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Polyunsaturated Fatty Acids in Serum Phospholipids and Risk of Breast Cancer: A Case–control Study from the Janus Serum Bank in Norway

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We have tested the hypothesis that specific polyunsaturated fatty acids (PUFA) of the *n*-3 and *n*-6 families, as measured in serum phospholipids, are negatively associated with the risk of breast cancer. The study is based on serum samples from women who have donated blood to the Janus serum bank at the University Hospital in Oslo, Norway. It consists of sera from 87 women who developed breast cancer (cases) subsequent to blood donation and 235 women who were free of any diagnosed cancer (controls), but were of similar age and had similar blood storage time as the cases. We measured fatty acids (monounsaturated, polyunsaturated and saturated) in serum phospholipids, and made comparisons between cases and controls. The results showed that there was an inverse relation between the *n*-6 PUFA linoleic acid (18 : 2*n*-6) and risk of breast cancer, but this association was restricted to women who were 55 years and younger. In this age group, the relative risk (odds ratio) of women in the highest quartile of linoleic acid was 0.4 (95% confidence limits, 0.2 and 1.0) compared with women in the lowest quartile, and there was a negative trend over quartiles of linoleic acid (Mantel's χ for trend = −2.49, $P < 0.02$). No association was noted between the *n*-3 PUFA of marine oil origin and breast cancer risk. If the measured concentration of linoleic acid in serum phospholipids reliably reflects dietary intake, these data suggest that linoleic acid in the diet may decrease breast cancer risk among women at premenopausal and perimenopausal age. No similar association with *n*-3 unsaturated fatty acids was observed. It is noteworthy that none of the measured fatty acids (saturated or unsaturated) showed a positive association with breast cancer risk.
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INTRODUCTION

MORE THAN 5-fold differences in incidence between countries [1], and marked increases in breast cancer risk in populations who have moved from low to high incidence areas [2], show that environmental factors, maybe particularly diet, influence the aetiology of breast cancer. International correlations [3] and animal studies [4] indicate that type [5, 6] and amount of dietary fat may be decisive factors. Case–control [7–12] and cohort [13–17] studies of humans, however, have been equivocal; in fact, contradictory results have not been uncommon. The issue of diet in breast cancer aetiology is under dispute [18–20], and one area of controversy has focused on whether it is calories ingested from fat or the total intake of calories which is the more important factor.

Biochemical indicators may measure dietary lipid intake with varying reliability [21]. Serum measurements of essential fatty acids, which cannot be endogenously synthesised, may be more closely correlated with dietary intake than measurements of other serum lipids. Among polyunsaturated fatty acids linoleic acid (18 : 2*n*-6) is a principal precursor of long-chain fatty acids in the *n*-6 family. Linoleic acid is primarily derived from plants and vegetables, and from oils refined from these sources.

Animal studies [22] have suggested that a certain amount of dietary linoleic acid is necessary for initiation and progression of mammary carcinomas. Most of the human studies, however, have either found no association [13], or there has been an inverse relation between linoleic acid or *n*-6 polyunsaturated fatty acid (PUFA) [23] in the diet and breast cancer risk. It has been hypothesised, and later verified [10], that the relatively low incidence of breast cancer in Mediterranean countries may be attributed to a high intake of vegetable oil. A recent study from Singapore [24] suggested that a high diet in soya beans and soya products may have a protective effect. To our knowledge, only one previous study has used biochemical measurements of fatty acids [25], and found lower values of linoleic acid in blood plasma among breast cancer patients than in controls. This study, however, was unable to take into account the possibility that lipid concentrations may be lowered by a preclinical effect of the disease.

