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Low alpha-linolenic acid content of adipose breast tissue is associated with an increased risk of breast cancer

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Abstract

Data derived from experimental studies suggest that alpha-linolenic acid may have a protective effect in breast cancer. Observations obtained from epidemiological studies have not allowed conclusions to be drawn about a potential protective effect of dietary alpha-linolenic acid on breast cancer, possibly because of methodological issues. This case–control study conducted in an homogeneous population from a central area in France was designed to explore the hypothesis that alpha-linolenic acid inhibits breast cancer, using fatty acid levels in adipose breast tissue as a biomarker of past qualitative dietary intake of fatty acids. Biopsies of adipose breast tissue at the time of diagnosis were obtained from 123 women with invasive non-metastatic breast carcinoma. 59 women with benign breast disease served as controls. Individual fatty acids were analysed by capillary gas chromatography. An unconditional logistic regression model was used to obtain odds ratio estimates whilst adjusting for age, menopausal status and body mass index (BMI). No association was found between fatty acids (saturates, monounsaturates, long-chain polyunsaturates n-6 or n-3) and the disease, except for alpha-linolenic acid which showed an inverse association with the risk of breast cancer. The relative risk of breast cancer for women in the highest quartile of adipose breast tissue alpha-linolenic acid level was 0.36 (95% confidence interval = 0.12–1.02) compared with those in the lowest quartile (P trend = 0.026), suggesting a protective effect of alpha-linolenic acid in the risk of breast cancer. The effects of dietary alpha-linolenic on the risk of breast cancer warrant further study.

Keywords: Alpha-linolenic acid; Adipose tissue; Breast cancer

1. Introduction

Data derived from animal experiments indicate that the tumour-promoting properties of high-fat diets may be more a function of differences in fatty acid composition than of fat content *per se* or of total caloric intake [1]. In several animal models, high-fat diets rich in n-6 polyunsaturated fatty acids (PUFA) generally stimulated mammary tumour development, growth and metastasis, whereas diets rich in n-3 PUFA appeared to inhibit tumour growth and metastasis [2]. However, the promotion phase of chemically induced carcinogenesis has been shown to be significantly suppressed when equal parts of high-fat diets rich in both n-6 and n-3 long-chain PUFA were fed [2]. Alpha-linolenic acid (18:3 n-3) is the main n-3 PUFA present in the Western diet and found in green vegetables and in several vegetable oils (rapeseed, soybean). Besides long-chain n-3 PUFA, the direct effects of this fatty acid on mammary tumour growth have also been investigated. High alpha-linolenic acid diets have been shown to inhibit the growth of spontaneous or carcinogen-induced mammary tumours [3–6], and the proliferation of human mammary tumour cells *in vitro*, independent of the production of long-chain n-3 PUFA [7].

Epidemiological studies in which alpha-linolenic acid intake has been assessed in individuals by dietary ques-

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tionnaire measurements are scarce and the findings inconsistent. Whilst one case–control study conducted in Italy showed a protective effect of unsaturated fatty acids, including alpha-linolenic acid, against breast cancer [8], another case–control study conducted in Uruguay reported an association between a high intake of dietary alpha-linolenic acid and an increased risk of breast cancer [9]. However, conclusive evidence for a role of alpha-linolenic acid in breast cancer risk may be precluded by the many methodological limitations in measurements of dietary intake of individual fatty acids [10].

In this regard, the use of reliable biochemical markers of relatively stable metabolic characteristics related to diet would be of major interest. Several biological markers of qualitative composition of dietary fat exist [11]. Among them, adipose tissue is particularly advantageous because its fatty acid composition reflects past qualitative dietary intake of fatty acids [12,13], and thus, would not be appreciably influenced by very recent modifications in diet. Among fatty acids, adipose tissue is known to best reflect dietary concentrations for PUFA [14,15]. Thus, adipose tissue PUFA composition may be appropriate to examine whether the type of PUFA is related to breast cancer risk. We have previously compared adipose tissue fatty acids levels with the risk of metastasis in patients treated for breast cancer [16], where an inverse association between the level of alpha-linolenic acid in breast fat and the development of metastasis subsequent to breast cancer treatment was reported. This suggests alpha-linolenic acid has a protective effect in the evolution of breast cancer [16]. However, the relationship of alpha-linolenic acid to the risk of breast cancer was not evaluated in this study.

