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ORIGINAL PAPER

Relations of omega-3 and omega-6 intake with mammographic breast density

Caroline Diorio · Isabelle Dumas

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Abstract
Purpose Omega-3 (n-3) and n-6 fatty acids (FA) intake could influence the occurrence of certain diseases such as breast cancer but little is known about their relation to mammographic density (MD). The purpose of this study is to examine the association of the intake of n-3 FA and n-6 FA with MD among 777 premenopausal and 783 postmenopausal women.
Methods In this cross-sectional study, FA intake was assessed with a self-administered food-frequency questionnaire and MD was measured using a computer-assisted method. Multivariate analyses were performed by using generalized linear models to evaluate the associations of quartiles of FA intake with MD.
Results For increasing quartiles of total long-chain n-3 FA intake (< 0.11, 0.11–0.20, 0.21–0.32, and ≥ 0.33 g/day), adjusted mean MD was 29, 29, 27, and 25 %, respectively ($P_{\text{trend}} = 0.005$). This association remained significant among postmenopausal ($P_{\text{trend}} = 0.006$) but not among premenopausal ($P_{\text{trend}} = 0.21$) women. No significant association was found between n-6 FA intake and MD. However, for increasing quartiles of the n-6 FA/long-chain n-3 FA ratio intake (< 31.75, 31.75–52.28, 52.29–94.28, and ≥ 94.29), adjusted mean MD was 26, 27, 29, and 29 %, respectively ($P_{\text{trend}} = 0.008$).
Conclusions Higher intake of long-chain n-3 FA was associated with lower MD, suggesting that increased long-chain n-3 FA intake could be a strategy for breast cancer prevention.

Keywords Omega-3 · Omega-6 · Polyunsaturated fatty acid · Breast density · Breast cancer

Introduction

Human beings evolved on a diet with a ratio of omega-6 fatty acids (n-6 FA)/omega-3 fatty acids (n-3 FA) of approximately one while today in Western diet, this ratio is 15/1 [1], and this change warrants consideration because growing evidence suggests that n-3 FA and n-6 FA could play a role in the etiology of breast cancer. Animal models have shown that while n-6 FA exhibit a strong tumor-enhancing effect on mammary tissue, n-3 FA show a protective effect [2]. Experimental studies indicate that consumption of n-3 FA leads to the partial replacement of n-6 FA especially arachidonic acid (AA), by n-3 FA in the membrane of probably all cells in the body [3]. Fatty acids are converted to prostaglandins via the cyclooxygenase or lipoxygenase pathways to form eicosanoids [4]. Several studies on human and animal models demonstrate that n-3 FA-derived eicosanoids have anti-inflammatory, antiproliferative, and apoptotic properties while n-6 FA-derived eicosanoids have proliferative and

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C. Diorio (✉) · I. Dumas
Axe oncologie, Centre de recherche du CHU de Québec, 1050 Chemin Sainte-Foy, Quebec City, QC G1S 4L8, Canada
e-mail: cdiorio@uresp.ulaval.ca; caroline.diorio@uresp.ulaval.ca

C. Diorio
Centre des maladies du sein Deschênes-Fabia, Hôpital du Saint-Sacrement, Quebec City, QC, Canada

C. Diorio
Département de médecine sociale et préventive, Centre de recherche sur le cancer, Université Laval, Quebec City, QC, Canada

proinflammatory properties [1, 5–9]. The potency to suppress n-6 FA-derived ecosanoids is believed to be five times higher for long-chain n-3 (LC n-3 FA) than for n-3 FA alpha-linolenic acid (ALA) [10]. Also, FA are suspected to play a role in estrogen metabolism, which has proliferative properties on breast cells [11].

Ecological studies revealed that high per capita fish consumption, a good source of LC n-3 FA, is correlated to a lower incidence of breast cancer [12–14]. While most cohort and case–control studies that evaluated the relationship between fat intake and breast cancer risk considered polyunsaturated fatty acids as a group [15], several examined the individual effect that n-3 FA or n-6 FA consumption might have on breast cancer risk. Several groups, although not all [16–18], observed an inverse association between LC n-3 FA [19–23] or n-3 FA [22] intake and breast cancer risk, while others observed a positive association between intake of n-6 FA [17] or n-6 FA/n-3 FA ratio and breast cancer risk [24].

Mammographic density (MD), measured at mammography, reflects the proportion of the breast occupied by fibroglandular tissue and is strongly associated with breast cancer risk [25, 26]. As MD increases, so does the risk of developing breast cancer [25, 26]. Elevated MD represents a higher proportion of fibroglandular cells in the breast and therefore reflects a higher proliferative activity within this tissue [27]. Since the concentrations of n-3 FA- and n-6 FA-derived eicosanoids have been, respectively, related to a decrease and increase in cell proliferation, it would be reasonable to speculate that the intake of n-3 FA, especially LC n-3 FA, should be associated to lower MD, whereas the intake of n-6 FA or n-6 FA/n-3 FA ratio should show a positive association to MD. Only a few groups have assessed the relation of n-3 FA, LC n-3 FA or n-6 FA intake with MD and they observed null results [28–31]. One group examined the association of ALA intake with MD and they found a borderline significant negative association [32]. To our knowledge, the association between n-6 FA/n-3 FA ratio intake and MD has not been examined to date. The purpose of this study is to evaluate the relationship between the intakes of n-3 FA, n-6 FA, and n-6 FA/n-3 FA ratio with MD among a population of premenopausal and postmenopausal women.

