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Erythrocyte Omega-6 and Omega-3 Fatty Acids and Mammographic Breast Density

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Abstract

Diets low in omega-6 (n-6) polyunsaturated fatty acids (PUFAs) and high in omega-3 (n-3) PUFAs may protect against breast cancer development. Associations of PUFA intake with mammographic density, an intermediate marker of breast cancer risk, have been inconsistent; however, prior studies have relied on self-reported dietary PUFA intake. We examined the association between circulating erythrocyte n-6 and n-3 PUFAs with mammographic density in 248 postmenopausal women who were not taking exogenous hormones. PUFAs in erythrocytes were measured by gas-liquid chromatography, and mammographic density was assessed quantitatively by planimetry. Spearman's correlation coefficients and generalized linear models were used to evaluate the relationships between PUFA measures and mammographic density. None of the erythrocyte n-6 or n-3 PUFA measures were associated with percent density or dense breast area.

INTRODUCTION

Mammographic density is acknowledged as one of the strongest risk factors for breast cancer (1, 2), and data suggest that diet may influence mammographic density (3–5).

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Identifying specific nutrients that can alter a women's breast cancer risk may suggest opportunities for breast cancer prevention through dietary modification or supplementation.

Numerous epidemiological studies report positive associations between omega-6 (n-6) polyunsaturated fatty acids (PUFAs) and inverse associations between omega-3 (n-3) PUFAs with breast cancer risk, though results are contradictory (5–11). The actual ratio of n-6 to n-3 PUFAs also has been related to breast cancer development, with higher ratios conferring greater risk (6, 10) in some, but not all (11, 12), studies. Despite numerous reports on the relationship between n-6 and n-3 PUFAs and breast cancer, the association between these PUFAs and mammographic density has been less studied. Although n-6 intake has been associated with increased density (13) and n-3 intake with reduced density (14), the findings are not consistent (13–16). We are unaware of any studies assessing the relationship between the ratio of n-6 to n-3 PUFAs and mammographic density.

Previous studies assessing PUFA intake and mammographic density have measured n-6 and n-3 fatty acid intake via self-report dietary assessment instruments, and limitations of these assessment tools are well-documented (17). n-3 and n-6 PUFAs are considered essential and are mostly obtained from the diet. Thus, the n-6 and n-3 PUFAs in erythrocyte membranes are considered relatively good biomarkers of what is consumed and also have the advantage of being free of error due to human memory (18, 19).

Therefore, we examined the associations between circulating levels of erythrocyte n-6 and n-3 PUFAs with mammographic density. We hypothesized that erythrocyte n-6 PUFAs would be positively associated and n-3 PUFAs negatively associated with mammographic density.

METHODS

Study Population

We selected controls enrolled in the Mammograms and Masses Study (MAMS) for the present analysis. MAMS is a breast cancer case-control study intended to study associations between hormones and mammographic density. The MAMS methods and study population have been reported previously (20, 21). MAMS participants were eligible for the present analysis if they were postmenopausal, had not used hormone therapy within 3 months of study enrollment, reported no use of vaginal estrogen cream, oral contraceptives, corticosteroids or selective estrogen receptor modulators (SERMs) at blood sampling, and had a mammogram within 120 days of blood draw (N=248).

Data Collection

Participants completed a questionnaire that collected information on demographics, reproductive history, current and past hormone use, and various lifestyle habits (e.g., smoking status and alcohol consumption). Height and weight were measured by a study nurse after participants removed shoes and heavy clothing and were used to calculate body mass index (BMI, weight in kg divided by height in meters squared (kg/m^2)). A 40mL non-fasting blood sample was donated by each participant. Samples were processed immediately on site according to a standardized protocol, and stored at or below −70°C until assayed.

Fatty Acid Measurement

Erythrocyte fatty acid concentrations were identified using gas-liquid chromatography. Samples were analyzed at the University of Pittsburgh's Heinz Laboratory. Total lipids (500μl of packed red blood cells) were extracted according to the general technique of Bligh and Dyer (22). Briefly, the samples were homogenized in 4 ml of methanol, 2 ml of

