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PREDIAGNOSTIC LEVEL OF FATTY ACIDS IN SERUM PHOSPHOLIPIDS: Ω-3 AND Ω-6 FATTY ACIDS AND THE RISK OF PROSTATE CANCER

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Ecological and case-control studies have demonstrated a positive correlation between consumption of fat and the risk of prostate cancer. Two recent human studies have focused on α-linolenic acid as a risk factor for prostate cancer. Animal experiments have shown that dietary ω-6 polyunsaturated fatty acids have generally stimulated tumour development, whereas ω-3 polyunsaturated fatty acids have diminished it. The aim of our study was to investigate the association between these fatty acids and the subsequent risk of prostate cancer. Blood donors to the Janus serum data bank in Norway, who later developed prostate cancer, were matched to blood donors without prostate cancer (141 matched sets); the proportional level of fatty acids measured before diagnosis in the donors' serum was examined. The risk of later prostate cancer was analysed by conditional logistic regression. Increasing risk for prostate cancer was found with increasing quartiles of palmitoleic, palmitic and α-linolenic acid. An inverse risk association was found with increasing levels of tetracosanoic acid, for the ratios of linoleic to α-linolenic acid and arachidonic to eicosapentaenoic acid. There was no clear association between the risk effect of total ω-3 and total ω-6 fatty acids. There were no indications of a relationship between fatty acids and more aggressive cancers. Our results verify recent findings of a positive association between α-linolenic acid and a negative association between the ratio of linoleic to α-linolenic acid and the risk of prostate cancer. *Int. J. Cancer* 71:545–551, 1997.

Ecological studies first demonstrated a correlation between consumption of fat and death from prostate cancer (Armstrong and Doll, 1975). Later, case-control studies added support to a positive association with dietary fat, particularly saturated and animal fat (Le Marchand *et al.*, 1994; Franceschi, 1994; Mettlin *et al.*, 1989). A large prospective study of 51,529 American men showed a positive association between α-linolenic acid (LNA $C_{18:3}$, ω-3) in the diet and prostate cancer (Giovannucci *et al.*, 1993). A nested case-control study on plasma lipid levels and the development of prostate cancer suggested that low plasma levels of α-linolenic acid might be associated with reduced risk of prostate cancer (Gann *et al.*, 1994). Animal studies indicate that some fatty acids (FAs) in the diet might influence the development of cancer. Wang *et al.* (1995) showed that lowering the quantity of fat as a proportion of total calories decreased the growth rate of human prostate adenocarcinoma cells in mice. *In vitro* studies of human prostate cancer cells (PC-3) showed stimulated growth in the presence of linoleic acid (LA, $C_{18:2}$, ω-6), whereas long-chain ω-3 FAs (*e.g.*, docosahexaenoic acid (DHA, $C_{22:6}$, ω-3) and eicosapentaenoic acid (EPA, $C_{20:5}$, ω-3) may inhibit tumorigenesis in these cell lines (Rose and Connolly, 1991). Possibly, ω-6 FAs might be necessary for the promotion of cancer in animals (Carroll, 1986*a*) and, in most cases, high ω-6 polyunsaturated FA diets have stimulated tumour development (Cave, 1991). Fish oils are generally rich in ω-3 FAs, and dietary supplements have been recommended in the prevention of cardiovascular disease. The direct effect of ω-3 FAs on cancer is not clear. Ecological studies have not shown any association between ω-3 FAs and prostate cancer. Varying results in cohort and case-control studies on diet and prostate cancer may be attributed to difficulties in the proper recording of dietary data. They might also signify a complex relationship between various FAs and prostate cancer.

The aim of our study was to investigate the association between serum phospholipid FA levels, particularly the long-chain polyunsaturated ω-3 and ω-6 FAs (PUFAs), and the subsequent risk of prostate cancer.

SUBJECTS AND METHODS

The candidate population (with prostate cancer) and the controls contributed serum to the Janus serum bank in Norway during 1973–1994; they were recruited from separate sources: 3 health surveys and 1 blood donor group. The study was carried out as a nested case-control study among men with no known prostate cancer at the time of blood sampling. Controls were matched to cases by place of residence (county), age (±1 year) and date of blood sample (±6 months), and they should have been alive at time of diagnosis for the cases. There were 2 controls to each case, resulting in 141 matched sets altogether. Prostate cancer cases were identified by matching the serum bank files with the Cancer Registry, using the unique 11-digit personal identification number that is allocated to every citizen of Norway. The Cancer Registry has registered new cases of cancer since 1953 through compulsory reporting from hospitals and pathology laboratories, and the completeness of prostate cancer reporting in Norway is close to 100%. The files of the Cancer Registry and Statistics Norway are also regularly matched in order to establish records of dates and causes of death.