Methods

Study population and data collection

The study design and methods were published previously [33, 34]. Briefly, study participants (777 premenopausal and 783 postmenopausal women) were recruited between February 2001 and March 2002 among women who received a screening mammogram at two private radiology clinics in Quebec City (Quebec, Canada). To be eligible, women had to be classified as premenopausal or postmenopausal according to the Nurses' Health Study's criteria [35]. Also, participants should have no personal history of cancer, breast reduction or implants, diabetes mellitus, dwarfism/acromegaly, thyroid, adrenal or hepatic disease, not be pregnant and have never taken tamoxifen or raloxifene, have not taken oral contraceptives or used hormone replacement therapy in the last 3 months before mammography. This study was reviewed and approved by the Research ethics committee of the Centre hospitalier universitaire de Québec, Quebec (QC), Canada. Study participants provided written informed consent.

Data collection

At the radiology clinic where the mammography was performed, women's weight (kg), height (cm), and waist and hip circumferences (cm) were measured by a trained research nurse who also collected the blood specimen (20 ml). Known or suspected breast cancer risk factors were documented by a telephone interview and included reproductive and menstrual history, family history of breast cancer, personal history of breast biopsies, past use of oral contraceptives and hormone replacement therapy, smoking status, alcohol intake, education, and physical activity. The level of physical activity, expressed as metabolic equivalent (METs)-h/week [36], was assessed using the Nurses' Health Study II Activity and Inactivity Questionnaire [37].

Diet was assessed with a self-administered semiquantitative food-frequency questionnaire (97 GP copyright Harvard University). In this questionnaire, women reported their intake of 161 specific food items in the past year. Estimation of the diet's nutrient content was performed at Harvard University, where dietary nutrient intake was calculated on the basis of the nutrient content of food derived from United States Department of Agriculture sources, supplemented with the data from food manufacturers and personal communications with laboratories. Women also provided the duration in number of years and daily dosage of fish oil supplements ($< 2{,}500$, 2,500–4,999, 5,000–9,999, $\geq 10{,}000$ mg) or if they currently used cod liver oil supplements at least once a week. The total nutrient intake was calculated by adding the amounts from fish or cod liver oil supplements to the intake from food.

Digitization of mammograms and the assessment of mammographic density

A craniocaudal view of a randomly selected breast was evaluated for each women after all mammograms were

digitized using a Kodak Lumiscan85 digitizer. Assessment of MD was performed by one trained author (CD) without any information on women, using a computer-assisted thresholding program (Cumulus) [38–40]. Variability in the assessment of MD was similar in premenopausal and in postmenopausal women: the within-batch intraclass correlation coefficient was 0.98 and 0.98 and the between-batch coefficient of variation was 4 and 5 % for percent and absolute density, respectively.

Statistical analysis

Univariate and multivariate generalized linear models were used to evaluate the associations between each quartile of a specific FA or of a group of FA and MD. Tests for trends (P_{trend}) were based on the F test of the linear contrast between quartiles of FA and MD. Analysis of covariance was used to provide adjusted estimates of the means of MD according to each quartile of nutrient intake. Both the absolute and percent MD were analyzed, but since results were similar for both, only those for the percent MD are presented. MD was square root-transformed for all analyses to obtain normal distribution. All analyses were performed among all women and by menopausal status. For each group of FA (n-3 FA, n-6 FA, LC n-3 FA, n-6 FA/n-3 FA, and n-6 FA/LC n-3 FA ratios), dietary nutrient intake and total nutrient intake (intake from food and supplements) were both considered separately as independent variables. Multivariate models were adjusted for potential confounders including age (years), body mass index (BMI) (kg/m^2), waist-to-hip ratio, alcohol intake in the past year (drinks/week), mean daily caloric intake in the past year (kcal/day), level of physical activity in the past year (METs-h/week), parity (yes/no), smoking status (non, former, or current smoker), age at menarche (years), number of full-term pregnancies, age at first full-term pregnancy (years), lactation (number of months), family history of breast cancer in a first-degree relative (yes/no), number of breast biopsies, education (highest completed degree: primary, secondary, college, university), duration of past oral contraceptives and hormonal replacement therapy uses (years) and height (cm). Analyses for all women combined were also adjusted for menopausal status. Further adjustment for the intake of saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates and mutual adjustment for the intake of LC n-3 FA, n-6 FA, and ALA when applicable were also evaluated. Univariate and multivariate nutrient density models, in which the nutrients are expressed in percent of energy, were also performed [41]. All statistical analyses were performed using the SAS software package (version 9.3; SAS institute Inc.). All tests were two-sided, and a p value < 0.05 was considered statistically significant.

Results

Characteristics of the study population are summarized in Table 1. The mean age at the time of mammography was 54.1 years (standard deviation (SD) 9.4 years). Among the 1,560 women, 777 were premenopausal and 783 were postmenopausal. The mean percent MD was 30.2 % (SD 24.0 %) for all women, 42.0 % (SD 24.3 %) for premenopausal women, and 18.5 % (SD 16.8 %) for postmenopausal women. Participants had a daily average intake of n-3 FA and LC n-3 FA of 1.44 g (SD 0.80 g) and 0.28 g (SD 0.33 g), respectively, and of this amount 1.39 g (SD 0.73 g) and 0.24 g (SD 0.19 g), respectively, derived from the diet. Almost all intakes of n-3 FA and n-6 FA originated from the diet as only 3.7 % of women in this study used n-3 FA or cod liver oil supplements. Superior amounts of n-6 FA were ingested on a daily basis by the women with a mean of 11.36 g (SD 5.55 g) coming from the diet. Because cod liver oil users were rare and because this type of supplement provides little n-6 FA, the total daily intake of n-6 FA remained the same as the daily dietary intake. The means of n-6 FA/LC n-3 FA ratio based on total and dietary daily intake were 84.90 (SD 115.00) and 87.07 (SD 116.00), respectively, whereas the means of n-6 FA/n-3 FA ratio based on total and dietary daily intake were 8.55 (SD 3.08) and 8.70 (SD 3.09), respectively. The daily intake of FA was somewhat similar among premenopausal and postmenopausal women; however, postmenopausal women appeared to ingest more LC n-3 FA than premenopausal women. The intake of n-6 FA/LC n-3 FA ratio was lower among postmenopausal [77.44 (SD 118.74)] than among premenopausal women [92.42 (SD 110.72)].

