2,n-6c, 18 : 3,n-6c (γ-linolenic), 20 : 3,n-6c (dihomo-γ-linolenic), 20 : 4,n-6c (arachidonic), 22 : 4,n-6c, and 22 : 5,n-6c.

n-3 PUFAs—18 : 3,n-3c (α-linolenic), 20 : 5,n-3c (eicosapentaenoic), 22 : 5,n-3c, and 22 : 6,n-3c (docosahexaenoic).

PUFAs—n-6 PUFAs and n-3 PUFAs.

We also determined the SI as the ratio between stearic acid and oleic acid *(25,26)* and the n-7 fatty acid SI (SI_{n-7}) as the ratio of palmitic acid to palmitoleic acid. The SI is an indicator of membrane fluidity; its reciprocal can be considered to be an index of the activity of the rate-limiting enzyme delta 9 desaturase (Δ9-d) that transforms the SFAs palmitic and stearic acids, respectively, into the MUFAs palmitoleic and oleic acid *(7)*.

Statistical Methods

Subjects were categorized into tertiles according to the percentage composition of each individual fatty acid; the tertiles were derived on the basis of the distribution in control subjects. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for each tertile with the use of the lowest tertile as a reference category. Conditional logistic regression analysis was performed with the use of Stata statistical software release 5.0 (1995; Stata Corp., College Station, TX). To assess the statistical significance of trends, we employed the likelihood ratio test between models that included and omitted the tertile variable as an ordinal variable.

BMI, WHR, age at menarche, age at first childbirth, age at menopause, months of lactation, parity, and educational level were investigated as potential confounders; since none showed a clear association with or exerted a major confounding effect on the relationship between fatty acid content and breast cancer risk, only unadjusted ORs are presented. All of the statistical tests were two-sided.

RESULTS

Table 1 shows clinical and other characteristics possibly linked to breast cancer risk (age at recruitment, BMI, WHR, age at menarche, age at first childbirth, parity, and age at menopause) for case and control subjects. The two groups differed only slightly with respect to these variables.

Table 2 shows the mean percentage fatty acid composition of red blood cell membranes in case and control subjects. In both groups, the six most abundant individual fatty acids were 16 : 0 (palmitic), 18 : 0 (stearic), 20 : 4,n-6c (arachidonic), 18 : 1,n-9c (oleic), 18 : 2,n-6c (linoleic), and 22 : 6, n-3c (docosahexaenoic). These compounds constitute about 88% of total membrane fatty acids. Compared with control subjects, case subjects had higher percentages of 18 : 1n-9c (oleic acid, 15% versus 14.5%) and total MUFA (17.5% versus 16.9%) and lower percentages of 20 : 2n-6c (0.2% versus 0.3%). Case subjects also had lower SI (18 : 0/18 : 1,n-9c) and SI_{n-7} (16 : 0/16 : 1,n-7c) than control subjects.

ORs for breast cancer by tertiles of proportions of various fatty acids in erythrocyte membranes are shown in Table 3. None of the SFAs (myristic, 15 : 0, palmitic, 17 : 0, stearic, and arachidic) was associated with breast cancer risk. This group is the predominant one in erythrocyte membranes, constituting 40% of total fatty acids.

The MUFAs oleic and palmitoleic were statistically significantly associated with breast cancer risk. For oleic acid, the OR of the highest tertile was 2.79 (95% CI = 1.24–6.28) compared with the lowest tertile. For palmitoleic acid, the OR of the highest tertile was 2.32 (95% CI = 1.03–5.20). When all of the MUFAs (palmitoleic, vaccenic, oleic, elaidic, and 20 : 1,n-9c) were considered together, the OR was 4.03 (95% CI = 1.52–10.67) for the middle tertile and 5.21 (95% CI = 1.95–13.91) for the highest tertile. The only *trans* fatty acid detected in membranes—the MUFA elaidic acid—was not associated with breast cancer risk.