Analogous to the relation between linoleic acid and the *n*-6 PUFA, alpha-linolenic acid (18 : 3*n*-3) is the precursor of long-chain fatty acids of the *n*-3 family, with a typically marine fat origin [21]. It has been shown that two major components in blood serum, eicosapentaenoic (EPA) (20 : 5*n*-3) and docosahexaenoic (DHA) (22 : 6*n*-3) acids are positively correlated with dietary intake of fish and seafood [26, 27]. Experimental studies in animals have found that a relatively large intake of *n*-3 fatty acids lowers the incidence and slows down the growth rate of mammary carcinomas in rodents [28]. Among the few human

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Table 1. The relation between reported number of meals of fish per week and n-3 *fatty acids in serum phospholipids measured in absolute values and as percentage of total fatty acids**

	Number of meals of fish per week					
	<1 $n = 14$	1 $n = 15$	3 $n = 14$	4+ $n = 15$	Spearman's r	(P value)
Fatty acid (mg/l)†						
Eicosapentaenoic (20 : 5*n*-3)	15	16	25	30	0.34	(0.01)
Docosahexaenoic (22 : 6*n*-3)	49	53	75	87	0.56	(0.0001)
Total *n*-3 PUFA	70	85	117	136	0.49	(0.0001)
Fatty acid (% total fatty acids)†						
Eicosapentaenoic (20 : 5*n*-3)	1.1	1.2	1.8	2.2	0.33	(0.01)
Docosahexaenoic (22 : 6*n*-3)	3.7	4.1	5.8	6.5	0.58	(0.0001)
Total *n*-3 PUFA	6.0	6.6	9.0	10.2	0.53	(0.0001)

*Validation study based on 58 women who reported fish intake, and donated blood for storage in 1976 and 1977 [31]. Fatty acid analysis in 1991.
†Measured in serum phospholipids.

studies which have been published, one correlation study suggested a negative association between fish consumption and breast cancer risk [29]. Analytic studies have either shown no relation [13, 30], or suggested a protective effect associated with fish consumption [17]. To date, no biochemical study has related *n*-3 PUFA to the risk of breast cancer in humans.

In this study we have measured fatty acids in phospholipids of blood sera among women who donated blood and subsequently developed breast cancer. These sera have been compared with those of women at similar age, who were free of cancer, and had donated blood at the same time as those who later were diagnosed with breast cancer. We wanted to test the hypothesis that polyunsaturated fatty acids of the *n*-3 and *n*-6 families as measured in serum phospholipids are negatively associated with the risk of developing breast cancer.

SUBJECTS AND METHODS

Subjects

This study was based on blood samples provided by the Janus serum bank, which previously has been described in detail [31, 32]. The serum bank was initiated in 1973 and comprises nearly 500 000 samples consolidated from approximately 170 000 donors who had no diagnosed cancer at the time of blood donation. The sera have been kept frozen at −25°C, and constitute on average four (range: 2–16) consecutive samples from each subject. The objective of the serum bank has been to search for factors in the serum (chemical, biochemical and immunological) which may indicate increased risk or early preclinical development of cancer.

This study has developed through three separate stages. In the first stage, we wanted to assess stability of fatty acid measurements in phospholipids of frozen sera. We arbitrarily selected 50 serum samples with more than 10 years storage time. A quantitative and qualitative assessment indicated that some degradation of fatty acids had taken place over time, and suggested that this may particularly apply to the preservation of *n*-3 fatty acids.

In the second stage, we wanted to correlate dietary intake of fish with measurements of *n*-3 fatty acids in serum phospholipids. This should provide further information on fatty acid stability and was possible by using sera which have been contributed to the Janus bank by the cardiovascular county studies of Norway [33]. In some of these studies dietary information was collected, and our analysis of 58 serum samples indicated a consistent positive association between the reported number of main meals of fish per week and the concentration of long-chain *n*-3 fatty acids in serum (Table 1), an association which has been documented by others [26, 27]. Moreover, the absolute values of *n*-3 fatty acids and the percentage of *n*-3 fatty acids of total fat similarly correlated with fish intake. Even though some *n*-3 fatty acids had been lost during storage, their concentration in serum phospholipids relative to the reported intake, indicated that the biochemical measurements reflected dietary intake of long chain *n*-3 fatty acids fairly well.