Among the foods or supplements listed in the food-frequency questionnaire, a few items were identified as explaining a large part of the variability in n-3 FA, LC n-3 FA, or n-6 FA. Mayonnaise and margarine accounted for 59, 56, and 62 % of the variance in n-3 FA intake among all, premenopausal, and postmenopausal women, respectively. Fish intake explained more than 97 % of the variance in LC n-3 intake among all, premenopausal, or postmenopausal women while mayonnaise explained about 41, 37 and 46 % of the variance in n-6 FA intake among all, premenopausal, and postmenopausal, respectively.

Data from the analyses of the relationships between FA intake and percent MD are presented in Table 2. In adjusted models, increasing quartiles of total and dietary LC n-3 FA intake (eicosapentaenoic acid (EPA) + docosapentaenoic acid (DPA) + docosahexaenoic acid (DHA)) were associated with lower MD among all ($P_{trend} = 0.005$ and 0.01, respectively) and postmenopausal women ($P_{trend} = 0.006$ and 0.01, respectively). Adjusted mean percent MD according to increasing quartiles of total LC n-3 intake was, respectively, 29, 29, 27, and 25 % among all women and 19, 19, 16, and

Table 1 Characteristics of the study population

	All (n = 1,560)	Premenopausal (n = 777)	Postmenopausal (n = 783)
Age [(year), mean ± SD]	54.1 ± 9.4	46.7 ± 4.6	61.4 ± 6.8
Age (year) at menarche (mean ± SD)	12.7 ± 1.6	12.8 ± 1.6	12.7 ± 1.6
Age (year) at first full-term pregnancy[a] (mean ± SD)	25.7 ± 4.2	26.3 ± 4.2	25.2 ± 4.1
Body mass index [(kg/m^2), mean ± SD]	26.1 ± 4.7	25.2 ± 4.5	27.1 ± 4.7
Waist-to-hip ratio (mean ± SD)	0.80 ± 0.06	0.78 ± 0.06	0.81 ± 0.06
Height [(cm), mean ± SD]	159.1 ± 5.9	160.5 ± 5.8	157.7 ± 5.6
Number of full-term pregnancy (mean ± SD)	1.8 ± 1.6	1.6 ± 1.1	2.1 ± 1.8
Lactation [(months)[a], mean ± SD]	3.4 ± 6.3	5.2 ± 7.6	1.5 ± 3.9
Physical activity [(metabolic equivalent h/week), mean ± SD]	26.3 ± 22.8	27.0 ± 22.3	25.7 ± 23.4
Contraceptive ever use (%)	72.6	91.8	53.6
Hormone replacement therapy ever use (%)	22.8	5.8	39.5
Family history of breast cancer in first-degree relative (%)	33.5	36.6	30.4
Personal history of breast biopsies (%)	15.3	14.4	16.1
Ex- or current smoker (%)	47.8	54.4	41.1
College or university diploma (%)	50.7	62.2	39.3
Omega-3 supplement and/or cod liver oil use (%)	3.7	2.4	4.9
Daily average intake, mean ± SD			
Dietary long-chain n-3 fatty acid (g)	0.24 ± 0.19	0.22 ± 0.17	0.25 ± 0.21
Total long-chain n-3 fatty acid (g)	0.28 ± 0.33	0.24 ± 0.24	0.31 ± 0.40
Dietary n-3 fatty acid (g)	1.39 ± 0.73	1.39 ± 0.68	1.40 ± 0.78
Total n-3 fatty acid (g)	1.44 ± 0.80	1.41 ± 0.70	1.46 ± 0.89
Dietary n-6 fatty acid (g)	11.36 ± 5.55	11.55 ± 5.19	11.17 ± 5.88
Total n-6 fatty acid (g)	11.36 ± 5.55	11.55 ± 5.19	11.17 ± 5.88
Dietary n-6 fatty acid-to-long-chain n-3 fatty acid ratio	87.07 ± 116.0	94.88 ± 113.27	79.33 ± 118.27
Total n-6 fatty acid-to-long-chain n-3 fatty acid ratio	84.90 ± 115.0	92.42 ± 110.72	77.44 ± 118.74
Dietary n-6 fatty acid-to-n-3 fatty acid ratio	8.70 ± 3.09	8.87 ± 2.99	8.52 ± 3.17
Total n-6 fatty acid-to-n-3 fatty acid ratio	8.55 ± 3.08	8.76 ± 2.92	8.33 ± 3.22
Energy intake (kcal)	1,942 ± 585	1,912 ± 521	1,971 ± 642
Alcohol intake [(drinks/week), mean ± SD]	3.0 ± 4.1	3.4 ± 3.8	2.5 ± 4.4
Mammographic density [(%), mean ± SD]	30.2 ± 24.0	42.0 ± 24.3	18.5 ± 16.8
Absolute density [(cm^2), mean ± SD]	34.9 ± 27.6	46.5 ± 28.7	23.3 ± 20.9
Absolute non-dense mammographic area [(cm^2), mean ± SD]	102.2 ± 64.9	79.6 ± 60.8	124.6 ± 61.0

[a] In parous women

16 % among postmenopausal women. No such trend was observed among premenopausal women (P_{trend} = 0.21 and 0.31, respectively). Assessment of the relationship between components of n-3 FA and MD showed that while ALA intake did not seem to be associated with MD, higher components of LC n-3 FA intake appeared to be related to lower MD. For instance, EPA and DHA intake was each related to lower MD among all (P_{trend} = 0.03 and 0.005, respectively) and postmenopausal women (P_{trend} = 0.02 and 0.03, respectively). DPA showed a similar trend, although associations were not statistically significant among all, premenopausal, and postmenopausal women (P_{trend} = 0.14, 0.28, and 0.37, respectively). The intake of total and dietary n-3 FA (ALA + EPA + DPA + DHA) was not associated to MD whether assessed among all, premenopausal, or postmenopausal women.