To test the hypothesis that alpha-linolenic acid may have a protective role in breast cancer risk, we conducted a case–control study among an homogeneous population of 182 women treated for a breast tumour at the University Hospital of Tours in France, using adipose breast tissue fatty acid levels as a biomarker of qualitative dietary fatty acids.

2. Patients and methods

2.1. Study population

Data from a population of 123 patients with non-metastatic invasive breast carcinoma (case patients) and 59 women with benign breast disease (control patients) were used for the study. Patients' files were selected when a specimen of adipose tissue obtained during initial surgery was available; when pathology, staging (TNM) and treatment had been performed at the University Hospital of Tours. The population was ethnically homogeneous (all were Caucasian women) and lived in a limited area in central France, a region without significant population migration.

The distribution of cases and controls by age, body mass index (BMI) and menopausal status is given in Table 1. Clinical characteristics of breast carcinomas are presented in Table 2. Ninety-seven tumours were of the ductal type, 12 were lobular and 14 were of other types or were undetermined. Pathology of benign breast disease were dystrophy, non-proliferative ductal ectasia, cytosteatonecrosis, papilloma, fibroadenoma, low grade phyllod tumours and fibrosis.

Table 1
Characteristics of patients

	Control patients ($n=59$) n (%)	Breast cancer patients ($n=123$) n (%)	P[a]
Age (years)			
< 44	20 (34)	22 (18)	
44–52	20 (34)	27 (22)	
52–62	13 (22)	34 (28)	
> 62	6 (10)	40 (33)	0.002
BMI			
< 20.8	19 (32)	30 (24)	
20.8–23.1	15 (25)	26 (21)	
23.2–26.4	17 (29)	30 (24)	
> 26.4	8 (14)	35 (28)	0.15
Unknown		2 (02)	
Menopausal status			
No	37 (63)	49 (40)	
Yes	22 (37)	74 (60)	0.004

BMI, body mass index.
[a] P value for chi-square test.

Table 2
Clinical characteristics of breast carcinomas

	Number of patients (*n*) (%)
Tumour size (mm) (TNM)	
T0	2 (2)
T1 (< 20)	40 (33)
T2 (20–50)	63 (51)
T3 (> 50)	11 (9)
T4	7 (6)
Nodal status	
Positive	44 (36)
Negative	49 (40)
NA	30 (24)
Grade[a]	
I	11 (9)
II	70 (57)
III	35 (28)
NA	7 (6)
Oestrogen receptor (fml/mg)	
⩾10	89 (72)
< 10	17 (14)
NA	17 (14)
Progesterone receptor (fml/mg)	
⩽10	30 (24)
> 10	76 (62)
NA	17 (14)

NA, not available.

2.2. Adipose tissue preparation and fatty acids analysis

A fragment of adipose tissue was removed from the piece of lumpectomy or mastectomy during initial surgery. This fragment was freed from epithelial breast or carcinoma tissue and kept frozen in liquid nitrogen until analysis. The procedures for preparation of fatty acids have been reported elsewhere [17]. In summary, total lipids of the adipose tissue were extracted and triglycerides purified on one-dimensional silica gel G thin-layer chromatographic plates. Fatty acids were analysed as methyl esters by gas chromatography on a fused-silica capillary column, using an on-column injector and a flame ionisation detector, under operating conditions already described [18]. Fatty acids were identified by using commercial standards of known retention times (Nu-Check-Prep, MN, USA). Fatty acids were expressed as a percentage of total area. Unidentified peaks accounted for less than 3%. Intra- and interassay coefficients of variation (CV) were less than 1% for large peaks and reached 10% for the smallest peaks.