The two initial stages of the study were evaluated as positive by the steering committee of the Janus serum bank, and we were allowed to proceed into a third stage, which is the basis of the present report. It has been acknowledged that serum from the blood donor component of the bank has a higher serum quality than the other components [31], which is mainly due to the shorter time interval between drawing of blood and storage of serum. In all, approximately 30 000 blood donors have contributed sera to the bank. Linkage to the national cancer registry by use of each subject's unique identification number enabled us to ascertain incident cases of breast cancer among female donors. From the initiation of the serum bank in 1973 until the end of June 1991, a total of 87 cases of breast cancer have occurred, on which high-quality serum samples were available. Thus, the case sera have been obtained from women who developed breast cancer up to several years subsequent (mean: 5 years; range: 0.5–16 years) to their donation of blood, which minimises the likelihood that a preclinical effect of the disease may influence the value of serum measurements.

For a woman to be eligible as a control, three restrictions were set. Firstly, she had to be free of any diagnosed cancer, and to be born within 1 year (either way) of a case. Simultaneously, she must have donated blood within 6 months of a case donation. Among women who fulfilled these criteria we decided to randomly select three controls for each case of breast cancer. However, the eligibility criteria were met by only 235 women, thus leaving this study with 87 incident cases of breast cancer, and a frequency matched sample of 235 controls. The overall objective of this procedure was to achieve comparability between cases and controls with respect to age distribution and storage time of their sera (Table 2).

Table 2. Distribution of year of birth and year of blood sampling among cases and controls in the study

			No. of participants Cases	Controls
			87	235
Year of birth		Mean:	1933	1933
	Percentiles:	25%:	1924	1924
		50%:	1932	1932
		75%:	1942	1942
Year of blood sampling		Mean:	1978	1978
	Percentiles:	25%:	1975	1974
		50%:	1976	1976
		75%:	1981	1981

Laboratory analyses

Fatty acids in serum phospholipids were analysed blinded to the subsequent disease state of each serum sample, and the analytic procedures have been described elsewhere [34]. Briefly, serum lipids were extracted with *n*-butanol (butyl alcohol). The phospholipids were isolated from these extracts by column chromatography after addition of the internal standard diheptadecanoyl-glycerophosphocholine and the antioxidant butylated hydroxytoluene (Sigma Chemical, Pool, U.K.). Then the phospholipids were transmethylated and quantitated by gas–liquid chromatography. A normal human serum sample was included as a control to monitor and secure analytical performance. The long-term precision of the fatty acid analyses was evaluated from the coefficient of variation, based on analyses of 147 serum samples. The validation showed 3.7, 4.7, 2.3, 4.0, 3.9, 3.4, 2.6 and 3.7% for each of the respective fatty acids 20 : 5*n*-3, 22 : 6*n*-3, 18 : 2*n*-6, 20 : 3*n*-6, 20 : 4*n*-6, 18 : 0, 18 : 1*n*-9, and total phospholipid fatty acids. The results were quantitated in milligrams of phospholipid fatty acid per liter of serum.

Statistical analyses

For each fatty acid the values of cases and controls combined were divided into quartiles. First, all participants were analysed as a whole, before the data were analysed among cases who were diagnosed with breast cancer at age 55 or younger, and controls who had donated blood before this age. 55 years was arbitrarily chosen as a dividing line to include pre- and perimenopausal women into one category of 65 cases and 195 controls. Differences in mean values of the measured fatty acids were compared for cases and controls. Then standard case–control analyses [35] were applied, where the odds of being exposed within a quartile of a specific fatty acid was related to the reference odds in the lowest quartile of that particular fatty acid. This provided odds ratios of exposure as a measure of relative risk. Precision of the odds ratio was assessed by 95% confidence limits, applying Mantel–Haenszel χ^2 statistics [35], and linear trends over categories of fatty acids were evaluated using Mantel's extended test for trend [35]. Also, we wanted to evaluate whether various fatty acids may jointly contribute to breast cancer risk, and applied a logistic regression analysis.

The general objective of the Janus serum bank [31] precludes the collection of detailed information on covariates which may be of importance for specific diseases. Thus, adjustment for risk factors for breast cancer, such as age at first birth, parity and age at menopause, were not possible in this study.