The SI was strongly and inversely associated with breast cancer risk (OR = 0.29 [95% CI = 0.13–0.64] for the highest tertile). The SI_{n-7} was also inversely but statistically nonsignificantly associated with breast cancer risk.

The analysis suggested an inverse association between total PUFAs and breast cancer risk (OR = 0.34 [95% CI = 0.15–0.79] for the highest tertile), but individual PUFAs behaved differently from one another: Linoleic acid was negatively associated with breast cancer risk, with OR = 0.44 (95% CI = 0.20–1.00) for the highest tertile, whereas arachidonic acid (the most abundant PUFA and the third most abundant fatty acid) was positively but not statistically significantly associated with breast cancer risk.

Neither the two n-6 PUFAs intermediate between linoleic and arachidonic acid (γ-linolenic acid and dihomo-γ-linolenic acid) nor the long-chain n-6 PUFAs (22 : 4,n-6c and 22 : 5,n-6c) were associated with breast cancer risk. However, 20 : 2,n-6c, a derivative of linoleic acid by an alternative elongation pathway, was inversely associated with the risk of breast cancer (OR = 0.49 [95% CI =

Table 1. Mean and standard deviation (SD) of some variables possibly related to breast cancer in case and control subjects

	Case subjects (n = 71)		Control subjects (n = 141)	
	Mean	SD	Mean	SD
Age at recruitment, y	58.10	5.40	57.10	5.30
Body mass index, kg/m^2	26.23	4.11	26.75	4.12
Waist-to-hip ratio	0.81	0.06	0.82	0.07
Age at menarche, y	13.24	1.63	13.09	1.97
Age at first childbirth, y	25.80	4.00	25.80	2.20
Parity, No.	1.76	1.01	1.98	1.55
Age at menopause, y	48.89	5.34	47.97	4.66

Table 2. Percentage fatty acid composition of red blood cell membranes in case and control subjects

Systematic name	Common name	Case subjects (n = 71) Mean	Case subjects (n = 71) SD	Control subjects (n = 141) Mean	Control subjects (n = 141) SD
14 : 0	Myristic acid	0.3	0.1	0.3	0.1
15 : 0		0.2	0.0	0.2	0.0
16 : 0	Palmitic acid	21.3	0.9	21.2	0.9
17 : 0		0.3	0.0	0.4	0.1
18 : 0	Stearic acid	17.8	0.7	18.0	0.6
20 : 0	Arachidic acid	0.1	0.0	0.1	0.0
16 : 1,n-7c	Palmitoleic acid	0.5	0.1	0.5	0.1
18 : 1,n-7c	Vaccenic acid	1.4	0.2	1.4	0.2
18 : 1,n-9c	Oleic acid	15.0	1.3	14.5	1.2
18 : 1,n-9t	Elaidic acid	0.2	0.1	0.2	0.1
20 : 1,n-9c		0.3	0.1	0.3	0.1
18 : 2,n-6c	Linoleic acid	10.8	1.5	11.1	1.6
20 : 2,n-6c		0.2	0.0	0.3	0.0
18 : 3,n-6c	γ-Linolenic acid	0.1	0.0	0.1	0.0
20 : 3,n-6c	Dihomo-γ-linolenic acid	2.0	0.4	2.1	0.4
20 : 4,n-6c	Arachidonic acid	17.4	1.3	17.3	1.3
22 : 4,n-6c		2.8	0.5	2.8	0.6
22 : 5,n-6c		0.8	0.2	0.8	0.2
18 : 3,n-3c	α-Linolenic acid	0.1	0.0	0.1	0.0
20 : 5,n-3c	Eicosapentaenoic acid	0.6	0.2	0.6	0.2
22 : 5,n-3c		2.1	0.3	2.1	0.3
22 : 6,n-3c	Docosahexaenoic acid	5.7	1.1	5.8	1.2
Saturated fatty acids*		40.1	0.7	40.1	0.9
Monounsaturated fatty acids†		17.5	1.3	16.9	1.4
Polyunsaturated fatty acids (PUFAs)‡		42.4	1.5	43.0	1.5
n-6 PUFAs§		34.1	1.7	34.4	2.0
n-3 PUFAs‖		8.5	1.2	8.6	1.3
n-3 long-chain PUFAs¶		8.3	1.2	8.5	1.3
Saturation index$_{n-7}$#		47.0	12.3	50.6	14.1
Saturation index**		1.20	0.13	1.25	0.13

*Saturated fatty acids = 14 : 0 + 15 : 0 + 16 : 0 + 17 : 0 + 18 : 0 + 20 : 0.

