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Research Article

Cancer Prevention Research

Influence of Obesity on Breast Density Reduction by Omega-3 Fatty Acids: Evidence from a Randomized Clinical Trial

Narinder Sandhu[1], Susann E. Schetter[2], Jason Liao[3], Terryl J. Hartman[4], John P. Richie[5], John McGinley[6], Henry J. Thompson[6], Bogdan Prokopczyk[5], Cynthia DuBrock[7], Carina Signori[1], Christopher Hamilton[8], Ana Calcagnotto[3], Neil Trushin[5], Cesar Aliaga[9], Laurence M. Demers[8], Karam El-Bayoumy[9], and Andrea Manni[1]

Abstract

Preclinical data indicate that omega-3 fatty acids (n-3FA) potentiate the chemopreventive effect of the antiestrogen (AE) tamoxifen against mammary carcinogenesis. The role of n-3FA in breast cancer prevention in humans is controversial. Preclinical and epidemiologic data suggest that n-3FA may be preferentially protective in obese subjects. To directly test the protective effect of n-3FA against breast cancer, we conducted a 2-year, open-label randomized clinical trial in 266 healthy postmenopausal women (50% normal weight, 30% overweight, 20% obese) with high breast density (BD; ≥25%) detected on their routine screening mammograms. Eligible women were randomized to one of the following five groups (i) no treatment, control; (ii) raloxifene 60 mg; (iii) raloxifene 30 mg; (iv) n-3FA lovaza 4 g; and (v) lovaza 4 g plus raloxifene 30 mg. The 2-year change in BD, a validated biomarker of breast cancer risk, was the primary endpoint of the study. In subset analysis, we tested the prespecified hypothesis that body mass index (BMI) influences the relationship between plasma n-3FA on BD. While none of the interventions affected BD in the intention-to-treat analysis, increase in plasma DHA was associated with a decrease in absolute breast density but only in participants with BMI >29. Our results suggest that obese women may preferentially experience breast cancer risk reduction from n-3FA administration.

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Introduction

Prevention is the best approach to reduce breast cancer morbidity and mortality. Although the antiestrogens, tamoxifen and raloxifene, have been shown to be effective chemopreventive agents (1, 2), they are poorly accepted even by women at high risk primarily because of concerns of side effects such as thromboembolic events which are felt to outweigh the benefit of breast cancer risk reduction (3, 4). Furthermore, both agents are ineffective against estrogen receptor–negative tumors, which are more aggressive and associated with shorter survival (1, 2).

Because multiple cellular pathways, in addition to the estrogen receptor, contribute to breast cancer development, we hypothesize that prevention can be improved by combining estrogen receptor antagonists with compounds having a complementary mechanism of action. Because such compounds are to be used in healthy women, they have to be safe without significant side effects. Our preclinical data in rodent models of mammary carcinogenesis have shown that fish oil, rich in omega-3FA, potentiated the chemopreventive effect of tamoxifen (5, 6). Furthermore, our signaling (7), genomic (8), and proteomic (9) studies suggested complementarity in the mechanism of antitumor action of tamoxifen and n-3FA. Importantly, the combined approach allowed us to use a lower dose of tamoxifen without losing chemopreventive efficacy (5). Therefore, we believe that in addition to its efficacy, an attractive feature of this approach is its safety because it may allow us to use a lower and potentially less toxic dose of antiestrogens in combination with n-3FA which may provide additional health benefits beyond protection against breast cancer (10). On the other hand, the role of n-3FA in reducing breast cancer in women remains unproven. Epidemiologic studies have given inconsistent results (11). We hypothesize that the discrepant results on the relationship between intake of n-3FA and breast cancer risk may at least in part be due to the heterogeneity of the populations studied. Both preclinical (12–15) and clinical (16) studies have indicated that n-3FA may be preferentially effective in the presence of a pro-inflammatory milieu such as in obesity. Therefore, it may be necessary to target

[1]Department of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania. [2]Department of Radiology, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania. [3]Department of Public Health Sciences, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania. [4]Rollins School of Public Health and Winship Cancer Institute, Emory University, Atlanta, Georgia. [5]Department of Pharmacology, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania. [6]Cancer Prevention Laboratory, Colorado State University, Fort Collins, Colorado. [7]Penn State Hershey Cancer Center. [8]Department of Pathology, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania. [9]Department of Biochemistry and Molecular Biology, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Corresponding Author: Andrea Manni, Division of Endocrinology, Hershey Medical Center, HO44, 500 University Drive, PO Box 850, Hershey, PA 17033. Phone: 717-531-8395; Fax: 717-531-5726; E-mail: amanni@hmc.psu.edu

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specific populations, such as overweight and obese women, to demonstrate the protective effect of n-3FA against breast cancer.