The total and dietary intake of n-6 FA (LA + AA) was not associated with percent MD whether assessed among all, premenopausal, or postmenopausal women (Table 2). The ratios of total or dietary n-6 FA/LC n-3 FA intake were each positively associated with MD among all (P_{trend} = 0.008 and 0.02, respectively), premenopausal (P_{trend} = 0.06 and 0.06, respectively), or postmenopausal women (P_{trend} = 0.08 and 0.13, respectively), although significant only among all women. The adjusted mean percent MD according to increasing quartiles of the ratio of total n-6/LC n-3 intake for all women were, respectively, 26, 27, 29, and 29 %. The ratios of total or dietary n-6 FA/n-3 FA intake showed no significant association to MD.

Table 2 Relations of n-3 and n-6 fatty acids intake with percent mammographic density

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]
Alpha-linolenic fatty acid (ALA)									
Quartile 1: < 0.69 g/day	389	31	28	176	47	43	213	19	17
Quartile 2: 0.69–0.99 g/day	388	31	27	207	40	38	181	19	18
Quartile 3: 1.00–1.45 g/day	391	30	27	199	41	40	192	19	17
Quartile 4: ≥ 1.46 g/day	392	29	28	195	40	41	197	17	17
$P^{a,b}_{trend}$		0.12	0.69		0.019	0.41		0.30	0.95
$P^{b,c}_{trend}$			0.45			0.54			0.64
Eicosapentaenoic fatty acid (EPA)									
Quartile 1: < 0.03 g/day	353	30	29	181	42	41	172	18	19
Quartile 2: 0.03–0.06 g/day	417	31	28	228	41	40	189	19	18
Quartile 3: 0.07–0.10 g/day	405	31	27	197	44	42	208	18	17
Quartile 4: ≥ 0.11 g/day	385	29	26	171	42	38	214	19	16
$P^{a,b}_{trend}$		0.53	0.026		0.64	0.47		0.80	0.020
$P^{b,d}_{trend}$			0.053			0.94			0.012
Docosapentaenoic fatty acid (DPA)									
Quartile 1: < 0.010 g/day	108	29	30	48	45	44	60	17	18
Quartile 2: 0.010–0.019 g/day	559	31	28	295	42	40	264	19	18
Quartile 3: 0.020–0.029 g/day	473	31	28	242	42	41	231	19	17
Quartile 4: ≥ 0.030 g/day	420	29	27	192	42	40	228	18	16
$P^{a,b}_{trend}$		0.85	0.14		0.39	0.28		0.67	0.37
$P^{b,d}_{trend}$			0.53			0.80			0.36
Docosahexaenoic fatty acid (DHA)									
Quartile 1: < 0.07 g/day	372	32	29	203	43	43	169	18	19
Quartile 2: 0.07–0.12 g/day	392	31	28	200	41	39	192	19	18
Quartile 3: 0.13–0.19 g/day	412	31	28	211	44	42	201	19	17
Quartile 4: ≥ 0.20 g/day	384	27	25	163	39	37	221	18	16
$P^{a,b}_{trend}$		0.018	0.005		0.32	0.064		0.70	0.032
$P^{b,d}_{trend}$			0.007			0.144			0.013
Long-chain n-3 fatty acid (EPA, DPA, and DHA)									
Quartile 1: < 0.11 g/day	394	32	29	217	42	41	177	19	19
Quartile 2: 0.11–0.20 g/day	375	31	29	187	42	40	188	19	19
Quartile 3: 0.21–0.32 g/day	400	31	27	202	43	42	198	18	16
Quartile 4: ≥ 0.33 g/day	391	28	25	171	41	38	220	18	16
$P^{a,b}_{trend}$		0.058	0.005		0.75	0.21		0.65	0.006
$P^{b,d}_{trend}$			0.009			0.52			0.002
Long-chain n-3 fatty acid from food only (EPA, DPA, and DHA)									
Quartile 1: < 0.11 g/day	405	32	29	224	42	41	181	19	19
Quartile 2: 0.11–0.20 g/day	387	30	28	189	41	40	198	19	18
Quartile 3: 0.21–0.31 g/day	383	31	27	190	44	42	193	19	16
Quartile 4: ≥ 0.32 g/day	385	28	26	174	41	38	211	18	16
$P^{a,b}_{trend}$		0.083	0.014		0.78	0.31		0.62	0.013
$P^{b,d}_{trend}$			0.061			0.89			0.011
N-3 fatty acid (ALA, EPA, DPA, and DHA)									
Quartile 1: < 0.91 g/day	386	32	28	188	46	44	198	18	16
Quartile 2: 0.91–1.26 g/day	390	32	28	211	42	39	179	19	19
Quartile 3: 1.27–1.75 g/day	393	30	27	188	42	40	205	18	17
Quartile 4: ≥ 1.76 g/day	391	28	28	190	38	39	201	18	18