The Janus serum bank is one of the largest collections of serum available, having been in operation since 1973. Its objectives have previously been described in detail (Jellum *et al.*, 1993). Patients with prior prostate cancer were excluded. The serum samples were frozen locally before transportation to the main storage room at a temperature of −25°C. If several blood samples were available, the oldest sample was used.

Laboratory analysis

Fatty acids were analysed blind, *i.e.*, the disease status of the donor was unknown. Serum lipids were extracted with *n*-butanol; the phospholipids were isolated by column chromatography after addition of the internal standard diheptadecanoylglycerophosphocholine and the antioxidant butylated hydroxytoluene (Sigma, Poole, UK). The phospholipids were transmethylated and quantified by gas-liquid chromatography. A normal human serum sample was included as a control to monitor and secure analytic performance. Results were primarily quantified in milligrams of phospholipid fatty acid per litre of serum (mg/l), but the statistical analysis was done on proportional content. The analytic procedures have been described in detail elsewhere (Bjerve *et al.*, 1993).

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TABLE I – MEAN VALUES AND STANDARD DEVIATIONS (SD) AND MEAN PROPORTION AND SD OF FATTY ACIDS IN SERUM PHOSPHOLIPIDS AMONG CASES AND CONTROLS SEPARATELY, AND *p* VALUES FOR DIFFERENCES AND UPPER QUARTILE LIMITS BASED ON CONTROLS[1]

Fatty acid	Cases and controls combined		Cases (n = 141), mean proportion		Controls (n = 282), mean proportion		*p* values[1]	Quartiles, upper limits		
	Mean (mg/l)	SD	(%)	SD	%	SD		1	2	3
Groups of FAs										
Saturated fat	624.7	108.6	47.5	2.48	47.2	2.25	0.50	45.93	47.03	48.43
Monounsaturated fat	177.0	16.1	13.5	2.00	13.2	1.81	0.96	11.98	13.10	14.37
Polyunsaturated fat (PUFA; ω-6)	421.6	91.1	31.5	3.63	32.0	3.60	0.29	29.53	31.99	34.61
Polyunsaturated fat (PUFA; ω-3)	100.5	47.9	7.4	3.21	7.5	3.15	0.39	5.23	6.78	9.07
Total percentage of FA groups			99.9		99.9					
Single FAs										
Myristic (C14:0)	5.4	2.1	0.4	0.14	0.4	0.13	0.18	0.32	0.38	0.47
Palmitic (C16:0)	353.1	66.8	27.9	2.36	26.1	1.46	<0.05	25.40	26.47	27.82
Palmitoleic (C16:1)	4.9	4.3	0.4	0.28	0.3	0.25	0.24	0.21	0.28	0.42
Stearic (C18:0)	193.3	31.3	14.7	1.26	14.7	1.14	0.62	13.87	14.65	15.36
Oleic (C18:1, ω-9)	131.1	36.5	10.0	1.78	9.8	1.60	0.62	8.63	9.68	10.75
Linoleic (LA; C18:2, ω-6)	303.4	68.1	22.9	3.80	23.1	3.60	0.58	20.51	22.88	25.38
α-linolenic (LNA; C18:3, ω-3)	2.0	1.1	0.2	0.08	0.1	0.08	0.02	0.11	0.15	0.19
Arachidic (C20:0)	10.5	2.9	0.8	0.21	0.8	0.18	0.06	0.68	0.80	0.91
Eicosenoic (C20:1)	3.3	1.9	0.3	0.14	0.3	0.15	0.46	0.17	0.21	0.29
Eicosadienoic (C20:2, ω-6)	7.1	2.8	0.5	0.18	0.5	0.16	0.33	0.43	0.50	0.62
Dihomo-γ-linoleic (C20:3, ω-6)	28.4	11.1	2.1	0.69	2.1	0.62	0.78	1.67	2.04	2.55
Arachidonic (AA; C20:4, ω-6)	78.3	25.9	5.7	1.39	5.9	1.43	0.24	4.86	5.68	6.68
Eicosapentaenoic (EPA; C20:5, ω-3)	22.1	18.4	1.6	1.23	1.7	1.36	0.52	0.81	1.24	2.00
Docosanoic (C22:0)	44.7	14.5	3.3	0.99	3.4	0.89	0.07	2.76	3.32	3.92
Docosapentanoic (DPA; C22:5, ω-3)	14.4	5.3	1.0	0.30	1.1	0.29	0.19	0.88	1.04	1.23
Docosahexaenoic (DHA; C22:6, ω-3)	62.1	27.7	4.6	1.76	4.7	1.80	0.55	3.29	4.30	5.67
Tetracosanoic (C24:0)	17.8	6.0	1.3	0.39	1.4	0.38	<0.01	1.08	1.31	1.61
Tetracosenoic (C24:1, ω-9)	36.3	12.7	2.7	0.81	2.8	0.82	0.09	2.17	2.67	3.19
Other FAs[2]	6.8		0.4		0.5					
Total percentage of single FAs			100.0		100.2					

[1]*p* values for differences between cases and controls on proportions of the various FAs.–[2]Other FAs include: C20:3, ω-9, C22:1, C22:4, ω-6, C22:5, ω-6 (not included in analysis).