†Monounsaturated fatty acids = 16 : 1,n-7c + 18 : 1,n-7c + 18 : 1,n-9c + 18 : 1,n-9t + 20 : 1,n-9c.

‡PUFAs = 18 : 2,n-6c + 20 : 2,n-6c + 18 : 3,n-6c + 20 : 3,n-6c + 20 : 4,n-6c + 22 : 4,n-6c + 22 : 5,n-6c + 18 : 3,n-3c + 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.

§n-6 PUFAs = 18 : 2,n-6c + 20 : 2,n-6c + 18 : 3,n-6c + 20 : 3,n-6c + 20 : 4,n-6c + 22 : 4,n-6c + 22 : 5,n-6c.

‖n-3 PUFAs = 18 : 3,n-3c + 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.

¶n-3 long-chain PUFAs = 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.

#Saturation index$_{n-7}$ = 16 : 0/16 : 1,n-7c.

**Saturation index = 18 : 0/18 : 1,n-9c.

0.24–0.98] for the middle versus the lowest tertile).

Among n-3 fatty acids, neither long-chain nor short-chain forms were consistently associated with breast cancer risk. High levels of docosahexaenoic acid, however, the most abundant n-3 PUFA in erythrocyte membranes, were associated with a reduced risk of breast cancer (OR = 0.44 [95% CI = 0.21–0.92] for the middle versus the lowest tertile).

Discussion

The most clear-cut findings of this study are that postmenopausal women who developed breast cancer had erythrocyte membranes characterized by higher levels of MUFAs (especially oleic acid) and a lower SI than those who did not develop breast cancer. Case women were also characterized by lower levels of the n-6 PUFA 20 : 2,n-6c and n-3 docosahexaenoic acid than women who remained disease free.

These differences in membrane composition are unlikely to be a preclinical effect of the cancer because the ORDET study is a prospective study and because all participants underwent mammography or clinical examination of the breast at recruitment. Tumors not picked up by these examinations would have been of such small size that they are highly unlikely to have caused detectable changes to extratumoral tissues. However, to take account of any potential preclinical effects of breast cancer, we performed a separate analysis in which the 10 breast cancer case subjects diagnosed within 6 months of blood sampling were excluded; the results did not differ from those obtained with the use of all of the case subjects.

Linoleic acid (18 : 2,n-6c). We found a small inverse association between erythrocyte membrane linoleic acid levels and the risk of breast cancer. After absorption, the essential fatty acid linoleic acid undergoes desaturation/saturation and chain elongation/shortening reactions, producing other n-6 fatty acids. A correlation study between diet and erythrocyte membrane content (Fuhrman B: unpublished data) suggest that our finding of reduced breast cancer risk in women with high levels of linoleic acid could be partially due to protection by a diet high in this fatty acid residue and partially due to its possibly low transformation rate into other n-6 PUFAs.

Other n-6 PUFAs. While high proportions of the fatty acids on the pathway from linoleic to arachidonic acid were not associated with breast cancer, the 20 : 2,n-6c fatty acid was statistically significantly protective and was the only long-chain residue to be so. 20 : 2,n-6c is derived from linoleic acid by a different elongation pathway from that leading to arachidonic acid. Hence, the possibility arises that diversion of linoleic acid metabolism away from arachidonic acid (and hence from arachidonic acid-derived prostaglandins) might be protective against breast cancer *(27);* this possibility might be worth investigating further. It has been found *(9)* that, even when long-chain n-6 PUFAs are a minor constituent of dietary triglycerides, erythrocyte phospholipids are constitutionally rich in them; therefore, they cannot be considered to be a reliable marker of dietary intake.