Table 2 continued

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]
$P_{trend}^{a,b}$		0.035	0.50		0.006	0.11		0.89	0.56
$P_{trend}^{b,e}$			0.39			0.14			0.58
N-3 fatty acid from food only (ALA, EPA, DPA, and DHA)									
Quartile 1: < 0.90 g/day	385	32	29	188	46	44	197	18	17
Quartile 2: 0.90–1.24 g/day	394	31	27	210	42	38	184	19	19
Quartile 3: 1.25–1.71 g/day	389	30	27	182	42	40	207	19	17
Quartile 4: ≥ 1.72 g/day	392	28	27	197	39	40	195	17	17
$P_{trend}^{a,b}$		0.023	0.41		0.006	0.20		0.46	0.99
$P_{trend}^{b,e}$			0.40			0.40			0.91
N-6 fatty acid									
Quartile 1: < 7.57 g/day	389	29	28	166	44	40	223	19	18
Quartile 2: 7.57–10.32 g/day	390	30	28	201	42	40	189	18	18
Quartile 3: 10.33–13.75 g/day	391	32	27	214	44	41	177	19	17
Quartile 4: ≥ 13.76 g/day	390	30	27	196	41	40	194	18	17
$P_{trend}^{a,b}$		0.52	0.66		0.34	0.98		0.82	0.63
$P_{trend}^{b,f}$			0.45			0.92			0.58
N-6 fatty acid from food only									
Quartile 1: < 7.56 g/day	389	29	28	165	44	40	224	19	18
Quartile 2: 7.56–10.32 g/day	390	30	28	202	42	40	188	18	18
Quartile 3: 10.33–13.75 g/day	391	32	27	214	42	41	177	19	17
Quartile 4: ≥ 13.76 g/day	390	30	27	196	41	40	194	18	17
$P_{trend}^{a,b}$		0.54	0.62		0.33	0.97		0.88	0.58
$P_{trend}^{b,f}$			0.41			0.90			0.53
N-6 fatty acid-to-n-3 fatty acid ratio									
Quartile 1: < 6.820	390	26	27	154	37	38	236	18	17
Quartile 2: 6.820–8.120	390	31	29	207	43	41	183	19	19
Quartile 3: 8.121–9.695	390	31	27	201	43	41	189	18	16
Quartile 4: ≥ 9.696	390	33	28	215	45	41	175	20	18
$P_{trend}^{a,b}$		<0.0001	0.49		0.008	0.21		0.19	0.74
$P_{trend}^{b,g}$			0.55			0.38			0.81
N-6 fatty acid-to-n-3 fatty acid ratio from food only									
Quartile 1: < 6.923	390	26	27	158	38	39	232	18	18
Quartile 2: 6.923–8.207	390	31	28	202	42	40	188	19	18
Quartile 3: 8.208–9.795	390	31	27	207	42	41	183	18	16
Quartile 4: ≥ 9.796	390	33	28	210	45	41	180	20	18
$P_{trend}^{a,b}$		<0.0001	0.69		0.011	0.27		0.19	0.59
$P_{trend}^{b,g}$			0.95			0.54			0.55
N-6 fatty acid-to-long-chain n-3 fatty acid ratio									
Quartile 1: < 31.75	390	27	26	164	40	38	226	18	16
Quartile 2: 31.75–52.28	390	30	27	182	43	40	208	19	16
Quartile 3: 52.29–94.28	390	31	29	213	42	41	177	19	19
Quartile 4: ≥ 94.29	390	32	29	218	43	42	172	18	18
$P_{trend}^{a,b}$		0.007	0.008		0.45	0.058		0.88	0.08
$P_{trend}^{b,h}$			0.017			0.142			0.05
N-6 fatty acid-to-long-chain n-3 fatty acid ratio from food only									
Quartile 1: < 32.74	390	27	26	160	40	37	230	18	16
Quartile 2: 32.74–54.48	390	31	27	190	43	41	200	19	17

Table 2 continued

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]
Quartile 3: 54.49–96.87	390	32	29	209	42	40	181	19	19
Quartile 4: ≥ 96.88	390	32	29	218	42	42	172	18	18
$P_{\text{trend}}^{\text{a,b}}$		0.006	0.016		0.46	0.056		0.83	0.13
$P_{\text{trend}}^{\text{b,h}}$			0.070			0.217			0.14

[a] Analyses are adjusted for age, body mass index, waist-to-hip ratio, alcohol intake, energy intake, physical activity, parity, smoking status, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, lactation, family history of breast cancer, number of breast biopsies, education, past use of oral contraceptive, past use of hormone replacement therapy, and height. Analyses for all women combined are also adjusted for menopausal status. Absolute non-dense area (cm^2) was square root-transformed for all analyses to obtain an approximate normal distribution. Means are presented as back-transformed values for these analyses

[b] Test for trends is an F test of the linear contrast

[c] The same as model[a] with adjustment for the intake of long-chain n-3 fatty acid, n-6 fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[d] The same as model[a] with adjustment for the intake of n-6 fatty acid, alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[e] The same as model[a] with adjustment for the intake of n-6 fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[f] The same as model[a] with adjustment for the intake of long-chain n-3 fatty acid, alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[g] The same as model[a] with adjustment for the intake of saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[h] The same as model[a] with adjustment for the intake of alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

Because absolute non-dense mammographic area has been recently suggested to be associated with breast cancer risk, we also examined the association of FA intake with non-dense mammographic area (Table 3). As for percent MD, similar associations between FA intake and non-dense mammographic area were observed but in the opposite direction and mostly limited to postmenopausal women. For instance, increasing quartiles of total and dietary LC n-3 FA, EPA, DPA, and DHA intakes were associated with higher absolute non-dense mammographic area among postmenopausal women (P_{trend} = 0.003, 0.012, 0.013, 0.16, and 0.012, respectively) only. Moreover, the ratios of total or dietary n-6 FA/LC n-3 FA intake were each negatively associated with absolute non-dense mammographic area among all (P_{trend} = 0.047 and 0.078, respectively) or postmenopausal women (P_{trend} = 0.009 and 0.039, respectively), but not among premenopausal women. All the associations remained statistically significant after further adjustment for absolute density (data not shown).

Further adjustment for the intake of saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates and mutual adjustment for the intake of LC n-3 FA, n-6 FA, and ALA (when applicable) did not materially alter the results (Tables 2 and 3).

Data from the nutrient density analyses of the relationships between FA intake and percent MD or absolute non-dense mammographic area provided similar results (Supplemental tables 1 and 2, respectively).