In the clinical trial reported here (NCT00723398), in addition to testing the individual and combined effects of n-3FA and the antiestrogens raloxifene in reducing breast density, a validated biomarker of breast cancer risk (17), we explored the hypothesis that body mass index (BMI) may influence the relationship between breast density and n-3FA. The study was conducted in healthy postmenopausal women at increased risk of breast cancer based on high breast density detected during their annual screening mammogram.

Materials and Methods

Subjects

Our study included healthy, post-menopausal women between the ages of 35 and 75 years who were found to have a breast density $\geq$25% as assessed by ACR-BIRADS (American College of Radiology Breast Imaging Reporting and Data System) at their yearly screening mammogram. Postmenopausal status was defined as history of at least 12 months without spontaneous menstrual bleeding or a documented hysterectomy and bilateral salpingo-oophorectomy. Additional eligibility criteria included no hormone replacement therapy for at least six months prior to entry into the study except for topically applied Vagifem, and being smoke free for more than 5 years. Exclusion criteria included history of stroke, pulmonary embolism or deep vein thrombosis, history of atherosclerotic heart disease, presence of hypercoagulabe state (congenital and acquired), uncontrolled hypertension (blood pressure $\geq$140/90), diabetes mellitus, history of allergy to fish, history of HIV, and presence of psychiatric conditions which would interfere with adherence to the protocol. Subjects were excluded if they had history of breast cancer (including ductal carcinoma *in situ* and lobular carcinoma *in situ*), other prior malignancies except for adequately treated basal cell and squamous cell carcinoma, *in situ* cervical cancer, and other cancers from which the patient has been disease free for at least 5 years. Women were also excluded if they were drinking alcohol more than one drink a day or were unwilling not to use n-3 FA outside of protocol.

Trial design

The open-label study was approved by the Institutional Review Board of the Penn State College of Medicine. After signing the informed consent, each study participant was randomly assigned with equal probability to one of the following five groups: group 1, no treatment, control; group 2, raloxifene 60 mg orally daily; group 3, raloxifene 30 mg orally daily; group 4, lovaza 4 g orally daily; and group 5, lovaza 4 g per day plus raloxifene 30 mg orally daily. Subjects were recruited between March 2009 and March 2012. Lovaza is the FDA-approved n-3FA formulation containing 465 mg of EPA and 375 mg of DHA per gram. A block randomization scheme was used to ensure balance treatment allocation during the course of enrollment. Upon entry, information was collected on parity, family history of breast cancer, and prior history of breast biopsies. In addition, anthropometric measures, including weight, height, and waist-to-hip ratio as well as blood samples for lipid profile and fatty acid analysis, were obtained at baseline and follow-up visits. Adverse events and compliance by pill count were also recorded at follow-up visits. Adverse events were assigned a grade from 0 to 5 as per NCI guidelines.

Assessment of dietary habits

Diet assessment methods have been previously described (18). Briefly, at baseline, 1-year and 2-year follow-up participants completed a modified version of the National Cancer Institute's (NCI) Diet History Questionnaire (DHQ) which queried dietary and supplement intake over the past year. Completed questionnaires were reviewed for completeness, scanned, and analyzed to estimate total energy and nutrient intakes using Diet*Calc version 1.4.3 (19) reconfigured for our modified questionnaires.

Assessment of physical activity

Energy expenditure due to physical activity was estimated using the International Physical Activity Questionnaire—or IPAQ as previously described (18). The IPAQ instrument and scoring methodology (20) is publicly available and has been validated (21). Respondents estimate physical activity in four domains (leisure-, domestic/yard-, employment- and transportation-related activities) allowing for the calculation of a total physical activity score expressed as metabolic equivalent task (METs)-min/week, which we used to estimate daily total physical activity (METs/d).

Breast density measurements

The methodology for volumetric measurement of breast density has been recently published by us (22). Briefly, volumetric assessment of breast density was achieved by exporting raw DICOM data from the craniocaudal views of each subject into the research version of Volpara 1.0.0 (Matakina). This software uses a mathematical model to calculate total breast volume, percent density volume, and absolute breast density volume based on breast thickness and the x-ray attenuation at each pixel of the image (23). Percent volumetric density has been shown to correlate with percent area density which we used to assess subject eligibility in the screening mammogram. However, percent density expressed volumetrically is numerically lower than percent area density as reported in the literature (24, 25).