2.3. Statistical analysis

Odd ratios (ORs) and corresponding confidence intervals (CIs) were derived by using an unconditional logistic regression model after adjustment for age, menopausal status and BMI [19]. Because the risk of breast cancer associated with increased age or BMI is not linear, these continuous exposure variables were categorised in groups, using the quartiles of distribution (of cases and controls combined) as cut-off points to delineate four groups. The categorical variables were entered as indicator variables in the multivariate regression model. Tests for trend were performed by using the means within each category as modalities of a categorical variable in the logistic regression model. All analyses of the fatty acids were adjusted for age (in four classes), menopausal status and BMI (in four classes). Multivariate adjusted ORs were accompanied by 95% CIs. All P values quoted are two-sided.

3. Results

3.1. Clinical characteristics of the population study

The 182 patients included 123 case patients with invasive non-metastatic breast carcinoma and 59 control patients with benign breast disease (Table 1). There were significant differences in age and menopausal status distribution of case and control patients, with a higher proportion of case patients > 52 years of age and a higher proportion were post-menopausal. Whilst age ($P = 0.002$) and menopausal status ($P = 0.004$) were both of significance, no difference in BMI distribution was found.

3.2. Fatty acid composition of adipose breast tissue

The fatty acid composition of adipose breast tissue in case and control patients is presented in Table 3. Major fatty acids were considered as individual fatty acid levels, whilst peaks accounting for less than 0.05% of total area were taken into account in the corresponding summed classes (total saturates, total monounsaturates, total long-chain PUFA n-6 and n-3, total PUFA n-6 and n-3). For each group of patients, major fatty acids in adipose breast tissue were monounsaturated fatty acids, mainly represented by oleic acid, 18:1 n-9, followed by saturated fatty acids, mainly palmitic acid, 16:0, and n-6 PUFA, mainly linoleic acid, 18:2 n-6, and n-3 PUFA, mainly alpha-linolenic acid, 18:3 n-3. The mean values of individual fatty acids as well as the summed classes in adipose breast tissue were not different between case and control patients.

3.3. Relative risks (ORs) of breast cancer by adipose breast tissue fatty acid levels

An unconditional logistic regression model was used to evaluate the relation between breast cancer and fatty acids whilst adjusting for age, menopausal status and BMI. In the model, major fatty acids, including alpha-

Table 3
Fatty acid composition of adipose breast tissue

Fatty acids	Control patients ($n=59$) Mean % (S.D.)	Case patients ($n=123$) Mean % (S.D.)
Saturated		
14:0	3.46 (0.72)	3.27 (0.76)
16:0	22.94 (1.92)	22.80 (2.41)
18:0	5.94 (1.29)	5.86 (1.35)
Total[a]	33.02 (2.95)	32.15 (3.55)
Monounsaturated		
14:1	0.30 (0.14)	0.25 (0.11)
16:1	4.01 (1.15)	4.12 (1.42)
18:1	41.90 (2.49)	42.17 (3.08)
Total[b]	47.24 (3.05)	47.61 (4.03)
n-6 PUFAs		
18:2 n-6	14.75 (3.65)	14.73 (4.60)
LC n-6[c]	0.80 (0.25)	0.89 (0.33)
Total n-6	15.60 (3.78)	15.67 (4.68)
n-3 PUFAs		
18.3 n-3	0.45 (0.14)	0.42 (0.15)
LC n-3[d]	0.32 (0.15)	0.41 (0.17)
Total n-3	0.82 (0.24)	0.93 (0.29)
Total PUFAs	16.42 (3.76)	16.60 (4.71)

S.D., standard deviation.
[a] Included: 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 22:0.
[b] Included: 14:1; 16:1; 18:1; 20.1; 22.1.
[c] Included: 20:2; 20:3; 20:4; 22:4.
[d] Included: 20:5; 22:5; 22:6.

linolenic acid, were considered as individual levels, and minor peaks were taken into account in the summed classes. Table 4 shows age-, BMI- and menopausal status-adjusted relative risks of breast cancer calculated for individual fatty acid levels in adipose breast tissue. There was no association between individual saturated (16:0, 18:0) or monounsaturated (16:1, 18:1) fatty acid level and breast cancer risk. In addition, n-6 PUFA, 18:2 n-6 level, long-chain n-6 PUFA, n-3 PUFA or long-chain n-3 PUFA were not associated with breast cancer risk. In contrast, 18:3 n-3 levels in adipose breast tissue were inversely associated with breast cancer risk. The OR for breast cancer among women in the highest quartile of adipose breast tissue alpha-linolenic acid level was 0.36 (95% CI = 0.12–1.02) compared with women in the lowest quartile (trend $P=0.026$).