α-Linolenic acid (18 : 3,n-3c). α-Linolenic acid, the other essential PUFA in humans, was not associated with breast cancer risk. The α-linolenic acid content of erythrocyte membranes is very low and is largely unrelated to dietary intake [*(9);* Fuhrman B: unpublished data].

Other n-3 PUFAs. Docosahexaenoic acid, the most abundant n-3 fatty acid in erythrocyte membranes, was inversely associated with breast cancer risk. Docosahexaenoic acid was the only fatty acid associated with fish consumption (r = .48; P<.000) in the ORDET cohort (Fuhrman B: unpublished data) in spite of the low intake of fish (average intake, 15 g daily).

MUFAs and SFAs. We found membrane MUFAs to be positively associated and the SI to be inversely associated with breast cancer risk, whereas there was no association with SFA levels. The idea that

Table 3. Associations between breast cancer and 95% confidence intervals (CIs) with percentage phospholipid fatty acids in erythrocyte membrane fatty acids among postmenopausal ORDET participants (71 case subjects, 141 controls subjects)

Systematic name	Common name		Odd ratios (95% CI) by tertiles			Two-sided *P* for trend
			I (referent)	II	III	
		Tertile cutpoints	<0.30	⩾0.30 to <0.36	⩾0.36	
14 : 0	Myristic acid		1	1.59 (0.72 to 3.48)	1.52 (0.65 to 3.55)	.37
		Tertile cutpoints	<0.16	⩾0.16 to <0.19	⩾0.19	
15 : 0			1	0.69 (0.32 to 1.46)	0.93 (0.46 to 1.89)	.86
		Tertile cutpoints	<20.72	⩾20.72 to <21.39	⩾21.39	
16 : 0	Palmitic acid		1	0.78 (0.38 to 1.62)	1.49 (0.75 to 2.96)	.27
		Tertile cutpoints	<0.33	⩾0.33 to <0.38	⩾0.38	
17 : 0			1	1.08 (0.55 to 2.12)	0.77 (0.38 to 1.58)	.48
		Tertile cutpoints	<17.63	⩾17.63 to <18.25	⩾18.25	
18 : 0	Stearic acid		1	0.85 (0.40 to 1.82)	0.68 (0.32 to 1.48)	.33
		Tertile cutpoints	<0.07	⩾0.07 to <0.08	⩾0.08	
20 : 0	Arachidic acid		1	0.46 (0.19 to 1.15)	0.66 (0.25 to 1.75)	.55
		Tertile cutpoints	<0.40	⩾0.40 to <0.49	⩾0.49	
16 : 1,n-7c	Palmitoleic acid		1	1.31 (0.60 to 2.85)	2.32 (1.03 to 5.20)	.04
		Tertile cutpoints	<1.27	⩾1.27 to <1.45	⩾1.45	
18 : 1,n-7c	Vaccenic acid		1	2.01 (0.92 to 4.39)	1.42 (0.57 to 3.55)	.50
		Tertile cutpoints	<13.97	⩾13.97 to <15.03	⩾15.03	
18 : 1,n-9c	Oleic acid		1	1.72 (0.74 to 3.98)	2.79 (1.24 to 6.28)	.01
		Tertile cutpoints	<0.17	⩾0.17 to <0.24	⩾0.24	
18 : 1,n-9t	Elaidic acid		1	0.81 (0.39 to 1.69)	0.71 (0.30 to 1.64)	.42
		Tertile cutpoints	<0.25	⩾0.25 to <0.36	⩾0.36	