Fatty acid analysis

Plasma fatty acid analysis was performed at baseline and years 1 and 2 according to the methodology previously published by us (5, 26).

Sample size calculations

A sample size of 50 subjects per group was selected to detect a difference in breast density of 6% between any two groups with 85% power. The reason for choosing a 6% difference was based upon the reported effects of tamoxifen on breast density (27). Furthermore, the difference is clinically relevant because a 6% reduction in breast density predicts an 11% reduction in breast cancer risk (2). The adjustment for multiple comparisons between groups was incorporated in the power calculations.

Statistical analysis

This is a randomized longitudinal study with data collected at baseline, month 12 and month 24 for each subject. The summary statistics are provided for all major variables at these three time points and for each of the five treatment groups. The change of various variables of interest from baseline to month 24 is compared between the treatment groups using regression analysis. Prespecified subset analysis of absolute breast density for different BMI levels was also performed. For better model fitting,

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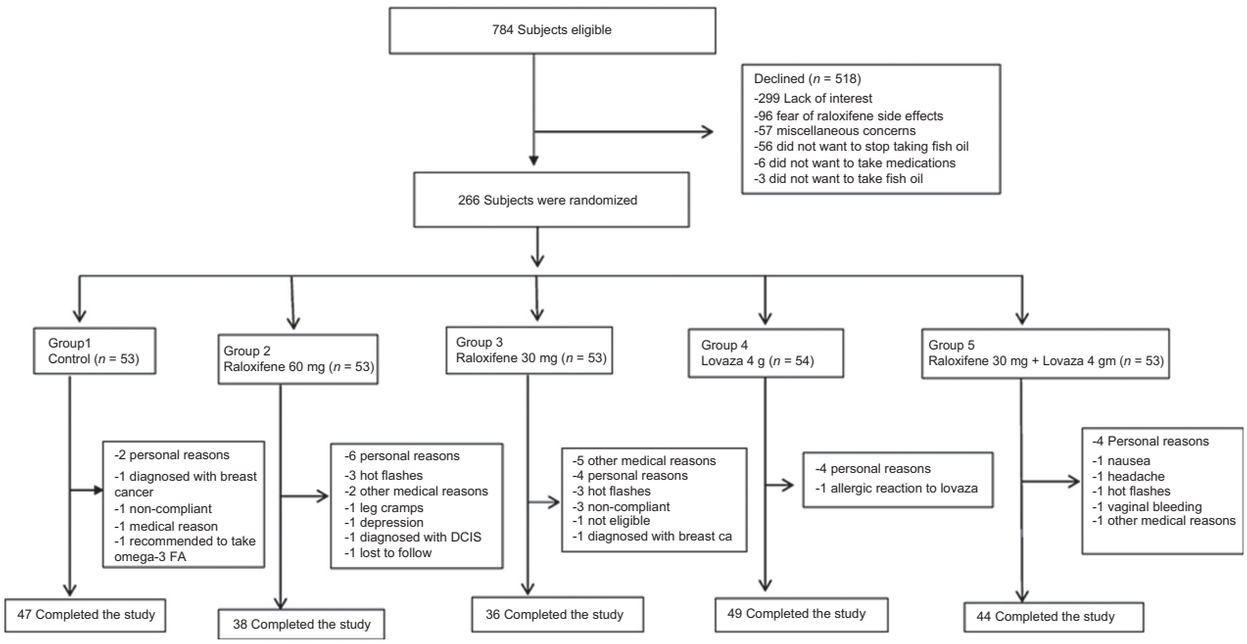

Figure 1.
Number of subjects who were eligible, randomized, and included in the final analysis.

log-transformation was applied to absolute and percentage breast densities and the square root-transformation applied for diet and physical activity variables in linear regression analysis. The correlation of baseline breast densities with baseline demographics is performed with and without adjusting for the BMI level. The relationship between absolute breast density at 24 months and plasma DHA and EPA was analyzed using a multivariate regression which adjusts for subject age, baseline breast density, BMI at 24 months, DHA and EPA at 24 months and treatment groups (28). Data management and analysis were conducted using R 3.1.