Data elements and statistical analyses

Twenty-two FAs were measured as well as sums of FA groups. Ratios between some of the FAs were also calculated. Analyses were done primarily on differences in the proportional content of the various FAs. For each FA, the values for cases and controls were divided into quartiles, based on the distribution of values among controls. Place of residence and dates of blood donation, diagnosis and death were registered. To carry out some subanalyses on prostate cancer risk, data were categorised for age (<60 years and ≥60 years), cause of death (prostate cancer; other causes; unknown), histological differentiation (adenocarcinoma, differentiation not given; well-differentiated; moderately well-differentiated; poorly differentiated; not histologically examined/unknown), metastasis (no metastasis; metastasis; unknown) and lag time, *i.e.*, time from blood donation to occurrence of prostate cancer (<10 years; ≥10 years).

Four FAs were present in very small amounts, and detectable values were found in less than half of the studied individuals: eicosatrienoic ($C_{20:3}$, ω-9), docosenoic ($C_{22:1}$), docosatetraenoic (adrenic, $C_{22:4}$, ω-6) and docosapentaenoic acid ($C_{22:5}$, ω-6). These FAs were excluded from further univariate regression analyses.

The risk of acquiring prostate cancer among the various FAs and FA groups was analysed by a conditional logistic regression model with multiplicative risk, using the EGRET statistical software package (Statistics and Epidemiology Research, Seattle, WA). Differences in average values between cases and controls were analysed by Wilcoxon's test for 2 related samples, using SPSS for Windows (Base System User Guide Release 6.0). The odds of being at risk for prostate cancer within the quartiles of FAs were estimated using the lowest quartile of the particular FA as the reference category. Confidence intervals (95%) were estimated for the odds ratios, and these were tested for linear trends across the quartiles of FAs, represented by values 1–4. Two-sided tests were used, and statistical significance was noted for $p \leq 0.05$.

RESULTS

The mean values of FAs in serum phospholipids in the total material are given in Table I. The 5 dominant FAs, accounting for 80.2% of the total, were: palmitic acid ($C_{16:0}$; 26.7%); linoleic acid ($C_{18:2}$, ω-6; 23.0%); stearic acid ($C_{18:0}$; 14.7%); oleic acid ($C_{18:1}$, ω-9; 9.9%); and arachidonic acid ($C_{20:4}$, ω-6; 5.9%).

Table I also shows the mean proportional values for the various FAs and the significance of the differences between cases and controls by *p* values. The mean values for LNA, palmitic and tetracosanoic ($C_{24:0}$) acid were different between cases and controls. Docosatetraenoic acid had detectable concentrations for 63.8% of cases but in only 46.1% of controls, with a higher mean proportion ($p < 0.01$) among controls (0.33%) than among cases (0.21%). A higher mean percentage of arachidic ($C_{20:0}$) and docosanoic ($C_{22:0}$) acid in cases than in controls was of borderline significance. Analyses were also carried out on the differences between FA values in the various geographic regions, in both absolute values and percentages, with no clear findings (data not shown).

The average time span from blood sampling to cancer diagnosis was 11.6 years (range, 3 months to 19.2 years). Average age at time of blood donation was 50 years.

Table II presents univariate analyses of each FA and its quartile values. Increasing odds ratios with increasing quartiles were found for palmitoleic acid, palmitic acid and LNA. An increasing risk trend of borderline significance was also found for myristic acid ($C_{14:0}$). Increasing levels of FAs were inversely associated with prostate cancer for tetracosanoic acid, but were statistically insignificant for arachidic, docosanoic and tetracosenoic acid ($C_{24:1}$, ω-9). Inverse associations were further found for the ratios between LA and LNA and between AA and EPA. Negative associations were found for total ω-6 PUFAs, as well as for the ratio between total ω-6 and total ω-3 PUFAs. The trends of the odds ratios for the FAs and the ratios mentioned above were almost consistently

present when analysed separately by stage, age, lag time and histological differentiation (data not shown).

Another study from the Janus serum bank on the prediagnostic content of serum FAs and cancer risk (Berg *et al.,* 1994) presented an inverse association between the sum of AA and DHA and the risk of thyroid cancer. In this study, we repeated the multivariate analyses of AA and DHA and their respective precursors LA and LNA, but did not find any inverse trend between these FAs and prostate cancer risk (not shown). Their analyses were, however, done on the basis of the absolute serum content and not of the proportion of FAs.

