

Influence of obesity on breast density reduction by omega-3 fatty
acids: Evidence from a randomized clinical trial

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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. Pre-clinical 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 two-year, open label randomized clinical trial in 266 healthy postmenopausal women (50% normal weight, 30% overweight, 20% obese) with high breast density (BD) ($\geq 25\%$) detected on their routine screening mammograms. Eligible women were randomized to one of the following five groups 1) No treatment, Control; 2) Raloxifene 60 mg; 3) Raloxifene 30 mg; 4) n-3FA Lovaza 4 gm and 5) Lovaza 4 gm plus Raloxifene 30 mg. The two-year change in BD, a validated biomarker of breast cancer risk was the primary endpoint of the study. In subset analysis, we tested the pre-specified 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.

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).

Since 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 since 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 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 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 hypercoagulable 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 five 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 were randomly assigned with equal probability to one of the following five groups: group 1- no treatment, control, group 2- Raloxifene 60mg orally daily, group 3- Raloxifene 30mg orally daily, Group 4- Lovaza 4gm orally daily, and group 5- Lovaza 4gm per day plus Raloxifene 30mg 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 gm. 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, one-year and two-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 publically 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, Wellington, New Zealand). 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 since 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 are compared between the treatment groups using regression analysis. Pre-specified subset analysis of absolute breast density for different BMI levels was also performed. For better model fitting, log-transform 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 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 Figure 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 ≥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 six 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 Figure 1. A total of 214 women (80%) completed the two-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 Supplemental Table 1. 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 to 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

Supplemental Table 2 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 number of births was also found to be negatively correlated with absolute breast density in addition to percent density (Supplemental Table 2).

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 (Supplemental Table 1). As can be seen in Figure 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 Supplemental Table 3 and Supplemental Table 4, 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).

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-value = 0.0076) whereas the regression coefficient on EPA was -0.46283 (p-value = 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-value = 0.59) and the coefficient on EPA was -0.0095 (p-value = 0.44). The log absolute breast density was used for this dataset for better fit of linear model although similar results were obtained by modelling 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, show the dependence of the absolute breast density (for BMI>29) (Panel A) or log absolute breast density (for BMI≤29) (Panel B) 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.

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 gm 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 HDL level. In contrast, Raloxifene 30mg alone did not have a significant effect on any of the lipid parameters, whereas Lovaza 4 gm significantly reduced triglyceride level at two years. Of note, Raloxifene at the conventional dose of 60mg significantly reduced LDL cholesterol but increased serum triglycerides at two years.

Adverse Events:

Overall our interventions were well tolerated. A summary of the adverse events is provided in Supplemental Table 5. As expected, vasomotor symptoms were more frequently reported by the women taking Raloxifene, a side effect which 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 (grade 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.

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 sub-group 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 (Figure 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 since it is the only validated non-invasive biomarker of breast cancer risk (17). In addition to being a biomarker of breast cancer risk, mammographic density is modified by interventions which influence breast cancer risk such as hormone replacement therapy (34, 35) and Tamoxifen (17, 36). Most importantly, a reduction in mammographic density after only 12-18 months administration of Tamoxifen to high-risk women has been shown 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., two 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 to 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 gm, 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 nine subjects withdrew from the study because of concerns of side effects and overall 80% of randomized subjects completed the two-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 sub-populations of obese women and should use DHA as the n-3FA of choice.