4. Discussion

In this study, the OR for breast cancer among women in the highest quartile of adipose breast tissue alpha-linolenic acid level compared with the lowest quartile was 0.36, suggesting a protective effect of alpha-linolenic acid on breast cancer risk. This observation on the risk of breast cancer is consistent with our previous observation, carried out in a cohort of 121 patients with an initially localised breast cancer, where we reported a link between a low level of alpha-linolenic measured in adipose breast tissue and an increased risk of metastatic development, subsequent to treatment [16]. We are aware of the limitations of our finding, because we designed our case–control study by adding a population of patients treated for a benign breast tumour, to the population of breast cancer patients previously used for the prognostic study [16]. Although all measures were performed in the same laboratory and using the same methods, methodological bias due to the time differ-

Table 4
Adjusted relative risks (ORs) and 95% CIs for breast cancer calculated for individual fatty acids levels in the adipose breast tissue among 182 women of the Tours area

	Category[a] (quartile)				
Fatty acid	1 (reference)	2	3	4	P^b
			Adjusted relative risks (95% CI)		
Saturated					
16:0	1[a]	0.49 (0.18–0.31)	0.82 (0.32–2.18)	0.90 (0.32–2.50)	0.869
18:0	1	0.81 (0.30–2.18)	1.72 (0.60–4.96)	2.01 (0.69–5.89)	0.115
Monounsaturated					
16:1	1	0.96 (0.37–2.49)	0.64 (0.26–1.61)	0.78 (0.29–2.08)	0.547
18:1	1	0.77 (0.31–1.90)	0.87 (0.34–2.21)	1.26 (0.48–3.34)	0.608
Polyunsaturated					
18:2 n-6	1	0.74 (0.28–1.93)	0.91 (0.35–2.39)	0.70 (0.27–1.82)	0.548
LC n-6	1	1.03 (0.42–2.49)	1.37 (0.51–3.66)	0.95 (0.30–3.08)	0.992
Total n-6	1	0.62 (0.24–1.64)	1.05 (0.39–2.87)	0.57 (0.22–1.49)	0.378
18:3 n-3	1	0.59 (0.21–1.66)	0.36 (0.14–0.93)	0.36 (0.12–1.02)	0.026
LC n-3	1	1.20 (0.48–2.96)	0.81 (0.30–2.24)	2.66 (0.80–8.89)	0.136
Total n-3	1	0.97 (0.39–2.39)	0.96 (0.39–2.37)	2.54 (0.88–7.29)	0.108

[a] Approximate quartiles for the entire study group were used. Values in parentheses are 95% confidence interval (CI). The lowest category served as the reference category. The reference group was subjects with non-proliferative benign breast disease. Results were adjusted for age. Body mass index (BMI) and menopausal status.
[b] Test for trend (*P*-value).

ences in the study cannot definitively be ruled out. To confirm the present finding, we are currently conducting a new, independent case–control study, in which we prospectively select new case and control patients.