Results

Subjects

Out of 784 eligible women, 518 (66%) declined to participate for a variety of reasons as shown in Fig. 1. Although lack of interest was the most common (58%), fear of side effects from raloxifene (19%) and unwillingness to stop taking fish oil (11%) were common reasons. Only three eligible subjects (less than 1%) declined to participate because they did not want to take the fish oil preparation. Two hundred and sixty-six women were randomized to the five experimental groups which were well balanced with regard to baseline subject demographics, including age, BMI, waist:hip ratio, parity, age of first child birth, family history of breast cancer, history of prior breast biopsies, as shown in Table 1. Relevant to one of the main objectives of our analysis, 50% of our subjects were normal weight (BMI < 25), 30% were overweight (BMI 25–29.9). and 20% were obese (BMI $\geq$ 30). After randomization but prior to starting the trial, one woman in group 1 and one in group 3 were diagnosed with breast cancer while one subject in group 2 was diagnosed with DCIS. One woman in group 5 was diagnosed with endometrial cancer less than 6 months after enrollment. Therefore, these four subjects were removed from the study. An additional 48 subjects withdrew while on study for various reasons as indicated in Fig. 1. A total of 214 women (80%) completed the 2-year trial. Baseline variables were similar between women who completed the trial and those that did not, except for BMI which was greater in the latter group.

Dietary and physical activity data

Data on diet and physical activity (PA) at baseline and during the trial are summarized in Supplementary Table S1. We included the dietary and activity data (diet/PA) for all participants who completed the questionnaires at each time point for completeness (for diet/PA, $n = 262/247$ at baseline, $n = 224/172$ at year 1 and $n = 212/139$ at year 2, respectively); however, change over the course of the trial was assessed only for those women who completed the trial with these data available. Overall, there were no statistically significant differences in energy expenditure, total energy consumption, macronutrients intake n-3FA, n-6FA, or the n-3FA:n-6FA ratio compared with the control group and across groups overall. Furthermore, none of these variables changed overtime for the group overall and in the individual groups.

Breast density correlations with baseline demographics

Supplementary Table S2 summarizes the correlations between baseline demographics and breast density in the whole group of 266 women, thus updating the data we have already published in the first 169 women accrued to the trial (22). Our updated results confirm our previously reported novel finding of a strong positive correlation between BMI and absolute breast density which is quite consistent with the well-known association between BMI and breast cancer (29–31). The remaining significant correlations between baseline demographics and breast density were also confirmed in this extended analysis with the addition that the

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Table 1. Baseline demographics of the study population ($n = 266$)[a]

Demographic	Overall ($n = 266$)	Control ($n = 53$)	Raloxifene 60 mg ($n = 53$)	Raloxifene 30 mg ($n = 53$)	Lovaza 4 g ($n = 54$)	Lovaza 4 g + raloxifene 30 mg ($n = 53$)	Overall difference *P*
Age (years)	57.46 ± 5.66	57.11 ± 5.9	58.15 ± 5.09	57.68 ± 5.1	56.56 ± 6.9	57.85 ± 5.1	0.612
BMI (kg/m^2)	26.12 ± 5.03	26.54 ± 5.8	26.05 ± 5.49[b]	26.16 ± 4.46[b]	25.71 ± 4.9	26.17 ± 4.5[b]	0.946
Waist:hip ratio	0.81 ± 0.09	0.81 ± 0.08	0.80 ± 0.08[b]	0.81 ± 0.07[b]	0.80 ± 0.07	0.820 ± 0.13[b]	0.815
Number of births							0.208
0	50 (19%)	8 (15%)	9 (17%)	9 (17%)	12 (22%)	12 (23%)	
1	37 (14%)	7 (13%)	7 (13%)	9 (17%)	9 (17%)	5 (9%)	
2	81 (30%)	13 (25%)	21 (40%)	14 (26%)	16 (30%)	17 (32%)	
≥3	98 (37%)	25 (47%)	16 (30%)	21 (40%)	17 (31%)	19 (36%)	
Age at first child birth (years)	25.93 ± 5.12 ($n = 205$)	24.3 ± 4.0 ($n = 44$)	26.4 ± 4.8 ($n = 41$)	26.6 ± 6.5 ($n = 42$)	27.1 ± 5.1 ($n = 40$)	25.4 ± 4.7 ($n = 38$)	0.105
Family history of breast cancer	140 (53%)	26/53 (49%)	26/53 (49%)	26/53 (49%)	33/54 (61%)	29/53 (55%)	0.634
History of prior breast biopsy	95 (36%)	14/53 (26%)	20/53 (38%)	26/53 (49%)	15/54 (28%)	20/53 (38%)	0.107

[a]Data represent mean ± SD.
[b]One subject in each of these groups did not get anthropometric measurements.

number of births was also found to be negatively correlated with absolute breast density in addition to percent density (Supplementary Table S2).