RESULTS

The mean values of fatty acids in serum phospholipids (Table 3) show that the concentration of fatty acids of the *n*-6 family was 81% of that of saturated fatty acids (ratio = 0.81, S.D. = 0.15). Analogously, the concentration of *n*-3 PUFA was 24% of that of the *n*-6 family (ratio = 0.24, S.D. = 0.11). Overall, there were no statistically significant differences between cases and controls in the mean values of any of the measured fatty acids (Table 4). Among women 55 years and younger the mean value of *n*-6 fatty acids was lower in cases than in controls ($P = 0.05$), which could wholly be attributed to the lower level of linoleic acid ($P = 0.014$).

The overall odds ratios of breast cancer according to levels of fatty acids in serum phospholipids (data not shown) did not show that fatty acid concentrations were associated (positively or negatively) with breast cancer risk. In relation to the primary component of the *n*-6 PUFA family, a negative association was suggested over quartiles of linoleic acid (χ for trend = -1.76, $P = 0.08$).

By restricting the analysis to women who were 55 years and younger (Table 5), the negative association with linoleic acid was strengthened. Women in the highest quartile (297 mg/l) had an odds ratio of 0.4 (95% confidence interval, 0.2 and 1.0) compared to women in the lowest quartile (225 mg/l) of linoleic acid. The inverse relation displayed a trend over quartiles of linoleic acid (χ for trend = -2.49, $P < 0.02$).

In this age group, there was a consistent tendency for a lower risk of breast cancer with increasing values for a number of fatty acids, although the trend was either not statistically significant, or it reached borderline significance. The latter was observed for total phospholipid fatty acids (all fatty acids combined, *P*

Table 3. Mean and quartile median values (in mg/l) of selected fatty acids in serum phospholipids, and ratios between selected combinations of fatty acids

Fatty acid	Mean (mg/l)	S.D.	Quartiles (median values) I	II	III	IV
Total	1189	213	950	1087	1233	1442
Saturated	570	93	476	536	586	667
Polyunsaturated (PUFA)	464	111	363	414	492	594
Monounsaturated	155	35	121	140	160	191
n-6 PUFA	375	83	293	354	410	476
n-3 PUFA	89	44	47	72	99	142
Ratio PUFA/saturated (P/S)	0.81	0.15	0.64	0.74	0.88	1.0
Ratio *n*-6/saturated	0.66	0.11	0.54	0.61	0.69	0.81
Ratio *n*-3/*n*-6	0.24	0.11	0.14	0.19	0.24	0.36
Linoleic (18 : 2*n*-6)	262	55	203	239	275	328
Dihomo-gamma-linolenic (20 : 3*n*-6)	25	9	17	22	27	36
Arachidonic (20 : 4*n*-6)	79	29	47	66	83	116
Alpha-linolenic (18 : 3*n*-3)	2.1	1.1	1.0	1.8	2.3	3.3
Eicosapentaenoic (20 : 5*n*-3)	18	15	6.3	10.4	16.1	35.2
Docosahexaenoic (22 : 6*n*-3)	57	27	28	43	62	90
Palmitic (16 : 0)	331	58	272	308	340	388
Stearic (18 : 0)	178	31	145	167	185	213
Oleic (18 : 1*n*-9)	118	27	92	106	121	145

Table 4. Mean values (mg/l) of selected fatty acids in serum phospholipids in cases and controls