Multiple regression analyses were not performed because of strong correlations between most of the relevant FAs.

DISCUSSION

In the present study, controls were matched to cases by age, date of blood sample and residence. Because of this, and as both cases and controls have been blood donors or have taken part in intervention studies, differences in possible confounders are likely to be reduced. Matching by place of residence may introduce a bias, because the groups represent both inland and coastal areas, and intake of fish oils differs. This could obscure the results if dietary fish oil is a determinant of risk. Separate analyses of the differences in levels of the various FAs between the different geographic areas do not indicate the presence of such a bias. It is therefore unlikely that our matching procedures would introduce overmatching and thus reduce the ability of the study to detect an association.

Measurement of FAs can be done in different ways, but it is possible that the single best analysis of essential FA status is the PUFA patterns of serum phospholipids (Holman, 1986). This lipid class is richest in PUFAs, it is the principal lipid component of membranes and it responds most markedly to changes in dietary FAs (Holman, 1986). Tests of the laboratory method applied in this study have indicated that analysis of total phospholipid ω-3 FA concentrations can be used to quantify indirectly the dietary intake of ω-3 FAs on an individual basis (Bjerve *et al.,* 1993). Even the measured values of EPA, DHA and total ω-3 FAs exhibit a linear association with dietary intake (Bjerve *et al.,* 1993). As both cancer incidence and mortality in humans have been reported as having a positive association with caloric intake, and caloric intake may be related to tumorigenesis in animals (Carroll, 1986*b*), the analyses of FAs were based on their proportional content. The reason for choosing this procedure was to take account of the fact that diets high in fat also tend to be rich in calories (Carroll *et al.,* 1986). The quality of FAs under storage at −25°C has remained surprisingly stable (Jellum *et al.,* 1993). The day-to-day precision expressed as the coefficient of variation (CV) was measured by analysing a human control serum stored at −70°C. The CV for $C_{18:2}$, ω-6, $C_{18:3}$, ω-3, $C_{20:4}$, ω-6, $C_{20:5}$, ω-3 and $C_{22:6}$, ω-3 were 3.0%, 6.9%, 4.0%, 4.0% and 5.3%, respectively, for 31 consecutive days of analysis, at the phospholipid fatty-acid concentrations found in the population.

Inverse associations with prostate cancer risk were consistently found for total ω-6 PUFAs when doing subanalyses for the various parameters, although the inverse risk trend for total ω-6 PUFAs was not significant. Fish oils are high in EPA and DHA, and the estimated cancer risk linked to these 2 FAs was inconsistent with both positive and negative risks across the subanalyses.

The most striking result is that our study verifies, in regard to both LNA and LA, the findings of the detailed prospective cohort study of fatty acids by nutrient analysis, carried out by Giovannucci *et al.* (1993). The positive association found between LNA and prostate cancer is of particular interest because LNA and LA are essential FAs which can only be obtained from dietary sources. In agreement with the results of Giovannucci *et al.* (1993), we also found an inverse association between LA and prostate cancer risk (not statistically significant). This may suggest that the effects of dietary LA and marine FAs observed in animals do not apply to human prostate cancer (Carroll *et al.,* 1986).

Prostate cancer risk was positively associated with the saturated myristic and palmitic acids. It is noteworthy that myristic acid showed a positive association over all subanalyses, just like LNA. Saturated arachidic, docosanoic and tetracosanoic acids exhibited a consistently inverse association with prostate cancer risk. These FAs might not, however, provide a simple index of dietary intake (Hunter, 1990).

Whether the difference between cases and controls for docosatetraenoic acid has any biological significance is difficult to interpret. From laboratory findings we know that this FA is increased in serum phospholipids when the level of essential FAs is low, and in animal experiments it is higher in protein-deficient rats (Holman, 1986). This acid represents such a small proportion of FAs (0.3%) that the results for this FA could be erroneous.

In this study there was no difference in the proportional, prediagnostic serum content of saturated and mono- or polyunsaturated FAs between prostate cancer cases and controls. Thus, we could not confirm the hypothesis that saturated fat may increase the risk of prostate cancer, and that unsaturated fat may reduce this risk. However, a major proportion of FAs are also synthesised endogenously, which makes them poor markers of dietary intake. It should be kept in mind that the study group had a mean age at blood donation of about 50 years. Whether this had any influence on the results, apart from providing a low incidence of prostate cancer, is unclear.