Treatment effects on plasma n-3FA profile

The baseline plasma n-3FA:n-6FA ratio in the overall population group ranged between 0.101 ± 0.022 and 0.110 ± 0.044. Remarkably, this ratio is very similar to that estimated based on the reported intake of n-3FA and n-6FA in the dietary questionnaires (Supplementary Table S1). As can be seen in Fig. 2, lovaza administration resulted in a sustained increase in the plasma n-3FA:n-6FA ratio (groups 4 and 5). In contrast, the ratio did not change in the control group (group 1) and in the groups receiving raloxifene only (groups 2 and 3). A detailed analysis of the plasma FA profiles in our subjects expressed as absolute or relative amount of total FA content is reported in Supplementary Tables S3 and S4, respectively. As can be seen, lovaza administration induced a 2- to 2.5-fold increase in plasma EPA and DHA, respectively, while the level of arachidonic acid (AA) was significantly reduced. Raloxifene treatment, on the other hand, did not have major influence on plasma FA profiles.

Treatment effects on breast density

As can be seen in Table 2, no significant difference in either percent or absolute density was observed at baseline among the different groups. Furthermore, in our intention-to-treat analysis, none of our interventions significantly affected breast density at either year 1 or 2 (the primary endpoint of our study).

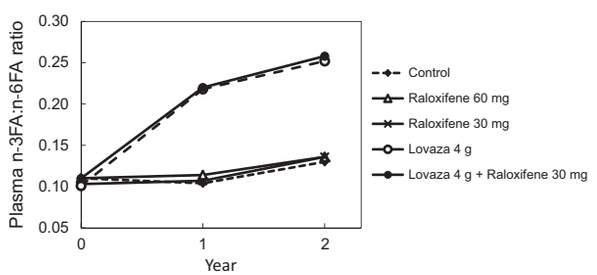

Figure 2.
Plasma n-3FA:n-6FA ratio in the five experimental groups. The effect of lovaza is significant at $P < 0.05$.

BMI affects the relationship between breast density and plasma n-3FA

A major goal of our study was to determine whether BMI affects the relationship between absolute breast density and n-3FA, in particular EPA and DHA. Initially, the effect of BMI on such relationships was assessed in an unadjusted regression model over a wide range of values from 18 to 35. That analysis showed that the magnitude of the inverse relationship between absolute breast density and percent DHA and EPA was greatest at a BMI of 29 (data not shown). Therefore, this value was selected to test whether the effect of BMI on the relationship between breast density and EPA and DHA would remain after adjustment for multiple variables. Linear regression analysis with careful model checking was conducted with the absolute breast density at month 24 as the outcome variable. The breast density at baseline, subject's age, BMI at month 24, DHA and EPA at month 24, and treatment groups were the predictors included in the multivariate regression models. We stratified the dataset into two subsets, one for subjects with BMI > 29 and the other for subjects with BMI ≤ 29. The regression analysis was conducted on each separately. For the dataset of BMI > 29, the regression coefficient of absolute breast density on DHA was −4.301 ($P = 0.0076$), whereas the regression coefficient on EPA was −0.46283 ($P = 0.77$) after adjusting for other predictors in the model. For the dataset of BMI ≤ 29, the regression coefficient of log breast density on DHA was −0.0080 ($P = 0.59$) and the coefficient on EPA was −0.0095 ($P = 0.44$). The log absolute breast density was used for this dataset for better fit of linear model, although similar results were obtained by modeling absolute density directly. These analyses suggest that DHA and absolute breast density are negatively associated in subjects with BMI > 29 but not on subjects with BMI ≤ 29. Figure 3, usually called partial regression plot or added variable plots, shows the dependence of the absolute breast density (for BMI > 29; Fig. 3A) or log absolute breast density (for BMI ≤ 29; Fig. 3B) on DHA after adjusting for other predictors in the regression model. No association between absolute breast density and EPA was found in either BMI levels.