chloroform and 1.1 ml of water. Two ml of chloroform and 2 ml of water were added to the samples after 15 min. The tubes were then centrifuged at 1200 g for 30 min at 16°C and the upper phase discarded. The lower phase was dried under nitrogen and resuspended in 1.5 ml 14% boron trifluoride methanol. The samples were heated at 90°C for 40 min and after cooling extracted with 4.0 ml pentane and 1.5 ml water. The mixtures were vortexed and the organic phase recovered (23). The extracts were dried under nitrogen, resuspended in 50 μl heptane and 2 ml injected into a capillary column (SP-2380, 105 m × 53 mm ID, 0.20 um film thickness). Gas chromatographic analyses were carried out on a Perkin Elmer Clarus 500 equipped with a flame ionization detector. Operating conditions were as follows: the oven temperatures were 140°C for 35 min; 8°C/min to 220°C, held for 12 min; injector and detector temperatures were both at 260°C; and helium, the carrier gas, was at 15 psi. Identification of fatty acids was by comparison of retention times with those of authentic standards (Sigma). A random subset of 27 samples was analyzed for reproducibility; laboratory personnel were blinded to duplicate samples and subject identification. The inter-assay coefficients of variation (CV) for the fatty acid measures reported ranged between 1.7–15.2%. CVs's were 4.6% for linoleic acid (LA), 3.4% for arachidonic acid (AA), and 1.7% for total n-6 fatty acids. CV's were higher for the n-3 fatty acids, with CV's of 15.2% for alpha-linoleic acid (ALA), 5.3% for eicosapentaenoic acid (EPA), 7.5% for docosahexaenoic acid (DHA) and 5.3% for total n-3 fatty acids. The CVs for the total n-6:n-3, LA:ALA, AA:EPA, and AA:EPA+DHA ratios were 5.2%, 11.1%, 4.5% and 5.7% respectively. The individual and total n-6 and n-3 fatty acids are expressed as a percentage by weight of the total erythrocyte fatty acid content. Though the primary focus of our analysis was on the PUFAs, data on additional erythrocyte fatty acids (14.0, 15.0, 16.0, 17.0, 18.0, 16:1n7t, 18:1 t,18:2n6tt, 16:1n7, 18:1n9, 18:1n7, 20:1n9, and 24:1n9) were available from the laboratory analysis.

Mammographic Density Measurement

The assessment of mammographic measures has been described in detail elsewhere (20). Briefly, mammographic density was measured quantitatively via planimetry using the craniocaudal view of the mammographic film corresponding closest to the participant's date of blood draw. Total breast area and all dense regions were measured using a compensating polar planimeter (LASICO). Dense breast area was defined as the sum of all dense regions; percent density was calculated by dividing dense breast area by total breast area and multiplying that by 100. Intra-rater reliability, assessed in a sample of 28 MAMS participants, was very high for all mammographic density measurements (intraclass correlation coefficients for dense area, total breast area and percent density were ρ=0.86, ρ=0.99, and ρ=0.89, respectively).

Statistical Analysis

Percent density and dense area were examined as continuous variables. Descriptive results for selected participant characteristics are expressed as a mean and standard deviation (SD) or as a frequency and percentage. Spearman's correlation coefficients were calculated between erythrocyte PUFA measures and mammographic density, both unadjusted and adjusted for age and body mass index (BMI). Adjusted geometric means for each mammographic density measure were calculated by PUFA tertile using generalized linear models. While the primary aim of our study was to examine n-6 and n-3 PUFAs in relation to density, as a secondary aim we examined the relation between individual erythrocyte fatty acids not of the n-6 or n-3 families and mammographic density using similar statistical approaches. A priori power calculations indicated 80% power to minimally detect a correlation coefficient of 0.18 as statistically significant with a two-sided α=0.05. Analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The mean (SD) age of the study population was 63.0 (8.3) years, mean BMI was 28.5 (6.1) kg/m^2, the mean time since menopause was 14.3 (10.1) years, and 93.6% were white. The median (interquartile range) of percent density was 23.7 (13.4 to 39.7) %, and of dense area was 35.8 (21.7 to 54.6) cm^2. The average time interval between the mammogram and blood collection was 35 days (range 8–114 days). The erythrocyte PUFA compositions are summarized in Table 1. The means (SD) of total n-6 PUFAs, total n-3 PUFAs, and the n-6:n-3 ratio were 38.3 (2.6) wt %, 7.9 (2.0) wt %, and 5.2 (1.5) wt %, respectively.