We found no indications from our subanalyses that dietary fat is associated with advanced (aggressive) cancer, as reported in the study of Giovannucci *et al.* (1993). If dietary fat has a promoting effect at a late stage in the development of prostate cancer, diet assessment could be made closer to the time of diagnosis (Franceschi, 1994) than in most cohort studies. This could explain the lack of any sign that PUFAs may be a stimulus to more aggressive tumours in our study, because the mean lag time to cancer development was 11.6 years. Examining blood nearer to a cancer diagnosis may, however, be disturbed by effects of the cancer itself on fat values and metabolism.

There was no association between deaths from prostate cancer and the various FAs, analysed by cause-specific mortality, and we interpret this as another missing indication of fat as a promoter of aggressive tumours.

Giovannucci *et al.* (1993) discussed the relationships between LA and LNA, and pointed out that red meat and dairy products, which have been associated with advanced prostate cancer, are relatively high in LNA and low in LA. They suggest that by-products of LNA formed during cooking of meat may have a carcinogenic effect, and that LA may counter the effect of LNA. The nested case-control study of plasma lipid levels carried out by Gann *et al.* (1994), based on the US Physicians' Health Study, examined LA, LNA and prostate cancer risk, and FAs were measured as percentages of total FAs. A positive association with prostate cancer, irrespective of stage, was found for LNA, and this association was greater among men with a low LA and a reduced meat intake. This is in line with our observed inverse cancer risk association with increasing ratios of LA to LNA, and may well suggest that low plasma levels of LNA can be associated with reduced risk of prostate cancer, independent of high meat intake.

It is difficult to find any biological explanation for the inverse association of the LA/LNA ratio, although a competition for key enzymes between these 2 essential fatty acids is an evident possibility (Gann *et al.,* 1994). When ω-3 PUFAs are ingested, they are incorporated into tissue lipids in place of ω-6 PUFAs (Ip *et al.,* 1986), and ω-3 PUFAs may exert their effects by competing with AA for the cyclooxygenase and lipoxygenase enzymes. These regulate the synthesis of several active metabolites including prostaglandins, which may promote tumorigenesis, *e.g.,* by influencing the immune response (Cave, 1991; Carroll *et al.,* 1986;

TABLE II – NUMBER OF CASES OF PROSTATE CANCER (PCa) AND CONTROLS BY QUARTILES (Q1–4) OF FATTY ACIDS IN SERUM PHOSPHOLIPIDS, WITH ODDS RATIO FOR PCa WITH 95% CONFIDENCE INTERVAL (c.i.), AND *p* VALUES FOR TRENDS

Fatty acid	Q1	Q2	Q3	Q4	Trend, *p* values
Total(FAs combined)					
Cases	37	29	37	38	
Controls	69	77	68	68	
Odds ratio	1.0	0.7	1.1	1.1	
95% c.i.		(0.4–1.3)	(0.6–1.9)	(0.6–1.9)	0.6
Saturated fat					
Cases	34	38	29	40	
Controls	72	68	76	66	
Odds ratio	1.0	1.2	0.8	1.6	
95% c.i.		(0.6–2.2)	(0.4–1.6)	(0.7–3.3)	0.6
Polyunsaturated fat (PUFA)					
Cases	37	35	29	40	
Controls	69	71	76	66	
Odds ratio	1.0	0.9	0.7	1.1	
95% c.i.		(0.5–1.7)	(0.4–1.3)	(0.6–2.1)	0.9
Monounsaturated fat					
Cases	36	33	30	42	
Controls	70	73	74	65	
Odds ratio	1.0	0.9	0.8	1.3	
95% c.i.		(0.5–1.6)	(0.4–1.5)	(0.7–2.4)	0.4
ω-6 PUFA					
Cases	39	39	30	33	
Controls	68	65	75	74	
Odds ratio	1.0	1.0	0.6	0.7	
95% c.i.		(0.6–1.7)	(0.3–1.2)	(0.3–1.3)	0.1
ω-3 PUFA					
Cases	33	38	35	35	
Controls	73	68	70	71	
Odds ratio	1.0	1.2	1.1	1.1	
95% c.i.		(0.7–2.2)	(0.6–2.1)	(0.6–2.1)	0.9
Ratio of PUFA/saturated fat					
Cases	32	36	31	42	
Controls	74	70	74	64	
Odds ratio	1.0	1.3	1.1	1.8	
95% c.i.		(0.7–2.4)	(0.6–2.0)	(0.9–3.6)	0.16
Ratio of PUFAs ω-6/ω-3					
Cases	35	36	39	31	
Controls	71	70	66	75	
Odds ratio	1.0	1.0	1.2	0.8	
95% c.i.		(0.6–1.9)	(0.7–2.2)	(0.4–1.6)	0.7
Myristic (C14:0)					
Cases	32	33	35	41	
Controls	80	69	72	61	
Odds ratio	1.0	1.2	1.2	1.8	
95% c.i.		(0.7–2.1)	(0.7–2.1)	(1.0–3.3)	0.08
Palmitic (C16:0)					
Cases	29	34	36	42	
Controls	78	72	68	64	
Odds ratio	1.0	1.2	1.4	2.3	
95% c.i.		(0.7–2.2)	(0.8–2.5)	(1.1–4.7)	0.02
Palmitoleic (C16:1)					
Cases	28	37	30	46	
Controls	85	65	75	57	
Odds ratio	1.0	1.7	1.2	2.8	
95% c.i.		(1.0–3.1)	(0.7–2.2)	(1.5–5.1)	0.01
Stearic (C18:0)					
Cases	39	29	29	44	
Controls	67	78	75	62	
Odds ratio	1.0	0.7	0.7	1.3	
95% c.i.		(0.4–1.2)	(0.4–1.2)	(0.7–2.5)	0.4
Oleic (C18:1, ω-9)					
Cases	32	40	24	45	
Controls	75	64	83	60	
Odds ratio	1.0	1.4	0.7	1.8	
95% c.i.		(0.8–2.6)	(0.3–1.2)	(1.0–3.3)	0.3
Linoleic (LA; C18:2, ω-6)					
Cases	42	30	29	40	
Controls	64	75	76	67	
Odds ratio	1.0	0.6	0.6	0.9	
95% c.i.		(0.3–1.0)	(0.3–1.0)	(0.5–1.7)	0.7