Data derived from studies on mammary tumours carried out in experimental animal systems fit well with our observations on the apparent protection effect of alpha-linolenic acid. Diets rich in 18:3 n-3 have been shown to inhibit the growth of mammary carcinomas in animals [3–6], and the proliferation of human mammary tumour cells *in vitro* [7]. Epidemiological studies on estimated dietary intake of alpha-linolenic acid and breast cancer are scarce and data inconsistent [8,9]. Few studies have examined the fatty acid composition of adipose tissue in relation to breast cancer risk. Two case–control studies compared fatty acid levels of subcutaneous fat between case patients with breast cancer and control patients with benign breast disease [20,21]. In these two studies, no consistent association between breast cancer risk and any individual fatty acid stored was found. However, these studies were undertaken in women living in North America, where dietary habits are very different from those of our population, which was ethnically and nutritionally homogeneous. One study conducted in Finland reported a lower percentage of docosahexaenoic acid (22:6 n-3) in breast adipose tissue of patients with breast cancer compared with those of control patients with benign breast disease among postmenopausal women [22]. In this study, no association was found between the alpha-linolenic acid level measured in adipose breast tissue and breast cancer risk [22]. However, in agreement with our observation, a study comparing the fatty acid composition of adipose breast tissue between patients with breast cancer and control patients with a fibroadenoma, reported lower levels of alpha-linolenic and stearic acids in the case patients [23]. An ecological study undertaken in five European populations, that differ greatly in their dietary fat intakes as well as breast cancer risks, found an inverse association for the long-chain n-3 to n-6 ratio in subcutaneous adipose tissue with breast cancer in four of the five centres [24]. In this study, alpha-linolenic acid showed an overall inverse association with disease in two centres (Ireland, Switzerland) and a positive association in the other three populations [24]. Thus, no conclusions can be drawn about the possible protective effect that dietary alpha-linolenic acid may have against breast cancer, and further epidemiological studies integrating biological markers of dietary fatty acids, along with dietary data, are needed.

The significance of a low level of alpha-linolenic acid in adipose breast tissue associated with an increased risk of breast cancer observed in our study is not known. Fatty acid composition of subcutaneous adipose tissue reflects past dietary intake of fatty acids [12,13]. We have previously reported in breast cancer patients that 18:3 n-3 levels were positively correlated between breast and subcutaneous adipose tissue, suggesting that the level of this fatty acid in the breast fat site might actually reflect the body reserves of 18:3 n-3 [17]. Therefore, a possible cause of depleted 18:3 n-3 in adipose breast tissue may be insufficient dietary intake of this fatty acid. However, in contrast to the detailed information available concerning the relationships between estimated dietary linoleic or long-chain n-3 PUFA intake and their levels in adipose tissue, few studies have examined the association between the estimated dietary intake of alpha-linolenic acid and its level in adipose tissue. In two studies, a positive relationship between estimated dietary alpha-linolenic and its proportion in adipose tissue has been reported [25,26]. In another study, a weak correlation between estimated dietary intake of 18:3 n-3 and its level in subcutaneous adipose tissue has been found, but a higher correlation was observed in the subpopulation of patients with stable weight [14]. In our population, we have no dietary data from questionnaires; therefore, the possibility exists that a low level of alpha-linolenic acid in adipose breast tissue reflected a reduced dietary intake of alpha-linolenic acid.

Factors other than diet may be associated with a depletion of alpha-linolenic acid in adipose breast tissue from breast cancer patients compared with control patients. Whether this depletion is related to the vicinity of the carcinoma is not known. Metabolic interactions have been described between adipose tissue and breast epithelium [27] and the carcinoma may alter alpha-linolenic acid metabolism, leading to a decrease of this fatty acid in adipose tissue. The lack of relationships between alpha-linolenic acid level in adipose breast tissue and the size, as well as other characteristics, of the carcinoma suggests that the disease was not responsible for the low alpha-linolenic acid level [16]. Furthermore, using an animal system where mammary tumours have been induced by NMU, we reported a lack of an association of 18:3 n-3 in adipose tissue and mammary tumour growth [28]. Therefore, a low level of 18:3 n-3 as a consequence of breast cancer is very unlikely. Interestingly, it has been reported that 18:3 n-3 is more rapidly oxidised than all the other 16- to 22-carbon fatty acids [29]. In a previous study conducted in a different population of patients, we found a higher level of peroxides and a lower level of alpha-tocopherol in adipose breast tissue from breast cancer patients compared with control patients with benign breast disease [30]. In this study, we had no measurement of fatty acids, but the possibility that the depletion in alpha-linolenic acid could be due to an increase in its rate of oxidation deserves consideration.

In conclusion, our current data support a possible protective effect of alpha-linolenic acid in breast cancer risk. Further epidemiological and experimental data are

needed to precisely identify the role of dietary alpha-linolenic acid in breast cancer development.

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