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Table 2. Changes in percent and absolute breast density over time in the five experimental groups[a]

	Baseline		1 year		2 years	
Experimental groups	**Percent density**	**Absolute density (cm^3)**	**Percent density**	**Absolute density (cm^3)**	**Percent density**	**Absolute density (cm^3)**
Group 1	9.79 ± 4.35 (3.25–22.44)	65.53 ± 59.43 (29.66–458.40)	8.93 ± 3.48 (4.21–17.33)	59.29 ± 40.72 (27.34–299.00)	8.86 ± 3.52 (4.23–18.41)	54.34 ± 20.11 (26.05–103.10)
Control	$n = 53$		$n = 48$		$n = 46$	
Group 2	10.98 ± 5.78 (2.97–30.35)	64.39 ± 39.95 (23.87–254.20)	10.34 ± 5.50 (3.79–33.79)	60.48 ± 38.89 (20.45–243.10)	9.65 ± 4.27 (4.25–27.30)	60.57 ± 35.10 (22.29–196.20)
Ral 60 mg	$n = 53$		$n = 41$		$n = 38$	
Group 3	10.76 ± 4.63 (3.31–22.08)	65.08 ± 34.47 (22.95–162.30)	10.84 ± 5.49 (4.83–25.52)	59.53 ± 30.32 (25.12–144.50)	10.56 ± 5.47 (4.33–25.63)	58.86 ± 27.93 (25.43–130.40)
Ral 30 mg	$n = 53$		$n = 41$		$n = 37$	
Group 4	10.91 ± 6.55 (4.20–31.99)	56.35 ± 22.61 (16.91–121.30)	11 ± 6.59 (4.70–30.44)	58.87 ± 22.21 (24.41–104.30)	10.90 ± 7.04 (4.43–31.87)	57.60 ± 20.77 (18.64–116.10)
Lovaza 4 g	$n = 54$		$n = 50$		$n = 48$	
Group 5	10.13 ± 5.05 (4.19–30.32)	63.81 ± 29.81 (19.53–165.66)	10.23 ± 4.69 (4.29–27.52)	60.93 ± 24.64 (21.55–149.40)	9.67 ± 4.26 (3.74–25.95)	58.53 ± 25.18 (21.36–133.90)
Lovaza 4 g + Ral 30 mg	$n = 53$		$n = 45$		$n = 44$	

[a]Data represent mean ± SD.

If we use a BMI cutoff of 30 (which is conventionally used to identify obesity), the regression coefficient of absolute breast density on DHA remains significant, although at a higher *P* value ($P = 0.0381$). This is likely due to the fact that at a cutoff point of 29, the two groups, below and above 29, are more balanced (169 and 43, respectively) than at a cutoff BMI of 30 (179 and 33, respectively).

Treatment effects on lipids

Table 3 shows the changes in serum lipid levels over time in the five groups of largely normolipemic women at baseline. The most striking finding was the beneficial effects of the combination of raloxifene 30 mg and lovaza 4 g which significantly reduced LDL cholesterol and triglycerides while increasing the level of HDL cholesterol. In none of the other groups there was a rise in the HDL level. In contrast, raloxifene 30 mg alone did not have a significant effect on any of the lipid parameters, whereas lovaza 4 g significantly reduced the triglyceride level at 2 years. Of note, raloxifene at the conventional dose of 60 mg significantly reduced LDL cholesterol but increased serum triglycerides at 2 years.

Adverse events

Overall, our interventions were well tolerated. A summary of the adverse events is provided in Supplementary Table S5. As expected, vasomotor symptoms were more frequently reported by the women taking raloxifene, a side effect that appeared to be dose dependent. Three subjects in groups 2 and 3 and one subject in group 5 withdrew from the study because of hot flashes. Leg cramps were also associated with raloxifene in a dose-dependent fashion, even though the association was of borderline statistical significance ($P = 0.0632$). Nevertheless, one woman in group 2 withdrew from the study because of this side effect. Gastrointestinal symptoms were observed more frequently in the combination treatment, although their relation to the interventions is uncertain. However, one woman in group 5 withdrew from the study because of nausea. All side effects were graded as mild or moderate (grades 1 and 2) except for hot flushes in a woman in group 5 which was graded severe (grade 3). This side effect was deemed to be definitely related to raloxifene administration. No episode of venous thromboembolism occurred in any group.