References

1. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *Journal of the National Cancer Institute*. 1998;90:1371-88.
2. Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. *Multiple Outcomes of Raloxifene Evaluation*. *Jama*. 1999;281:2189-97.
3. Ropka ME, Keim J, Philbrick JT. Patient decisions about breast cancer chemoprevention: a systematic review and meta-analysis. *J Clin Oncol*. 2010;28:3090-5.
4. Waters EA, Cronin KA, Graubard BI, Han PK, Freedman AN. Prevalence of tamoxifen use for breast cancer chemoprevention among U.S. women. *Cancer Epidemiol Biomarkers Prev*. 2010;19:443-6.
5. Manni A, Xu H, Washington S, Aliaga C, Cooper T, Richie JP, Jr., et al. The impact of fish oil on the chemopreventive efficacy of tamoxifen against development of N-methyl-N-nitrosourea-induced rat mammary carcinogenesis. *Cancer Prev Res*. 2010;3:322-30.
6. Manni A, Richie JP, Jr., Xu H, Washington S, Aliaga C, Bruggeman R, et al. Influence of omega-3 fatty acids on Tamoxifen-induced suppression of rat mammary carcinogenesis. *International journal of cancer*. 2014;134:1549-57.
7. Jiang W, Zhu Z, McGinley JN, El-Bayoumy K, Manni A, Thompson HJ. Identification of a Molecular Signature Underlying Inhibition of Mammary Carcinoma Growth by Dietary N-3 Fatty Acids. *Cancer research*. 2012;72:3795-806.
8. Bidinotto LT, de Cicco RL, Vanegas JE, Santucci-Pereira J, Vanden Heuvel JP, Washington S, et al. Fish oil alters tamoxifen-modulated expression of mRNAs that encode genes related to differentiation, proliferation, metastasis, and immune response in rat mammary tumors. *Nutrition and cancer*. 2012;64:991-9.
9. Skibinski CG, Thompson HJ, Das A, Manni A, Bortner JD, Stanley A, et al. Proteomic changes induced by effective chemopreventive ratios of n-3:n-6 fatty acids and tamoxifen against MNU-induced mammary cancer in the rat. *Cancer Prev Res (Phila)*. 2013;6:979-88.
10. Ruxton CH, Reed SC, Simpson MJ, Millington KJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet*. 2007;20:275-85.
11. Signori C, El-Bayoumy K, Russo J, Thompson HJ, Richie JP, Hartman TJ, et al. Chemoprevention of breast cancer by fish oil in preclinical models: trials and tribulations. *Cancer research*. 2011;71:6091-6.
12. Gonzalez-Periz A, Horrillo R, Ferre N, Gronert K, Dong B, Moran-Salvador E, et al. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J*. 2009;23:1946-57.
13. White PJ, Arita M, Taguchi R, Kang JX, Marette A. Transgenic restoration of long-chain n-3 fatty acids in insulin target tissues improves resolution capacity and alleviates obesity-linked inflammation and insulin resistance in high-fat-fed mice. *Diabetes*. 2010;59:3066-73.
14. Flachs P, Ruhl R, Hensler M, Janovska P, Zouhar P, Kus V, et al. Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids. *Diabetologia*. 2011;54:2626-38.
15. Puglisi MJ, Hasty AH, Saraswathi V. The role of adipose tissue in mediating the beneficial effects of dietary fish oil. *J Nutr Biochem*. 2010;22:101-8.
16. Chajes V, Torres-Mejia G, Biessy C, Ortega-Olvera C, Angeles-Llerenas A, Ferrari P, et al. omega-3 and omega-6 Polyunsaturated fatty acid intakes and the risk of breast cancer in Mexican women: impact of obesity status. *Cancer Epidemiol Biomarkers Prev*. 2012;21:319-26.

17. Cuzick J, Warwick J, Pinney E, Duffy SW, Cawthorn S, Howell A, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. *Journal of the National Cancer Institute*. 2011;103:744-52.
18. Signori C, DuBrock C, Richie JP, Prokopczyk B, Demers LM, Hamilton C, et al. Administration of omega-3 fatty acids and Raloxifene to women at high risk of breast cancer: interim feasibility and biomarkers analysis from a clinical trial. *Eur J Clin Nutr*. 2012;66:878-84.
19. NCI. •Diet*Calc Analysis Program, Version 1.4.3. National Cancer Institute, Applied Research Program. November 2005. 2005; Available from: <http://riskfactor.cancer.gov/DHQ/dietcalc/>
20. International Physical Activity Questionnaire. Available from: <https://sites.google.com/site/theipaq/>
21. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35:1381-95.
22. Schetter SE, Hartman TJ, Liao J, Richie JP, Prokopczyk B, DuBrock C, et al. Differential impact of body mass index on absolute and percent breast density: implications regarding their use as breast cancer risk biomarkers. *Breast cancer research and treatment*. 2014;146:355-63.
23. Highnam B, Yaffe, Karssemeijer, Harvey. Robust breast composition measures - Volpara™. *Lecture Notes on Computer Science*. 2010;6136:342-9.
24. Mart J, Oliver A, Freixenet J, Mart R, Jeffreys M, Harvey J, et al. Comparing a New Volumetric Breast Density Method (Volpara™) to Cumulus. *Digital Mammography*: Springer Berlin Heidelberg; 2010. p. 408-13.
25. Woolcott CG, Courneya KS, Boyd NF, Yaffe MJ, Terry T, McTiernan A, et al. Mammographic density change with 1 year of aerobic exercise among postmenopausal women: a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev*. 2010;19:1112-21.
26. Manni A, Richie JP, Jr., Xu H, Washington S, Aliaga C, Cooper TK, et al. Effects of fish oil and Tamoxifen on preneoplastic lesion development and biomarkers of oxidative stress in the early stages of N-methyl-N-nitrosourea-induced rat mammary carcinogenesis. *International journal of oncology*. 2011;39:1153-64.
27. Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. *Journal of the National Cancer Institute*. 2004;96:621-8.
28. Fox J, and Weisberg, S. *An R companion to applied regression*, Chapter 6. Second ed: Sage; 2011.
29. Lawlor DA, Okasha M, Gunnell D, Smith GD, Ebrahim S. Associations of adult measures of childhood growth with breast cancer: findings from the British Women's Heart and Health Study. *British journal of cancer*. 2003;89:81-7.
30. Hunter DJ, Willett WC. Diet, body size, and breast cancer. *Epidemiol Rev*. 1993;15:110-32.
31. Ekblom A, Thurfjell E, Hsieh CC, Trichopoulos D, Adami HO. Perinatal characteristics and adult mammographic patterns. *International journal of cancer*. 1995;61:177-80.
32. Noguchi M, Minami M, Yagasaki R, Kinoshita K, Earashi M, Kitagawa H, et al. Chemoprevention of DMBA-induced mammary carcinogenesis in rats by low-dose EPA and DHA. *British journal of cancer*. 1997;75:348-53.
33. Yuri T, Danbara N, Tsujita-Kyutoku M, Fukunaga K, Takada H, Inoue Y, et al. Dietary docosahexaenoic acid suppresses N-methyl-N-nitrosourea-induced mammary carcinogenesis in rats more effectively than eicosapentaenoic acid. *Nutrition and cancer*. 2003;45:211-7.