20 : 1,n-9c			1	2.35 (1.00 to 5.49)	1.56 (0.49 to 4.93)	.42
		Tertile cutpoints	<10.31	⩾10.31 to <10.67	⩾10.67	
18 : 2,n-6c	Linoleic acid		1	0.99 (0.51 to 1.92)	0.44 (0.20 to 1.00)	.06
		Tertile cutpoints	<0.24	⩾0.24 to <0.27	⩾0.27	
20 : 2,n-6c			1	0.49 (0.24 to 0.98)	0.52 (0.26 to 1.02)	.04
		Tertile cutpoints	<0.06	⩾0.06 to <0.08	⩾0.08	
18 : 3,n-6c	γ-Linolenic acid		1	0.90 (0.46 to 1.82)	0.98 (0.46 to 2.07)	.95
		Tertile cutpoints	<1.88	⩾1.88 to <2.15	⩾2.15	
20 : 3,n-6c	Dihomo-γ-linolenic acid		1	0.76 (0.38 to 1.53)	0.68 (0.33 to 1.41)	.29
		Tertile cutpoints	<16.67	⩾16.67 to <17.94	⩾17.94	
20 : 4,n-6c	Arachidonic acid		1	1.76 (0.88 to 3.53)	1.40 (0.64 to 3.10)	.42
		Tertile cutpoints	<2.56	⩾2.56 to <3.09	⩾3.09	
22 : 4,n-6c			1	1.26 (0.62 to 2.59)	0.76 (0.35 to 1.63)	.49
		Tertile cutpoints	<0.71	⩾0.71 to <0.82	⩾0.82	
22 : 5,n-6c			1	1.86 (0.86 to 3.95)	1.65 (0.74 to 3.64)	.27
		Tertile cutpoints	<0.09	⩾0.09 to <0.11	⩾0.11	
18 : 3,n-3c	α-Linolenic acid		1	0.91 (0.45 to 1.84)	1.38 (0.70 to 2.70)	.35
		Tertile cutpoints	<0.50	⩾0.50 to <0.66	⩾0.66	
20 : 5,n-3c	Eicosapentaenoic acid		1	1.34 (0.66 to 2.73)	0.76 (0.35 to 1.62)	.46
		Tertile cutpoints	<1.98	⩾1.98 to <2.19	⩾2.19	
22 : 5,n-3c			1	1.12 (0.57 to 2.20)	0.83 (0.40 to 1.73)	.65
		Tertile cutpoints	<5.31	⩾5.31 to <6.15	⩾6.15	
22 : 6,n-3c	Docosahexaenoic acid		1	0.44 (0.21 to 0.92)	0.48 (0.23 to 1.00)	.05
		Tertile cutpoints	<39.68	⩾39.68 to <40.31	⩾40.31	
Saturated fatty acids*			1	1.34 (0.67 to 2.69)	1.01 (0.45 to 2.29)	.89
		Tertile cutpoints	<16.16	⩾16.16 to <17.42	⩾17.42	
Monounsaturated fatty acids†			1	4.03 (1.52 to 10.67)	5.21 (1.95 to 13.91)	.00
		Tertile cutpoints	<42.44	⩾42.44 to <43.61	⩾43.61	
Polyunsaturated fatty acids (PUFAs)‡			1	0.61 (0.30 to 1.25)	0.34 (0.15 to 0.79)	.01
		Tertile cutpoints	<33.69	⩾33.69 to <35.16	⩾35.16	
n-6 PUFAs§			1	0.90 (0.46 to 1.79)	0.49 (0.22 to 1.06)	.08
		Tertile cutpoints	<8.06	⩾8.06 to <8.97	⩾8.97	
n-3 PUFAs‖			1	0.53 (0.26 to 1.10)	0.53 (0.26 to 1.08)	.07
		Tertile cutpoints	<7.98	⩾7.98 to <8.89	⩾8.89	
n-3 long-chain PUFAs¶			1	0.48 (0.23 to 1.01)	0.51 (0.25 to 1.04)	.06
		Tertile cutpoints	<42.99	⩾42.99 to <53.01	⩾53.01	
Saturation index$_{n-7}$#			1	0.71 (0.37 to 1.37)	0.55 (0.26 to 1.17)	.11
		Tertile cutpoints	<1.19	⩾1.19 to <1.30	⩾1.30	
Saturation index**			1	0.44 (0.21 to 0.91)	0.29 (0.13 to 0.64)	.00