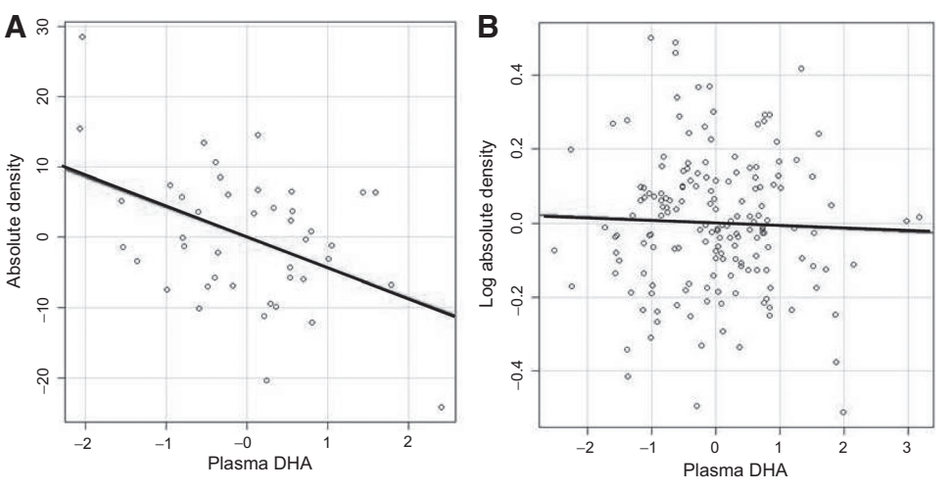

Figure 3.
Partial regression plot of the dependence of absolute breast density on DHA after adjusting for other predictors for BMI > 29 (A) and ≤29 (B). For details of data presentation in these partial regression plots, refer to reference (28).

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Table 3. Serum lipid levels over time in the five experimental groups[a]

Experimental groups	Number of subjects	Total cholesterol (mg/dL)	LDL cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)
Group 1. Control					
Baseline	53	207.3 ± 43.18	114 ± 38.07	68.75 ± 18.83	122.7 ± 54.57
12 months	48	208.8 ± 34.39	115.1 ± 31.99	70.71 ± 18.87	114.5 ± 62.95
24 months	47	207.5 ± 36.41	115.3 ± 29.21	70.19 ± 19.35	110.1 ± 44.25
Group 2. Raloxifene 60 mg					
Baseline	51	203.6 ± 29.98	114.7 ± 27.53	66.18 ± 15.47	113.2 ± 48.39
12 months	42	198.3 ± 29.33	106.8 ± 25.98[b]	68.88 ± 14.06	113.2 ± 55.08
24 months	38	196.6 ± 30.64	104.7 ± 28.13[c]	68.63 ± 15.04	116.9 ± 50.17[b]
Group 3. Raloxifene 30 mg					
Baseline	52	204.3± 36.29	111.2 ± 31.83	70.92 ± 18.54	110.6 ± 50.49
12 months	41	199.6 ± 28.43	106.2 ± 24.38	70.59 ± 16.63	113.7 ± 49.76
24 months	36	202.3 ± 25.58	106.1 ± 25.4	73.17 ± 18.01	115.8 ± 58.48
Group 4. Lovaza 4 g					
Baseline	54	197.7 ± 33.2	106.6 ± 31.96	68.06 ± 16.89	115.1 ± 52.33
12 months	51	199.6 ± 30.45	109.7 ± 29.22	70.59 ± 18.31	96.22 ± 42.94
24 months	49	200.2 ± 34.55	110.4 ± 29.2	70.67 ± 19.38	95.41 ± 49.6[b]
Group 5. Lovaza 4 g plus raloxifene 30 mg					
Baseline	52	197.6 ± 38.68	108.1 ± 35.87	68.9 ± 17.68	103.6 ± 38.79
12 months	45	189.4 ± 33.45[c]	96.58 ± 26.37[c]	76.11 ± 18.61[b]	83.71 ± 31.08[c]
24 months	44	192.6 ± 30.02	99.48 ± 25.2[b]	75.77 ± 17.8[b]	86.43 ± 35[b]

[a]Data represent mean ± SD.
[b]$P < 0.05$ versus control.
[c]$P < 0.01$ versus control.

Discussion

In the overall cohort of healthy postmenopausal women, the administration of n-3FA (a combination of 1,860 mg of EPA and 1,500 mg of DHA daily) alone or in combination with the antiestrogen raloxifene did not reduce breast density, a well-established biomarker of breast cancer risk (17). We believe that these findings can be largely explained by the demographics of our subjects, with only 20% being obese. The subgroup analysis addressing the hypothesis that n-3FA would be preferentially protective in obese subjects is consistent with this possibility. In support of our hypothesis, using an adjusted statistical model, we show a significant negative correlation between plasma DHA breast density only in women with BMI > 29 (Fig. 3). Such negative correlation was not observed with EPA which is in line with preclinical data showing the superiority of DHA in inhibiting mammary carcinogenesis (32, 33). This finding clearly points to obese women as the target population for further investigation of the chemopreventive effects of n-3FA and that the intervention agent should be DHA. The apparent importance of BMI in influencing the beneficial effect of n-3FA in reducing breast cancer risk is also strongly suggested by the literature. Preclinical studies have indicated that n-3FA ameliorate obesity-linked inflammation and insulin resistance (12, 13). Dietary n-3FA and mild dietary energy restriction have been shown to synergistically reduce the degree of inflammation of the white adipose tissue (14). n-3FA have been found to alter adipokines in a tumor-protective mode by increasing the plasma level of adiponectin and decreasing plasma leptin concentrations (15). The possible preferential protective effect of n-3FA in obese subjects has also been suggested by a recently published epidemiologic study (16). However, the results of this study collected in a very specific ethnic group may not be generalizable to the population at large.