Discussion

These findings support our a priori hypothesis that a higher intake of LC n-3 FA is associated with lower MD. As we evaluated the associations between n-3 FA or n-6 FA intake and MD, we found that LC n-3 FA, EPA, or DHA intakes were inversely associated with MD among all and postmenopausal women but not among premenopausal women. Our analyses also showed that an intake of a higher n-6 FA/LC n-3 FA ratio was related to higher MD regardless of the menopausal status. The observed 3–4 % difference in percent MD between the lower and the upper quartiles of LC n-3 FA intake or its components is significant in terms of breast cancer risk. For example, it was shown that among healthy women at risk of developing breast cancer, those who received 54 months of tamoxifen had an absolute reduction of 6.4 % in MD compared to placebo [42]; in high-risk women, tamoxifen has been shown to reduce the risk of breast cancer by 30–50 % [43, 44].

To our knowledge, the association between the intake of EPA, DPA or DHA, and MD has not been evaluated. So far, only one group recently examined circulating erythrocyte

Table 3 Relations of n-3 and n-6 fatty acids intake with non-dense mammographic area

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]
Alpha-linolenic fatty acid (ALA)									
Quartile 1: < 0.69 g/day	389	98	93	176	69	66	213	122	118
Quartile 2: 0.69–0.99 g/day	388	102	98	207	83	77	181	122	114
Quartile 3: 1.00–1.45 g/day	391	102	95	199	84	76	192	118	110
Quartile 4: ≥ 1.46 g/day	392	108	96	195	81	74	197	135	117
$P_{\text{trend}}^{\text{a, b}}$		0.03	0.58		0.029	0.12		0.05	0.68
$P_{\text{trend}}^{\text{b, c}}$			0.37			0.20			0.92
Eicosapentaenoic fatty acid (EPA)									
Quartile 1: < 0.03 g/day	353	106	94	181	84	75	172	130	116
Quartile 2: 0.03–0.06 g/day	417	102	95	228	84	76	189	123	116
Quartile 3: 0.07–0.10 g/day	405	100	96	197	75	70	208	125	126
Quartile 4: ≥ 0.11 g/day	385	101	98	171	75	73	214	122	125
$P_{\text{trend}}^{\text{a, b}}$		0.30	0.23		0.057	0.42		0.33	0.013
$P_{\text{trend}}^{\text{b, d}}$			0.31			0.35			0.012
Docosapentaenoic fatty acid (DPA)									
Quartile 1: < 0.010 g/day	108	108	92	48	79	71	60	132	117
Quartile 2: 0.010–0.019 g/day	559	100	95	295	80	75	264	122	118
Quartile 3: 0.020–0.029 g/day	473	102	96	242	81	74	231	124	120
Quartile 4: ≥ 0.030 g/day	420	104	97	192	77	71	228	127	126
$P_{\text{trend}}^{\text{a, b}}$		0.65	0.26		0.91	0.94		0.62	0.16
$P_{\text{trend}}^{\text{b, d}}$			0.66			0.36			0.13
Docosahexaenoic fatty acid (DHA)									
Quartile 1: < 0.07 g/day	372	101	92	203	81	72	169	126	116
Quartile 2: 0.07–0.12 g/day	392	102	96	200	82	77	192	122	119
Quartile 3: 0.13–0.19 g/day	412	99	95	211	77	72	201	123	121
Quartile 4: ≥ 0.20 g/day	384	107	99	163	79	73	221	128	128
$P_{\text{trend}}^{\text{a, b}}$		0.29	0.051		0.59	0.92		0.78	0.012
$P_{\text{trend}}^{\text{b, d}}$			0.067			0.89			0.007
Long-chain n-3 fatty acid (EPA, DPA, and DHA)									
Quartile 1: < 0.11 g/day	394	101	93	217	81	73	177	126	115
Quartile 2: 0.11–0.20 g/day	375	103	94	187	82	76	188	122	115
Quartile 3: 0.21–0.32 g/day	400	100	96	202	77	71	198	124	125
Quartile 4: ≥ 0.33 g/day	391	105	99	171	78	74	220	127	127
$P_{\text{trend}}^{\text{a, b}}$		0.54	0.042		0.34	0.93		0.88	0.003
$P_{\text{trend}}^{\text{b, d}}$			0.055			0.94			0.002
Long-chain n-3 fatty acid from food only (EPA, DPA, and DHA)									
Quartile 1: < 0.11 g/day	405	101	94	224	81	73	181	126	116
Quartile 2: 0.11–0.20 g/day	387	103	94	189	82	76	198	123	116
Quartile 3: 0.21–0.31 g/day	383	100	96	190	76	71	193	123	125
Quartile 4: ≥ 0.32 g/day	385	105	98	174	78	74	211	127	126
$P_{\text{trend}}^{\text{a, b}}$		0.57	0.09		0.42	0.89		0.88	0.012
$P_{\text{trend}}^{\text{b, d}}$			0.23			0.67			0.012
N-3 fatty acid (ALA, EPA, DPA, and DHA)									
Quartile 1: < 0.91 g/day	386	98	93	188	75	68	198	120	122
Quartile 2: 0.91–1.26 g/day	390	101	97	211	80	76	179	127	120
Quartile 3: 1.27–1.75 g/day	393	102	97	188	80	75	205	122	122
Quartile 4: ≥ 1.76 g/day	391	108	95	190	85	74	201	130	120