TABLE II – NUMBER OF CASES OF PROSTATE CANCER (PCa)AND CONTROLS BY QUARTILES (Q1–4) OF FATTY ACIDS IN SERUM PHOSPHOLIPIDS, WITH ODDS RATIO FOR PCa WITH 95% CONFIDENCE INTERVAL (c.i.), AND *p* VALUES FOR TRENDS (Continued)

Fatty acid	Q1	Q2	Q3	Q4	Trend, *p* values
α-linolenic (LNA; C18:3, ω-3)					
Cases	30	38	35	38	
Controls	84	78	65	55	
Odds ratio	1.0	1.4	1.5	2.0	
95% c.i.		(0.8–2.5)	(0.9–2.7)	(1.1–3.6)	0.03
Arachidic (C20:0)					
Cases	45	37	24	35	
Controls	64	71	25	72	
Odds ratio	1.0	0.8	0.4	0.7	
95% c.i.		(0.4–1.3)	(0.2–0.8)	(0.4–1.2)	0.07
Eicosenoic (C20:1)					
Cases	33	39	37	32	
Controls	76	64	78	64	
Odds ratio	1.0	1.4	1.1	1.2	
95% c.i.		(0.8–2.5)	(0.6–2.0)	(0.6–2.2)	0.8
Eicosadienoic (C20:2, ω-6)					
Cases	35	38	35	33	
Controls	78	63	67	74	
Odds ratio	1.0	1.4	1.2	1.0	
95% c.i.		(0.8–2.6)	(0.7–2.3)	(0.5–1.9)	0.9
Dihomo-γ-linoleic (C20:3, ω-6)					
Cases	39	31	30	41	
Controls	67	74	76	65	
Odds ratio	1.0	0.7	0.7	1.1	
95% c.i.		(0.4–1.3)	(0.4–1.2)	(0.6–1.9)	0.9
Arachidonic (AA; C20:4, ω-6)					
Cases	35	36	39	31	
Controls	72	68	67	75	
Odds ratio	1.0	1.1	1.2	0.8	
95% c.i.		(0.6–1.9)	(0.7–2.1)	(0.4–1.5)	0.6
Eicosapentaenoic (EPA; C20:5, ω-3)					
Cases	32	37	37	35	
Controls	74	69	68	71	
Odds ratio	1.0	1.3	1.3	1.2	
95% c.i.		(0.7–2.2)	(0.7–2.4)	(0.6–2.1)	0.6
Docosanoic (C22:0)					
Cases	43	36	26	36	
Controls	64	70	77	71	
Odds ratio	1.0	0.7	0.5	0.7	
95% c.i.		(0.4–1.3)	(0.2–0.9)	(0.4–1.3)	0.1
Docosapentaenoic (DPA; 22:5, ω-3)					
Cases	42	37	28	34	
Controls	66	70	78	68	
Odds ratio	1.0	0.7	0.5	0.7	
95% c.i.		(0.4–1.4)	(0.3–0.9)	(0.3–1.3)	0.1
Docosahexaenoic (DHA; C22:6, ω-3)					
Cases	34	39	34	34	
Controls	72	67	72	71	
Odds ratio	1.0	1.2	1.0	1.0	
95% c.i.		(0.7–2.1)	(0.5–1.8)	(0.5–1.8)	0.8
Tetracosanoic (C24:0)					
Cases	43	40	30	28	
Controls	64	67	73	78	
Odds ratio	1.0	0.8	0.6	0.5	
95% c.i.		(0.5–1.5)	(0.3–1.0)	(0.3–0.9)	0.01
Tetracosenoic (C24:1, ω-9)					
Cases	41	36	31	33	
Controls	66	72	71	73	
Odds ratio	1.0	0.8	0.7	0.7	
95% c.i.		(0.4–1.3)	(0.4–1.2)	(0.4–1.3)	0.2
Ratio of LA/EPA (C18:2, ω-6/C20:5, ω-3)					
Cases	34	38	36	31	
Controls	73	64	70	75	
Odds ratio	1.0	1.3	1.1	0.8	
95% c.i.		(0.7–2.4)	(0.6–2.0)	(0.4–1.6)	0.5