Of course, other possibilities could account for the lack of effect of our interventions on breast density in the intention-to-treat analysis. We selected breast density as the primary endpoint because it is the only validated noninvasive biomarker of breast cancer risk (17). In addition to being a biomarker of breast cancer risk, mammographic density is modified by interventions that influence breast cancer risk such as hormone replacement therapy (34, 35) and tamoxifen (17, 36). Most importantly, a reduction in mammographic density after only 12 to 18 months of administration of tamoxifen to high-risk women has been show to accurately predict long-term reduction in breast cancer risk (17). Similarly, a reduction in mammographic density after only 13 months of adjuvant endocrine therapy has been shown to be a significant predictor of long-term recurrence in women with estrogen receptor–positive tumors (37). Therefore, the duration of our trial, e.g., 2 years, should have been of sufficient length to detect an effect of our intervention on breast density. However, evidence in the literature indicates that not all effective interventions against breast cancer, either in the prevention or therapeutic setting, reduce breast density. For instance, raloxifene, an effective chemopreventive agent, although to a lesser degree than tamoxifen (38), has been shown to have either no effect or to cause a minimal statistically insignificant reduction in breast density when compared with placebo (39–42). In our study, we also did not observe an effect of raloxifene on breast density. Furthermore, aromatase inhibitors, which are more effective than tamoxifen for adjuvant therapy of breast cancer (43, 44) and for treatment of metastatic disease (45), have not been found to reduce mammographic density (46).

An interesting finding of our study is the beneficial effects on lipids of the combination of raloxifene 30 mg (half the conventional dose) and lovaza 4 g, the FDA-approved dose for treatment of hypertriglyceridemia (Table 3). Whereas raloxifene alone at 30 mg had no effect on its own on lipids and lovaza only reduced triglycerides at year 2, the combination reduced LDL cholesterol and triglycerides and increased HDL cholesterol significantly at all time points. If confirmed by future studies, such combination may prove to be very useful for treatment of hyperlipidemia given its beneficial influence on multiple lipid parameters.

Our interventions were well tolerated. As expected, vasomotor symptoms and perhaps leg cramps were observed more frequently in the groups receiving raloxifene. Gastrointestinal manifestations occurred more frequently in the combination groups, although their relation to the interventions is difficult to establish. Only 9 subjects withdrew from the study because of concerns of side effects, and overall 80% of randomized subjects completed the 2-year trial. Importantly, no subject developed any episodes of venous thromboembolism.

In conclusion, our results highlight the importance of BMI in affecting the relationship between n-3FA and breast density. Our data suggest that future clinical trials investigating the protective effects of n-3FA on breast cancer risk should be targeted to the subpopulations of obese women and should use DHA as the n-3FA of choice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.P. Richie, L.M. Demers, K. El-Bayoumy, A. Manni
Development of methodology: S.E. Schetter, J.P. Richie, L.M. Demers, A. Manni
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.E. Schetter, T.J. Hartman, C. DuBrock, A. Calcagnotto, N. Trushin, B. Prokopczyk, A. Manni
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.J. Hartman, J.P. Richie, J. McGinley, H.J. Thompson, A. Calcagnotto, L.M. Demers, K. El-Bayoumy, J. Liao, A. Manni
Writing, review, and/or revision of the manuscript: S.E. Schetter, T.J. Hartman, J.P. Richie, H.J. Thompson, K. El-Bayoumy, A. Manni
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.E. Schetter, T.J. Hartman, J. McGinley, C. Signori, C. Hamilton, A. Calcagnotto, C. Aliaga, A. Manni
Study supervision: A. Manni

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Influence of Obesity on Breast Density Reduction by Omega-3 Fatty Acids: Evidence from a Randomized Clinical Trial

Narinder Sandhu, Susann E. Schetter, Jason Liao, et al.

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