Fatty acid	≤ 55 years		> 55 years		Total			
	Cases (n = 65)	Controls (n = 195)	Cases (n = 22)	Controls (n = 40)	Cases (n = 87)	S.D.	Controls (n = 235)	S.D.
Total fat	1118	1167	1351	1318	1177	228	1192	208
Saturated	539	559	648	627	567	103	571	90
Polyunsaturated	431	457	520	513	454	113	467	110
Monounsaturated	147	150	182	176	156	42	154	33
n-6 PUFA	350	373*	409	400	365	86	378	82
n-3 PUFA	81	84	111	113	89	44	89	44
Linoleic	244	263†	285	277	254	58	265	53
Dihomo-gamma-linolenic	23	25	30	28	25	9	26	8
Arachidonic	76	78	85	87	78	30	79	29
Alpha-linolenic	1.7	2.0	2.6	2.6	2.0	1.3	2.1	1.1
Eicosapentaenoic	16	16	25	27	18	15	18	15
Docosahexaenoic	52	54	69	68	56	27	57	27
Palmitic	316	325	374	362	331	67	331	54
Stearic	170	174	203	199	178	31	179	31
Oleic	113	115	139	131	119	33	117	24

*P = 0.049; †P = 0.014.

trend = 0.06); saturated fatty acids (P trend = 0.06); and n-6 PUFA (P trend = 0.05).

The n-3 PUFA family and its specific components EPA and DHA acids did not show any association (positive or negative) with breast cancer risk in these data (Tables 4 and 5). We also transformed fatty acid concentrations into weight per cent, as well as into logarithmic form; the latter to examine a possible non-linear association with n-3 PUFA, but this did not change the impression of no association. Also, there was no apparent relation with the ratio between polyunsaturated and saturated fat (P/S ratio), or with the ratio between n-3 and n-6 PUFA (n-3/n-6 ratio).

We also assessed the joint contribution of the various fatty acids to breast cancer risk among women 55 years and younger in a logistic regression analysis. The results confirmed the previous analysis, showing an inverse relation between linoleic acid and breast cancer risk, also after adjustment for n-3 fatty acids, saturated fat and total fat in serum phospholipids.

DISCUSSION

The most striking finding of this study was the inverse relation between linoleic acid in serum phospholipids and risk of breast cancer. Linoleic acid cannot be synthesised endogenously, and it is the principal precursor of the n-6 family of unsaturated fatty acids. It is mainly obtained from vegetables and vegetable oils in the diet. Measurements of linoleic acid in serum phospholipids may reliably reflect their dietary intake [36], implying that vegetables and their oils may have a protective effect on the risk of developing breast cancer. It should be noted that the inverse relation with linoleic acid was confined to women who were 55 years and younger, a finding which is in agreement with that of recently published studies [23–25].

Moreover, the inverse relation between linoleic acid and breast cancer risk among young women confirms the result of the only (to our knowledge) previous study which has used biochemical indicators of fat intake [25]. In contrast to our study, it was based on sera from newly diagnosed breast cancer cases, and could not exclude the possibility that fatty acid measurements may be lower among cases due to a preclinical effect of the disease.

The superior method of measuring long-term dietary intake of n-6 PUFA may be analysis of subcutaneous fat biopsies [21], whereas measurements in serum phospholipids to a greater extent may be affected by short-term fluctuations in the diet and to internal metabolic regulation. It seems unlikely, however, that such factors would distort measurements of serum from cases differently from that of controls. The non-differential misclassification which may typically arise would tend to produce conservative estimates of effect [35], and indicates that the estimated negative association with linoleic acid in this study may actually be weaker than the real effect.

In several of the human studies of dietary intake of n-6 PUFA and breast cancer, a negative, but weak association has been found [7–11], although a meta-analysis of 12 case–control studies [12], and a cohort study [17] suggest a positive relationship between fat intake and breast cancer, including polyunsaturated fat. Further support for an inverse relation has been suggested by studies of special populations [14, 37]. These have found individuals who primarily eat a vegetarian diet to be at approximately 10% lower risk of breast cancer than what would be expected from the national rates. These studies, however, included older women, and did not make any attempt to distinguish between possible age-specific effects. Hence, if diet affects breast cancer risk differently among pre- and postmenopausal women, such effects may not have been allowed to appear. In contrast to human studies, experiments have consistently shown a positive association between dietary intake of linoleic acid and mammary carcinoma in rodents [4, 5], and further suggested that linoleic acid is a necessary requirement for tumour growth [22].