*Saturated fatty acids = 14 : 0 + 15 : 0 + 16 : 0 + 17 : 0 + 18 : 0 + 20 : 0.
†Monounsaturated fatty acids = 16 : 1,n-7c + 18 : 1,n-7c + 18 : 1,n-9c + 18 : 1,n-9t + 20 : 1,n-9c.
‡PUFAs = 18 : 2,n-6c + 20 : 2,n-6c + 18 : 3,n-6c + 20 : 3,n-6c + 20 : 4,n-6c + 22 : 4,n-6c + 22 : 5,n-6c + 18 : 3,n-3c + 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.
§n-6 PUFAs = 18 : 2,n-6c + 20 : 2,n-6c + 18 : 3,n-6c + 20 : 3,n-6c + 20 : 4,n-6c + 22 : 4,n-6c + 22 : 5,n-6c.
‖n-3 PUFAs = 18 : 3,n-3c + 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.
¶n-3 long-chain PUFAs = 20 : 5,n-3c + 22 : 5,n-3c + 22 : 6,n-3c.
#Saturation index$_{n-7}$ = 16 : 0/16 : 1,n-7c.
**Saturation index = 18 : 0/18 :1,n-9c.

low-erythrocyte-membrane SI is associated with increased risk of breast and other cancers has been tested in several case–control studies *(10–13,28)* with conflicting results, although all of the studies were small and had cross-sectional designs. Our finding of an inverse association between red blood cell membrane SI and postmenopausal breast cancer risk is consistent with the results of a Swedish cohort study *(14)* in which high plasma SI was found to be inversely associated with breast cancer risk. In that study, however, the apparent protective effect of high membrane SI was due to high levels of stearic acid (SI numerator), whereas the apparent protective effect was due to low levels of oleic acid (SI denominator) in the present study. These findings suggest that, rather than the individual components, the ratio between the two fatty acids may be more directly related to breast cancer risk.

A diet high in MUFAs is probably one determinant—but not the major one—of erythrocyte membrane MUFAs *(8,29,30),* which are extensively synthesized in the body *(7,31).* Therefore, the association of high levels of membrane oleic acid and low SI with increased breast cancer risk might be related to factors other than diet. Most oleic acid in mammalian tissue is derived from the saturated stearic acid residue *(7,32).* This key conversion is controlled by the enzyme Δ9-d (Fig. 1), which also regulates the transformation of the other common SFAs (myristic and palmitic) to their corresponding monounsaturated forms (myristoleic and palmitoleic). Several studies have suggested a link between Δ9-d activity and tumor growth. Thus, high levels of MUFAs are required by mouse mammary carcinoma cells *(18),* hepatoma cells *(33),* and human leukemia and lymphoma cells *(34).* In all of these cases, the required MUFA levels are assured by overexpression of the genes encoding Δ9-d. Inhibition of Δ9-d and, consequently, of oleic acid biosynthesis blocks the growth of transplanted mammary tumor in rats *(19).* Furthermore, *in vitro* inhibition of mammary carcinogenesis has been achieved by the addition of an inhibitor of Δ9-d *(20).*

The fat content of the diet has an important effect on Δ9-d activity. High levels of SFAs increase Δ9-d activity by twofold to threefold, whereas PUFAs decrease it *(35–40);* as a consequence, high levels of oleic acid and low SI in tissues may be the result of a diet poor in PUFAs.