TABLE II – NUMBER OF CASES OF PROSTATE CANCER (PCa) AND CONTROLS BY QUARTILES (Q1–4) OF FATTY ACIDS IN SERUM PHOSPHOLIPIDS, WITH ODDS RATIO FOR PCa WITH 95% CONFIDENCE INTERVAL (c.i.), AND *p* VALUES FOR TRENDS (Continued)

Fatty acid	Q1	Q2	Q3	Q4	Trend, *p* values
Ratio of LA/LNA (C18:2, ω-6/C18:3, ω-3)					
Cases	25	38	33	30	
Controls	26	68	72	77	
Odds ratio	1.0	0.5	0.4	0.3	
95% c.i.		(0.3–1.1)	(0.2–0.9)	(0.2–0.8)	0.01
Ratio of AA/EPA (C20:4, ω-6/C20:5, ω-3)					
Cases	35	48	25	31	
Controls	68	58	81	74	
Odds ratio	1.0	1.6	0.6	0.8	
95% c.i.		(0.9–3.0)	(0.3–1.2)	(0.4–1.5)	0.05
Ratio of stearic/oleic (C18:0/C18:1, ω-9)					
Cases	43	27	35	36	
Controls	63	79	70	70	
Odds ratio	1.0	0.5	0.7	0.8	
95% c.i.		(0.2–0.9)	(0.4–1.2)	(0.4–1.3)	0.6

Chaudry *et al.*, 1991). LA also serves as a precursor for AA, and by attenuating the biosynthesis of AA to prostaglandins, fish oils could influence the cancer process. The calculated ratios of LA/LNA, LA/EPA and AA/EPA all express ratios between ω-6 to ω-3 PUFAs, and they were all inversely associated with prostate cancer risk, possibly due to the mechanisms outlined above. A higher EPA/AA ratio has been linked to increased intake of marine oils and positively associated with cancer of the thyroid (Berg *et al.*, 1994). This is consistent with a negative association between the ratio of AA/EPA and prostate cancer in our study. EPA has also been shown to inhibit AA metabolism by both cyclooxygenase and lipoxygenase enzymes (Lee *et al.*, 1985), and may thus inhibit the formation of prostaglandins. The observed inverse risk association with the AA/EPA ratio could be consistent with this effect of EPA.

Several other hypotheses on biological mechanisms, apart from eicosanoid synthesis, have been proposed (Cave, 1991; Gann *et al.*, 1994) to explain how FAs could affect prostate cancer development. Our data do not allow further speculation on these issues.

A Norwegian study of PUFAs in serum and the risk of breast cancer (Vatten *et al.*, 1993) found an inverse association between LA and breast cancer risk in women aged 55 years and younger. No association was found between either ω-3 or ω-6 PUFAs and breast cancer risk. In contrast to the results in our study, they found an inverse association between both total fatty acid content and saturated fat, and risk of breast cancer (of borderline significance).

It has been suggested that a reduction in the stearic to oleic acid ratio may be related to malignant disease (Chaudry *et al.*, 1991), but our results did not support this hypothesis. Moreover, the original observations were in cancer patient groups, and might therefore be a consequence of the disease, rather than a diagnostic indicator.

CONCLUSIONS

Increasing risk for prostate cancer was found with increasing quartiles of palmitoleic, palmitic and α-linolenic acid. An inverse risk association was found with increasing levels of tetracosanoic acid, and for the ratios of LA/LNA and AA/EPA. There was no difference between prostate cancer cases and controls in the prediagnostic serum content of total saturated and mono- or polyunsaturated fatty acids. The relationships to the synthesis of prostaglandins, which may promote cancer, have been discussed. Generally, clear biological explanations are lacking. Studies of dietary fat and cancer risk retain great importance, particularly since so little is known about causal mechanisms.