Another intriguing question is whether the inverse relation with linoleic acid in the human studies may reflect an association with *cis* (and not *trans*) fatty acids [6]. Linoleic acid in the *trans* position is derived from processed vegetables, such as margarine, in contrast to the natural *cis* form. In the present study, the analysis of linoleic acid separated *cis* and *trans* isomers. Thus,

Table 5. Odds ratio of breast cancer in women 55 years or younger, according to quartiles of fatty acids in serum phospholipids

	Q-I	Q-II	Q-III	Q-IV	χ trend (*P* value)
Total					
Cases	20	20	13	12	
Controls	47	44	51	53	
Odds ratio	1.0	1.1	0.6	0.5	−1.87
95% C.I.		(0.5–2.3)	(0.3–1.3)	(0.2–1.2)	(0.06)
Saturated fat					
Cases	21	19	12	13	
Controls	46	45	53	51	
Odds ratio	1.0	0.9	0.5	0.6	−1.85
95% C.I.		(0.4–2.0)	(0.2–1.1)	(0.3–1.2)	(0.07)
Polyunsaturated fatty acids (PUFA)					
Cases	21	16	15	13	
Controls	46	48	50	51	
Odds ratio	1.0	0.7	0.7	0.6	−1.47
95% C.I.		(0.3–1.6)	(0.3–1.4)	(0.3–1.2)	(0.14)
Monounsaturated fatty acids					
Cases	23	14	12	16	
Controls	44	50	53	48	
Odds ratio	1.0	0.5	0.4	0.6	−1.34
95% C.I.		(0.35–1.2)	(0.2–1.0)	(0.3–1.4)	(0.18)
n-6 PUFA					
Cases	25	13	13	14	
Controls	42	51	52	50	
Odds ratio	1.0	0.4	0.4	0.5	−1.97
95% C.I.		(0.2–0.9)	(0.2–0.9)	(0.2–1.0)	(0.05)
n-3 PUFA					
Cases	20	13	17	15	
Controls	47	51	48	49	
Odds ratio	1.0	0.6	0.8	0.7	−0.57
95% C.I.		(0.3–1.3)	(0.4–1.8)	(0.3–1.6)	(0.57)
Ratio between PUFA and saturated (P/S)					
Cases	19	15	19	12	
Controls	48	49	45	53	
Odds ratio	1.0	0.8	1.1	0.6	−0.98
95% C.I.		(0.4–1.7)	(0.5–2.3)	(0.3–1.3)	(0.33)
Ratio between *n*-3 and *n*-6 PUFAs (*n*-3/*n*-6)					
Cases	18	12	18	17	
Controls	49	52	46	48	
Odds ratio	1.0	0.6	1.1	1.0	−0.32
95% C.I.		(0.3–1.4)	(0.5–2.3)	(0.4–2.1)	(0.75)
Linoleic acid (18 : 2*n*-6)					
Cases	26	15	10	14	
Controls	41	49	55	50	
Odds ratio	1.0	0.5	0.3	0.4	−2.49
95% C.I.		(0.2–1.0)	(0.1–0.7)	(0.2–1.0)	(0.02)
Dihomo-gamma-linolenic acid (20 : 3*n*-6)					
Cases	21	17	15	12	
Controls	46	47	50	52	
Odds ratio	1.0	0.8	0.7	0.5	−1.72
95% C.I.		(0.4–1.7)	(0.3–1.4)	(0.2–1.1)	(0.09)
Arachidonic acid (20 : 4*n*-6)					
Cases	19	15	17	14	
Controls	48	49	48	50	
Odds ratio	1.0	0.8	0.9	0.7	−0.70
95% C.I.		(0.4–1.7)	(0.4–1.9)	(0.3–1.6)	(0.48)
Alpha-linolenic acid (18 : 3*n*-3)					
Cases	23	15	11	16	
Controls	44	50	53	48	
Odds ratio	1.0	0.6	0.4	0.6	−1.44
95% C.I.		(0.3–1.2)	(0.2–0.9)	(0.3–1.4)	(0.15)
Eicosapentaneoic acid (EPA; 22 : 5*n*-3)					
Cases	18	15	16	16	
Controls	49	49	49	48	
Odds ratio	1.0	0.8	0.9	0.9	−0.19
95% C.I.		(0.4–1.8)	(0.9–0.4)	(0.4–2.0)	(0.85)
Docosahexaenoic acid (22 : 6*n*-3)					
Cases	20	13	19	13	
Controls	47	51	46	51	
Odds ratio	1.0	0.6	1.0	0.6	−0.83
95% C.I.		(0.3–1.3)	(0.5–2.1)	(0.3–1.3)	(0.41)
Palmitic acid (16 : 0)					
Cases	21	17	14	13	
Controls	46	47	51	51	
Odds ratio	1.0	0.8	0.6	0.6	−1.59
95% C.I.		(0.4–1.7)	(0.3–1.3)	(0.3–1.2)	(0.11)
Stearic acid (18 : 0)					
Cases	22	15	12	16	
Controls	45	49	53	48	
Odds ratio	1.0	0.6	0.5	0.7	−1.21
95% C.I.		(0.3–1.4)	0.2–1.0)	(0.3–1.5)	(0.23)
Oleic acid (18 : 1*n*-9)					
Cases	25	11	12	17	
Controls	42	53	53	47	
Odds ratio	1.0	0.3	0.4	0.6	−1.34
95% C.I.		(0.2–0.8)	(0.2–0.8)	(0.3–1.3)	(0.18)