Table 3 continued

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]	*N*	Crude	Adjusted[a]
$P_{trend}^{a, b}$		0.025	0.61		0.085	0.26		0.18	0.85
$P_{trend}^{b, e}$			0.53			0.29			0.91
N-3 fatty acid from food only (ALA, EPA, DPA, and DHA)									
Quartile 1: < 0.90 g/day	385	97	93	188	74	68	197	120	122
Quartile 2: 0.90–1.24 g/day	394	101	97	210	79	77	184	127	120
Quartile 3: 1.25–1.71 g/day	389	102	96	182	81	75	207	119	120
Quartile 4: ≥ 1.72 g/day	392	109	95	197	84	73	195	134	122
$P_{trend}^{a, b}$		0.014	0.67		0.055	0.32		0.08	0.95
$P_{trend}^{b, e}$			0.79			0.65			0.96
N-6 fatty acid									
Quartile 1: < 7.57 g/day	389	105	96	166	78	73	223	124	122
Quartile 2: 7.57–10.32 g/day	390	101	92	201	78	71	189	127	116
Quartile 3: 10.33–13.75 g/day	391	100	98	214	83	74	177	120	124
Quartile 4: ≥ 13.76 g/day	390	103	97	196	80	75	194	127	123
$P_{trend}^{a, b}$		0.69	0.38		0.56	0.48		0.90	0.51
$P_{trend}^{b, f}$			0.23			0.29			0.64
N-6 fatty acid from food only									
Quartile 1: < 7.56 g/day	389	104	96	165	78	73	224	123	121
Quartile 2: 7.56–10.32 g/day	390	102	92	202	78	71	188	128	116
Quartile 3: 10.33–13.75 g/day	391	100	98	214	83	74	177	120	124
Quartile 4: ≥ 13.76 g/day	390	103	97	196	80	76	194	127	123
$P_{trend}^{a, b}$		0.74	0.34		0.55	0.44		0.84	0.47
$P_{trend}^{b, f}$			0.19			0.26			0.58
N-6 fatty acid-to-n-3 fatty acid ratio									
Quartile 1: < 6.820	390	114	96	154	89	75	236	128	120
Quartile 2: 6.820–8.120	390	100	95	207	76	72	183	128	121
Quartile 3: 8.121–9.695	390	102	97	201	80	74	189	126	124
Quartile 4: ≥ 9.696	390	93	95	215	76	74	175	114	118
$P_{trend}^{a, b}$		<0.0001	0.96		0.056	0.98		0.01	0.80
$P_{trend}^{b, g}$			0.95			0.80			0.66
N-6 fatty acid-to-n-3 fatty acid ratio from food only									
Quartile 1: < 6.923	390	114	96	158	89	75	232	130	120
Quartile 2: 6.923–8.207	390	101	95	202	77	73	188	127	121
Quartile 3: 8.208–9.795	390	101	97	207	80	74	183	124	124
Quartile 4: ≥ 9.796	390	94	95	210	75	73	180	116	120
$P_{trend}^{a, b}$		<0.0001	0.95		0.034	0.82		0.01	0.96
$P_{trend}^{b, g}$			0.81			0.90			0.95
N-6 fatty acid-to-long-chain n-3 fatty acid ratio									
Quartile 1: < 31.75	390	107	99	164	78	72	226	129	127
Quartile 2: 31.75–52.28	390	99	96	182	77	73	208	118	121
Quartile 3: 52.29–94.28	390	103	96	213	83	77	177	128	119
Quartile 4: ≥ 94.29	390	100	92	218	80	71	172	124	116
$P_{trend}^{a, b}$		0.20	0.047		0.45	0.95		0.92	0.009
$P_{trend}^{b, h}$			0.058			0.93			0.003
N-6 fatty acid-to-long-chain n-3 fatty acid ratio from food only									
Quartile 1: < 32.74	390	109	99	160	79	74	230	129	126
Quartile 2: 32.74–54.48	390	97	95	190	76	72	200	118	122

Table 3 continued

	All (n = 1,560)			Premenopausal (n = 777)			Postmenopausal (n = 783)		
	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]	N	Crude	Adjusted[a]
Quartile 3: 54.49–96.87	390	103	96	209	83	77	181	125	117
Quartile 4: ≥ 96.88	390	100	93	218	80	71	172	126	118
$P^{a,\ b}_{trend}$		0.16	0.078		0.59	0.85		0.98	0.039
$P^{b,\ h}_{trend}$			0.163			0.98			0.034

[a] Analyses are adjusted for age, body mass index, waist-to-hip ratio, alcohol intake, energy intake, physical activity, parity, smoking status, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, lactation, family history of breast cancer, number of breast biopsies, education, past use of oral contraceptive, past use of hormone replacement therapy, and height. Analyses for all women combined are also adjusted for menopausal status. Percent mammographic density was square root-transformed for all analyses to obtain an approximate normal distribution. Means are presented as back-transformed values for these analyses

[b] Test for trends is an F test of the linear contrast

[c] The same as model[a] with adjustment for the intake of long-chain n-3 fatty acid, n-6 fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[d] The same as model[a] with adjustment for the intake of n-6 fatty acid, alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[e] The same as model[a] with adjustment for the intake of n-6 fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[f] The same as model[a] with adjustment for the intake of long-chain n-3 fatty acid, alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein and carbohydrates

[g] The same as model[a] with adjustment for the intake of saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

[h] The same as model[a] with adjustment for the intake of alpha-linolenic fatty acid, saturated fat, monounsaturated fat, polyunsaturated fat, animal fat, vegetable fat, protein, and carbohydrates