REFERENCES

ARMSTRONG, B. and DOLL, R., Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer,* **15,** 617–631 (1975).

BERG, J.P., GLATTRE, E., HALDORSEN, T., HØSTMARK, A.T., BAY, I.G., JOHANSEN, A.F. and JELLUM, E., Long chain serum fatty acids and risk of thyroid cancer: a population-based case-control study in Norway. *Cancer Causes Control,* **5,** 433–439 (1994).

BJERVE, K.S., BRUBAKK, A.M., FOUGNER, K.J., JOHNSEN, H., MIDTHJELL, K. and VIK, T., Omega-3 fatty acids: essential fatty acids with important biological effects, and serum phospholipid fatty acids as markers of dietary ω3-fatty acid intake. *Amer. J. clin. Nutr.,* **57** (Suppl.), 801–806 (1993).

CARROLL, K.K., Biological effects of fish oils in relation to chronic diseases. *Lipids,* **21,** 731–732 (1986*a*).

CARROLL, K.K., Experimental studies on dietary fat and cancer in relation to epidemiological data. *Progr. clin. biol. Res.,* **222,** 231–248 (1986*b*).

CARROLL, K.K., BRADEN, L.M., BELL, J.A. and KALAMEGHAM, R., Fat and cancer. *Cancer,* **58** (Suppl.), 1818–1825 (1986).

CAVE, W.T., JR., Dietary n-3 (ω-3) polyunsaturated fatty acid effects on animal tumorigenesis. *FASEB J.,* **5,** 2160–2166 (1991).

CHAUDRY, A., MCCLINTON, S., MOFFAT, L.E.F. and WAHLE, K.W.J., Essential fatty acid distribution in the plasma and tissue phospholipids of patients with benign and malignant prostatic disease. *Brit. J. Cancer,* **64,** 1157–1160 (1991).

FRANCESCHI, S., Fat and prostate cancer (editorial). *Epidemiology,* **5,** 271–273 (1994).

GANN, P.H., HENNEKENS, C.H., SACKS, F.M., GRODSTEIN, F., GIOVANNUCCI, E.L. and STAMPFER, M.J., Prospective study of plasma fatty acids and risk of prostate cancer. *J. nat. Cancer Inst.,* **86,** 281–286 (1994).

GIOVANNUCCI, E., RIMM, E.B., COLDITZ, G.A., STAMPFER, M.J., ASCHERIO, A., CHUTE, C.C. and WILLETT, W.C., A prospective study of dietary fat and risk of prostate cancer. *J. nat. Cancer Inst.,* **85,** 1571–1579 (1993).

HOLMAN, R.T., Nutritional and functional requirements for essential fatty acids. *Progr. clin. biol. Res.,* **222,** 211–228 (1986).

HUNTER, D., Biochemical indicators of dietary intake. *In:* W.C. Willett (ed.),

Nutritional Epidemiology, pp. 143–216, Oxford, Oxford University Press (1990).

IP, C., IP, M.M. and SYLVESTER, P., Relevance of trans fatty acids and fish oil in animal tumorigenesis studies. *Progr. clin. biol. Res.,* **222,** 283–294 (1986).

JELLUM, E., ANDERSEN, A., LUND-LARSEN, P., THEODORSEN, L. and ØRJASÆTER, H., The Janus serum bank. *Sci. Total Environ.,* **139/140,** 527–535 (1993).

LEE, T.H., HOOVER, R.L., WILLIAMS, J.D., SPERLING, R.I., RAVALESE, J., III, SPUR, B.W., ROBINSON, D.R., COREY, E.J., LEWIS, R.A. and AUSTEN, K.F., Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.,* **312,** 1217–1224 (1985).

LE MARCHAND, L., KOLONEL, L.N., WILKENS, L.R., MYERS, B.C. and HIROHATA, T., Animal fat consumption and prostate cancer: a prospective study in Hawaii. *Epidemiology,* **5,** 276–282 (1994).

METTLIN, C., SELENSKAS, S., NATARAJAN, N. and HUBEN, R., Beta-carotene and animal fats and their relationship to prostate cancer risk. *Cancer,* **64,** 605–612 (1989).

ROSE, D.P. and CONNOLLY, J.M., Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. *Prostate,* **18,** 243–254 (1991).

VATTEN, L.J., BJERVE, K.S., ANDERSEN, A. and JELLUM, E., Polyunsaturated fatty acids in serum phospholipids and risk of breast cancer: a case-control study from the Janus serum bank in Norway. *Europ. J. Cancer,* **29A,** 532–538 (1993).

WANG, Y., CORR, J.G., THALER, H.T., TAO, Y., FAIR, W.R. and HESTON, W.D.W., Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. *J. nat. Cancer Inst.,* **87,** 1456–1462 (1995).