C.I. = Confidence interval.

our finding of a negative association between linoleic acid in serum phospholipids and risk of breast cancer among women 55 years and younger is based on measurements of the *cis* form of linoleic acid, and not on the *trans* isomer. Insofar as phospholipid linoleic acid reflects dietary intake, our finding might further suggest that a protective effect of linoleic acid may be present among women who primarily use vegetable oil in cooking, and in general have a higher intake of vegetables. Alternatively, our finding may suggest that these women benefit from the absence of high intakes of other, saturated fatty acids, maybe having more fruit and vegetables, which provide a range of micronutrients with a putative cancer prevention effect.

We made particular effort to insure comparability between cases and controls with respect to age and storage time of blood sera, in order to avoid bias in selection of subjects, and in misclassification of fatty acid measurements. The fact that sera of cases were obtained up to several years before breast cancer was diagnosed may be particularly important in this respect, since it minimises the possibility that a preclinical effect of the disease has systematically lowered fatty acid levels among cases. Several years of storage time may distort biochemical measurement of the various fatty acids, but such changes are likely to have affected cases and controls in similar ways, and not to have seriously disturbed the relation between them.

A shortcoming of this study is our inability to control for well-known risk factors for breast cancer in the statistical analysis. However, we are not aware of studies which have shown a correlation between such factors (e.g. menarcheal age, age at first birth, parity) and polyunsaturated fatty acids in blood serum. Consequently, one would not anticipate confounding between such risk factors of breast cancer and serum fatty acids based on prior knowledge. On the other hand, greater attention

should be paid to the unresolved area of how to evaluate the inter-relationship between various fatty acids [21]. Thus, our general ignorance of basic biological interactions between serum phospholipid fatty acids may be a greater threat to the validity of this study.

Animal studies have suggested that supplementing large amounts of marine oil (*n*-3 PUFA) in the diet may decrease incidence and inhibit growth rate of mammary carcinomas in rodents [28]. Some epidemiological studies have given support to a negative association between fish consumption and breast cancer risk [16, 29], although the evidence is relatively weak. In this study, we measured *n*-3 fatty acids in serum phospholipids, a method which may be superior to most other approaches [21]. Moreover, it has been shown that EPA and DHA acids in serum correlate fairly well with reported fish intake [26, 27]. Nonetheless, we found no association (positive or negative) with breast cancer risk in this study.