concentration of EPA or DHA with MD and found no association among a population of 248 postmenopausal women [45]. However, the relationship between the intake of these combined components, namely LC n-3 FA, and MD has been assessed by one group other than ours. In this study, LC n-3 FA intake showed no association with MD among premenopausal (n = 348) or postmenopausal (n = 253) women, which is dissimilar from our findings that LC n-3 FA was associated with lower MD among all and postmenopausal women [30]. In contrast to our study, their population of postmenopausal women was relatively small in size and they did not assess the association of LC n-3 FA with MD among all women combined. Furthermore, their manner of evaluating MD differed from ours as we relied on a craniocaudal view of the breast to quantify MD and they used the mediolateral oblique view, which is reported to generate lower MD estimates than those deriving from the craniocaudal view [39]. Heterogeneity between studies' population could also explain the discrepancy in findings as their analyses were among Japanese women while ours were held among Caucasian women [46]. Not only the diet is different between those two populations, but their hormonal and reproductive histories are also distinct. For example, the daily average intake of LC n-3 FA is 934 mg among postmenopausal women from their cohort while it is 310 mg among postmenopausal women of ours. Postmenopausal women from the Nagata study were also younger, leaner, and had a later menarche. These factors are all estrogen-related and since the relationship between LC n-3 FA and MD could be influenced by a woman's level of estrogen, those disparities could in part explain the discrepancy in the results. In our study, the inverse associations of EPA, DHA, or LC n-3 FA intake with MD among all women were also observed among postmenopausal women but not among premenopausal women. It is interesting to observe that several studies on the relationship between fish intake and breast cancer risk that have stratified their analyses by menopausal status found an inverse association among postmenopausal but not among premenopausal women [23, 47, 48]. Others found similar associations between the intake of LC n-3 FA, EPA or DHA among postmenopausal but not premenopausal women [22, 49]. The possible modifying effect of menopausal status on the relationship between LC n-3 FA and MD reinforces the idea that one of the mechanisms by which these dietary factors affect MD could be through estrogens. For instance, it is suggested that high intake of n-3 FA relative to that of n-6 FA may decrease endogenous estrogen production via inhibition of aromatase activity/expression [10, 50, 51]. In vitro studies also suggest that estrogen could regulate the biosynthesis of LC n-3 FA by influencing delta-5 desaturase activity [52], but the relationship between dietary n-3 FA synthesis and endogenous steroid hormones remains to be

investigated in humans. Our finding of a lack of association between ALA intake and MD was also suggested in another study, although in the latter, a borderline significant inverse relationship was observed [32]. Their analyses were dissimilar to ours as they were conducted among a Mediterranean population whose diet is particular and their assessment of MD was of qualitative nature.

Only five studies examined the associations between n-3 FA or n-6 FA intake and MD as other evaluations focused on polyunsaturated fatty acids as a group [53–56]. Study analyses failed to show statistically significant associations between n-3 FA [28, 29, 31] or n-6 FA [29, 31] intake and MD. Their findings were similar to ours concerning the relationship between n-3 FA or n-6 FA intake and MD as they observed null results among premenopausal [28, 31], postmenopausal women [28, 29, 31] or both combined [28, 29]. Circulating erythrocyte concentration of n-3 FA or n-6 FA also showed no association to postmenopausal MD [45]. Similarly, most of the studies evaluating associations between n-3 FA or n-6 FA intake and breast cancer risk yielded null results [16, 18, 20, 21].

In the present study, we observed a positive association between the intake of n-6 FA/LC n-3 FA ratio and MD. To our knowledge, this relationship was not assessed in another study but one group evaluated the circulating erythrocytes n-6 FA/LC n-3 FA ratio and reported no association to MD among postmenopausal women [45]. Since the proportion of fatty acids (n-3 FA or n-6 FA) in cells membrane is dependent on the diet and n-3 FA compete with n-6 FA for enzymes desaturases and elongases for the biosynthesis of derived eicosanoids, it would be expected that the n-6 FA/n-3 or n-6 FA/LC n-3 FA ratio could influence MD. Of the studies that evaluated the relationship between the intake of n-6 FA/n-3 FA [16–18, 20, 21, 24, 57] or n-6 FA/LC n-3 FA [21, 57] ratio and breast cancer risk, only one found a positive association between the intake of n-6 FA/n-3 ratio and the risk of breast cancer [24].

In our exploratory analysis, we observed among postmenopausal women that intake of higher LC n-3 FA and lower n-6 FA/LC n-3 FA ratio was associated with higher non-dense area of the breast that is mainly occupied by fat tissue. Based on the biological roles of such FA, these results suggested that mammary fat tissue may have a protective role in breast carcinogenesis. However, little is known about the relationship between non-dense mammographic area and breast cancer risk [58–62]. While one group observed a positive association [58], others found a negative association of non-dense mammographic area with the risk of developing breast cancer [59–62], associations that remained significant after adjustment for absolute density [58, 59].

This study has several strengths and weaknesses. Firstly, the quality of the mammographic images was maximized. Almost all mammograms were done in the same clinic with the same equipment (mammography units, LORAD M4) that was accredited by the Canadian Association of Radiology in addition to satisfying the high-quality standards of the Quebec breast cancer screening program. Secondly, quantitative measures of MD were obtained without any information on women, using a computer-assisted method, by one reader whose reliability of reading was shown to be high. Although the density of only one breast was measured, the concordance of the measures between right and left breasts in this study was high [63]. Thus, the misclassification of MD should be relatively small, most likely be at random and therefore should not have biased our results. Furthermore, dietary FA intake is believed to reflect tissue or plasma composition of FA [64, 65]. Food-frequency questionnaires may lead to overestimation of the range of intake and may also lead to attenuation of the associations [66] because of non-differential misclassification. However, the low within-population variability in n-3 FA intake limits the statistical power to detect associations. Another limit of this type of study is that food-frequency questionnaires' data reflect the dietary intake throughout the previous year and do not account for nutriments uptake at a younger age, which may be of relevance for the associations studied [67]. We cannot exclude that our findings may be due to chance because we evaluated several associations. Type I error or false-positive results are therefore possible. Since this is a cross-sectional study, temporal association cannot be formulated, although it is improbable that MD could influence FA intake.

Higher LC n-3 FA intake appears to be associated with lower MD among all and postmenopausal women; however, this relationship should be investigated by further research. These findings may contribute to a better understanding of the role that LC n-3 FA have on MD and may therefore provide insight into the etiology of breast cancer and eventually lead to identifying new prevention strategies.

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