Unadjusted and adjusted Spearman rank correlation coefficients are presented in Table 2. We observed no significant associations between any of the erythrocyte n-6 or n-3 PUFA measures with percent density or dense breast area in either the unadjusted or adjusted correlation estimates. The lack of association persisted after controlling for various lifestyle and reproductive variables in generalized linear models (Table 3). We did observe a trend toward increased percent density with increased arachidonic acid (AA; 20:4 n-6) (p=0.07), total n-6:n-3 ratio (p=0.12), and the ratio of AA to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 20:6n-3) (AA:EPA+DHA, p=0.12), though these relationships did not achieve statistical significance. Similar results were obtained when excluding subjects whose mammogram was taken >30 days before blood sampling (data not shown).

We did not observe any strong correlations between erythrocyte fatty acids not of the n-6 or n-3 families and density. While all correlation coefficients were weak and less than the minimally detectable correlation of +/− 0.18 indicated by power calculations, in general, fatty acid 17.0 appeared to be associated with increased density whereas 18:1t and 18:2n6tt were inversely associated with percent density and dense area, respectively (Supplementary Table 1). These weak relationships were attenuated and did not reach statistical significance after further adjustment for age, BMI, and other potential confounders in generalized linear models (data not shown).

DISCUSSION

In this cross-sectional analysis of postmenopausal women, we assessed the association between erythrocyte n-6 and n-3 PUFAs and mammographic density. In contrast with what we had predicted, no association was found between any of the PUFA measures and either percent density or dense breast area. The lack of association persisted in adjusted analyses and in analyses limiting the study population to women whose mammograms were taken within 30 days of blood draw.

Numerous studies have investigated associations between PUFAs and breast cancer risk. In general, studies report contradictory findings with respect to dietary intake of n-6 PUFAs and breast cancer risk (6–12). Some studies observed a decreased breast cancer risk associated with increased n-3 PUFA intake (6, 8–10), though this effect may be dependent upon the level of n-6 PUFA intake (10–12). Thus, there is some evidence that PUFAs may affect breast cancer risk.

Few epidemiological studies have reported on the association between either total or individual n-6 and n-3 PUFAs and mammographic density, and these prior studies relied on self-reported dietary PUFA intake. Women with the highest mammographic density pattern reported significantly higher consumption of n-6 PUFAs when compared to women with the lowest mammographic pattern (13); however, another study reported no association between intake of the n-6 PUFA linoleic acid (18:2 n-6) and mammographic density (14). One study investigated the effects of n-3 PUFA alpha-linolenic acid (18:3 n-3) with mammographic

density, and a significant inverse association was observed (14). However, no difference in mammographic density was observed when comparing quartiles of total n-3 intake or long chain n-3 PUFAs (13, 15, 16). Our results, based on circulating measures of PUFAs, provide further evidence that mammographic density is not influenced by n-6 and n-3 intake.

Eicosanoids (e.g., prostaglandins, leukotrienes) derived from n-6 PUFAs (e.g. AA) are involved in several carcinogenic processes including tumor promotion and progression. n-3 PUFAs (particularly long chain n-3) competitively inhibit the production of eicosanoids derived from n-6 PUFAs. In western societies n-6 PUFA intake tends to be much higher than n-3 PUFA intake (24–26), therefore, n-6 PUFA levels in our population may have been too high to observe any effects that the n-3 PUFAs have on mammographic density (e.g., n-6 may have been above or n-3 below a threshold needed to observe an association if one existed). Indeed, the study by Shannon et al (6), which found a significant association between total n-3 PUFAs in erythrocytes and breast cancer risk, had much lower mean n-6 PUFA levels than our population (20.5 wt % vs. 38.3 wt %, respectively).

A possible limitation of our study is the use of non-fasting blood samples. However, we expect the effect of recent food intake on erythrocyte fatty acid levels to be minimal. The non-fasting samples would mainly differ from fasting samples by the presence of chylomicrons that contain dietary triglycerides. The fatty acids of the triglycerides would be rapidly taken up by various tissues, in particular, adipose tissue and muscle. There would be very little interaction between the fatty acids of triglycerides with the erythrocytes. Thus we believe that our methods provided a valid assessment of erythrocyte fatty acid levels.

Another potential limitation of our study is that the range of n-6 and n-3 PUFAs within our study population may have been too similar to detect a difference in mammographic features. Although we analyzed associations between mammographic density measures and each fatty acid separately, along with a few pre-specified fatty acid ratios, we did not assess if interactions between fatty acids might be related to mammographic density. Further, we utilized only a single measurement of erythrocyte fatty acid levels, which may not reflect usual exposure over extended time periods. Additionally, the weak, borderline significant correlations we observed between fatty acids not of the n-6 or n-3 families and mammographic density may have been due to multiple comparisons.