One should not exclude the possibility that the lack of an association between *n*-3 PUFA in serum phospholipids and breast cancer risk may be due to methodological difficulties. The general problem of more rapid degradation (peroxidation) of *n*-3 PUFA than, e.g. *n*-6 PUFA during long periods of sample storage [38] provides one such potential explanation. Another may be the deterioration of serum phospholipids over time, either by auto-oxidation or by lipase and/or phospholipase activity present in the samples [39, 40]. It seems conceivable that such mechanisms might have distorted the relative concentration of *n*-3 PUFA between cases and controls in such a way as to produce a null finding.

Another possibility might be that a beneficial effect of *n*-3 PUFA on breast cancer risk may require a relatively high intake of fish. Thus, fish consumption in this population may be insufficient to achieve any benefit, or the range between high and low consumers may be too narrow to allow a risk benefit to appear. The latter possibility seems unlikely, however, since the measured difference between the lowest and the highest quartile of *n*-3 fatty acids are close to what has previously been shown in an experimental study, supplementing the diet with 5 g per day of a highly purified fish oil [34].

Several studies have indicated that linoleic acid, both measured with dietary [23, 24] and biochemical [25] methods, appears to be negatively associated with breast cancer risk among women at premenopausal age, whereas the relation after the menopause is unclear. In males, it has been shown that overall cancer mortality may be unaffected by reported intake of *n*-6 or *n*-3 fatty acids [41], whereas the inter-relation between them (the *n*-3/*n*-6 ratio) was negatively associated with the overall mortality of cancer. Since different cancers represent separate aetiological entities which may be further modified by age and gender, we find it difficult to relate linoleic acid to any general association with cancer.

In fact, the results of this study indicate a specific negative association between linoleic acid and breast cancer risk, which may be confined to pre- and perimenopausal women. Possibly, this suggests that there may be a biological interaction between linoleic acid and ovarian hormones, indicating that high consumption of linoleic acid may somehow reduce the net effects of oestrogen in breast tissue [24].

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Urban–Rural Variation in Cancer Incidence in Denmark 1943–1987

Søren Friis and Hans H. Storm

Urban and rural cancer incidence in Denmark in 1943–1987 was analysed. A consistent urban excess was found for all sites combined for individuals of each sex, irrespective of age at diagnosis. The capital:rural incidence ratio was 1.42 for men and 1.25 for women, and these ratios were not affected to any great extent using another definition of urban areas. Urban:rural ratios were highest for cancers of the respiratory, urinary and upper digestive tracts. The differences cannot be explained by tobacco and alcohol consumption alone. Other risk factors linked to urbanisation may contribute importantly to the "urban factor", and analytical studies of data at an individual level are required to establish their relative importance. Our findings contradict the generally accepted view that there is no urban–rural difference in cancer incidence in the relatively small, homogeneous population of Denmark.
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INTRODUCTION

IN MOST countries for which data are available, clear differences in cancer incidence exist between urban and rural populations [1]. In general, cancer is more frequent in urban populations, and morbidity and mortality rates for many cancer types increase concurrently with increasing degrees of urbanisation. Only a few cancers (e.g. lip and stomach) have been reported to occur at higher rates in rural areas.

Urban–rural variations in cancer incidence have been studied since the phenomenon was first observed, in the 1930s, as the basis for hypotheses on the aetiology of cancer. These are that environmental changes and different social and cultural behavioural patterns linked to urbanisation are responsible for the excess risk of developing cancer in urban areas. Difficulties still exist, however, in disentangling the specific aetiological factors responsible for variations in cancer occurrence between urban and rural populations.

In Denmark, urban–rural differences in cancer incidence were explored systematically at the Danish Cancer Registry until 1977 [2]. It was discontinued when annual incidence reports emerged, due both to difficulties in achieving comparable data with other countries for reasons outlined by Roginski [3] and to the generally accepted view that Denmark of today is a fairly uniform area in terms of urbanisation. Moreover, the effects of urban life would be expected to be lessened due to higher mobility of the population, including migration of healthy people from rural to urban areas. In order to test whether these assumptions are true, we considered it of interest to analyse the trends in urban–rural differences in cancer incidence in Denmark for the entire period for which incidence data are

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