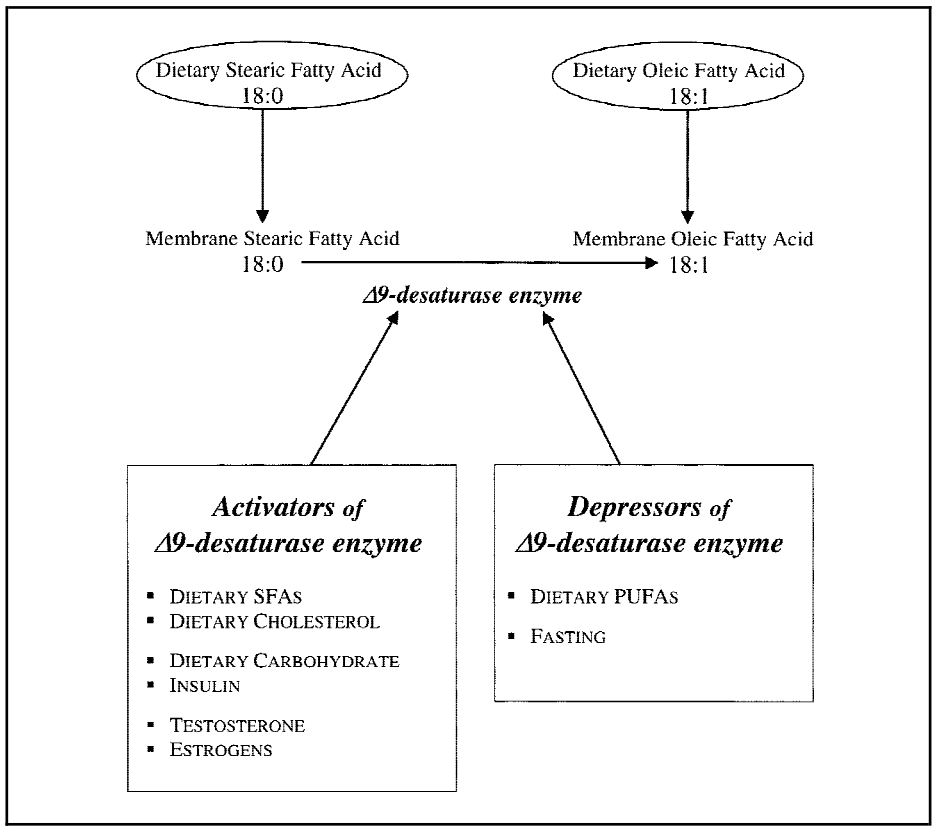

Fig. 1. Principal activators and depressors of the enzyme Δ9-desaturase, which catalyzes the conversion of stearic acid to oleic acid in mammalian tissues. SFAs = saturated fatty acids; PUFAs = polyunsaturated fatty acids.

A correlation study on the ORDET cohort (Fuhrman B: unpublished data) provided results consistent with this hypothesis, finding a negative correlation ($r = -.25$; $P = .009$) between erythrocyte membrane oleic acid (the main product of Δ9-d) and dietary PUFAs. A high-cholesterol diet also seems to increase Δ9-d activity, resulting in increased monounsaturation of the membrane fatty acids in rat liver to partially compensate for the "rigidizing" effect of cholesterol incorporation in the membrane *(31).*

Δ9-d is activated by carbohydrate administration *(37),* and it is well established that insulin enhances Δ9-d activity *(39–44).* The possible relationships among dietary carbohydrate, insulin resistance, and breast cancer risk is receiving increasing attention *(45–51).*

Δ9-d activity might be also enhanced by estrogens *(52,53)* and testosterone *(31).* Estradiol has been shown to markedly lower the SI of erythrocyte membranes in premenopausal and postmenopausal women *(54),* suggesting a role of this hormone in the regulation of membrane fluidity. There is strong evidence that high levels of these hormones increase the risk of breast cancer *(21).*

Thus, our finding of an inverse relationship between SI and breast cancer risk could also be related to hormonal and metabolic factors and to interactions between them, as well as to dietary factors.

We have shown that the fatty acid composition of erythrocyte membranes is associated with breast cancer risk. The composition seems to be influenced by the fatty acid composition of the diet and by the endogenous synthesis and metabolism of fatty acid residues, in which dietary, hormonal, and other factors may intervene in ways that are incompletely understood. Clearly, further studies are required to clarify these intriguing aspects of the relationship between red blood cell membrane fatty acid composition and breast cancer pathogenesis.

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NOTES

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