Despite these limitations, our results are strengthened by our use of circulating levels of n-6 and n-3 PUFAs, rather than self-report. This objective measurement of fatty acids reduces the potential for exposure misclassification. Further, we had 80% power to detect a minimum correlation of 0.18 as statistically significant, which provides confidence that there is truly no association between erythrocyte PUFA levels and mammographic density in our population.

In conclusion, in the present study, we found no evidence of an association between n-6 or n-3 PUFAs and mammographic density in postmenopausal women. We did observe an indication that percent density may increase with increased AA, total n-6:n-3 ratio, and the AA:EPA+DHA ratio. Though these associations did not achieve statistical significance in our study, the observed trends suggest that further evaluation of these relationships is warranted. Given this study's limitations, future studies, perhaps with repeated fatty acid and mammographic density measurements, are needed to either confirm or refute our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Mean PUFA composition in erythrocytes

Fatty Acids (wt %)	mean (SD)
n-6 PUFAs	
Total n-6 [a]	38.3 (2.6)
18:2n-6 (LA)	15.8 (2.4)
20:4n-6 (AA)	16.1 (1.9)
n-3 PUFAs	
Total n-3 [b]	7.9 (2.0)
18:3n-3 (ALA)	0.2 (0.1)
20:5n-3 (EPA)	0.9 (0.4)
22:6n-3 (DHA)	4.5 (1.5)
6:3 PUFA Ratios	
Total n-6:n-3	5.2 (1.5)
LA:ALA	72.8 (19.5)
AA:EPA	21.7 (8.9)
AA:EPA+DHA	3.3 (1.2)

NOTE: N=248. Data are expressed as mean (SD). Fatty acids are reported as a percentage by weight of the total fatty acids (weight percent, wt %). PUFA, polyunsaturated fatty acid; n-6, omega-6; LA, linoleic acid; AA, arachidonic acid; n-3, omega-3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid.

[a] 18:2n-6+18:3n-6+20:2n-6+20:3n-6+20:4n-6+22:4n-6+22:5n-6

[b] 18:3n-3+20:4n-3+20:5n-3+22:5n-3+22:6n-3

Table 2

Unadjusted and adjusted Spearman correlation coefficients between erythrocyte n-6 and n-3 PUFAs and mammographic density (n=248)[a]

	Percent Density		Dense Breast Area	
Fatty Acids	**Unadjusted r (P value)**	**Adjusted[b] r (P value)**	**Unadjusted r (P value)**	**Adjusted[b] r (P value)**
n-6 PUFAs				
Total n-6 [c]	−0.006 (0.93)	0.02 (0.75)	0.001 (0.99)	−0.01 (0.87)
18:2n-6 (LA)	0.08 (0.22)	−0.02 (0.78)	−0.04 (0.51)	−0.06 (0.34)
20:4n-6 (AA)	0.01 (0.85)	0.08 (0.20)	0.07 (0.25)	0.08 (0.22)
n-3 PUFAs				
Total n-3 [d]	0.10 (0.11)	0.02 (0.77)	0.01 (0.88)	0.02 (0.77)
18:3n-3 (ALA)	0.04 (0.52)	−0.04 (0.55)	−0.05 (0.46)	−0.06 (0.39)
20:5n-3 (EPA)	0.07 (0.28)	−0.02 (0.70)	−0.04 (0.48)	−0.04 (0.51)
22:6n-3 (DHA)	0.10 (0.12)	0.03 (0.64)	0.04 (0.57)	0.05 (0.47)
6:3 PUFA Ratios				
Total n-6:n-3	−0.09 (0.15)	−0.01 (0.84)	−0.007 (0.91)	−0.02 (0.78)
LA:ALA	−0.02 (0.75)	0.02 (0.74)	0.03 (0.61)	0.03 (0.63)
AA:EPA+DHA	−0.08 (0.18)	0.01 (0.88)	0.003 (0.96)	−0.003 (0.96)

[a] Abbreviations: n-6, omega-6; n-3, omega-3; PUFA, polyunsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid

[b] Adjusted for age and body mass index

[c] 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6

[d] 18:3n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3

Table 3

Geometric mean (95% confidence interval) breast density according to tertile of erythrocyte PUFA composition[a]

Fatty acids (wt %)	Percent Density[b]	P trend[c]	Dense Breast Area[b]	P trend[c]
n-6 PUFAs				
Total n-6 [d]		0.26		0.66
≤37.28	24.2 (20.6, 28.0)		35.8 (30.3, 41.8)	
37.29–39.49	23.6 (20.2, 27.3)		33.9 (28.7, 39.6)	
≥39.50	27.4 (23.6, 31.4)		37.7 (32.1, 43.9)	
18:2n-6 (LA)		0.30		0.18
≤14.69	27.3 (23.5, 31.4)		38.6 (32.9, 44.8)	
14.70–16.77	23.5 (20.1, 27.2)		36.0 (30.5, 41.8)	
≥16.78	24.4 (20.8, 28.1)		33.0 (27.8, 38.7)	
20:4n-6 (AA)		0.07		0.16
≤15.31	22.8 (19.5, 26.4)		32.1 (27.0, 37.6)	
15.32–16.57	24.6 (21.1, 28.4)		37.9 (32.4, 43.9)	
≥16.58	27.7 (23.9, 31.8)		37.5 (31.9, 43.6)	
n-3 PUFAs				
Total n-3 [e]		0.36		0.47
≤6.68	25.7 (22.0, 29.7)		37.3 (31.6, 43.5)	
6.69–8.42	26.3 (22.7, 30.1)		36.1 (30.7, 41.9)	
≥8.43	23.2 (19.7, 26.9)		34.2 (28.7, 40.0)	
18:3n-3 (ALA)		0.20		0.25
≤0.19	27.1 (23.5, 31.1)		38.3 (32.8, 44.3)	
0.20–0.25	24.2 (20.7, 27.9)		35.4 (30.0, 41.2)	
≥0.26	23.7 (20.3, 27.4)		33.7 (28.5, 39.4)	
20:5n-3 (EPA)		0.17		0.17
≤0.66	25.0 (21.5, 28.8)		36.8 (31.3, 42.8)	
0.67–0.91	29.0 (25.2, 33.1)		39.9 (34.2, 46.0)	
≥0.92	21.4 (18.1, 24.9)		31.1 (26.1, 36.7)	
22:6n-3 (DHA)		0.58		0.99
≤3.67	25.0 (21.4, 28.9)		35.3 (29.8, 41.3)	
3.68–4.93	26.5 (22.8, 30.4)		36.8 (31.4, 42.7)	
≥4.94	23.6 (20.1, 27.3)		35.3 (29.9, 41.2)	
6:3 PUFA Ratios				
Total n-6:n-3		0.12		0.41
≤4.47	21.9 (18.5, 25.6)		33.4 (28.1, 39.3)	
4.48–5.73	26.9 (23.3, 30.8)		37.0 (31.6, 42.9)	
≥5.74	26.2 (22.5, 30.2)		37.0 (31.3, 43.1)	
LA:ALA		0.45		0.57
≤64.25	24.7 (21.2, 28.4)		36.0 (30.6, 41.8)	
64.26–78.43	23.8 (20.3, 27.4)		33.3 (28.1, 38.9)	

Fatty acids (wt %)	Percent Density[b]	P trend[c]	Dense Breast Area[b]	P trend[c]
≥78.44	26.7 (23.0, 30.7)		38.4 (32.7, 44.5)	
AA:EPA+DHA		0.12		0.23
≤2.67	22.3 (18.9, 26.0)		33.4 (28.0, 39.2)	
2.68–3.72	26.3 (22.7, 30.1)		35.6 (30.3, 41.4)	
≥3.73	26.5 (22.8, 30.5)		38.5 (32.8, 44.6)	

[a]Abbreviations: PUFA, polyunsaturated fatty acid; n-6, omega-6; LA, linoleic acid; AA, arachidonic acid; n-3, omega-3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid.

[b]Square root transformation was applied to percent density and dense breast area. Values were adjusted for age (continuous), BMI (continuous), age at menopause (continuous), age at menarche (<12, 12–13, 14+), alcohol intake (none, <12g/day, ≥12g/day), current smoker (yes vs. no), previous breast biopsy (yes vs. no), nulliparous (yes vs. no), ever breastfed for > 1 month (yes vs. no), and past hormone therapy use (yes vs. no). 7 participants were excluded for missing variables.

[c]Linear trend tests were performed by treating the fatty acid tertile groups as ordinal variables

[d]18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6

[e]18:3n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3