34. Greendale GA, Reboussin BA, Slone S, Wasilauskas C, Pike MC, Ursin G. Postmenopausal hormone therapy and change in mammographic density. *Journal of the National Cancer Institute*. 2003;95:30-7.
35. Stuedal A, Ma H, Bjorndal H, Ursin G. Postmenopausal hormone therapy with estradiol and norethisterone acetate and mammographic density: findings from a cross-sectional study among Norwegian women. *Climacteric*. 2009;12:248-58.
36. Atkinson C, Warren R, Bingham SA, Day NE. Mammographic patterns as a predictive biomarker of breast cancer risk: effect of tamoxifen. *Cancer Epidemiol Biomarkers Prev*. 1999;8:863-6.
37. Kim J, Han W, Moon HG, Ahn SK, Shin HC, You JM, et al. Breast density change as a predictive surrogate for response to adjuvant endocrine therapy in hormone receptor positive breast cancer. *Breast Cancer Res*. 2012;14:R102.
38. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: Preventing breast cancer. *Cancer Prev Res*. 2010;3:696-706.
39. Eilertsen AL, Karssemeijer N, Skaane P, Qvigstad E, Sandset PM. Differential impact of conventional and low-dose oral hormone therapy, tibolone and raloxifene on mammographic breast density, assessed by an automated quantitative method. *BJOG*. 2008;115:773-9.
40. Jackson VP, San Martin JA, Secrest RJ, McNabb M, Carranza-Lira S, Figueroa-Casas P, et al. Comparison of the effect of raloxifene and continuous-combined hormone therapy on mammographic breast density and breast tenderness in postmenopausal women. *Am J Obstet Gynecol*. 2003;188:389-94.
41. Freedman M, San Martin J, O'Gorman J, Eckert S, Lippman ME, Lo SC, et al. Digitized mammography: a clinical trial of postmenopausal women randomly assigned to receive raloxifene, estrogen, or placebo. *Journal of the National Cancer Institute*. 2001;93:51-6.
42. Christodoulakos GE, Lambrinouadaki IV, Vourtsi AD, Panoulis KP, Kelekis DA, Creatsas GC. Mammographic changes associated with raloxifene and tibolone therapy in postmenopausal women: a prospective study. *Menopause*. 2002;9:110-6.
43. Burstein HJ, Prestrud AA, Seidenfeld J, Anderson H, Buchholz TA, Davidson NE, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*. 2010;28:3784-96.
44. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol*. 2013;24:2206-23.
45. Mauri D, Pavlidis N, Polyzos NP, Ioannidis JP. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. *Journal of the National Cancer Institute*. 2006;98:1285-91.
46. Vachon CM, Suman VJ, Brandt KR, Kosel ML, Buzdar AU, Olson JE, et al. Mammographic breast density response to aromatase inhibition. *Clin Cancer Res*. 2013;19:2144-53.

Figure Legends

- Figure 1 Number of subjects who were eligible, randomized and included in the final analysis
- Figure 2 Plasma n-3FA:n-6FA ratio in the five experimental groups. The effect of Lovaza is significant at p-value <0.05.
- Figure 3 Partial regression plot of the dependence of absolute breast density on DHA after adjusting for other predictors for BMI>29 (Panel A) and ≤29 (Panel B). For details of data presentation in these partial regression plots, refer to reference (28).

Table 1: Baseline Demographics of the study population (n= 266)^a

Demographic	Overall (n=266)	Control (n=53)	Raloxifene 60mg (n=53)	Raloxifene 30mg (n=53)	Lovaza 4g (n=54)	Lovaza 4g + Raloxifene 30 mg (n=53)	Overall Difference P value
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.

Table 2: Changes in percent and absolute breast density over time in the five experimental groups ^a

Experimental groups	Baseline		1 year		2 years	
	Percent density	Absolute Density (cm ³)	Percent density	Absolute Density (cm ³)	Percent density	Absolute Density (cm ³)
Group 1 Control	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)
	n = 53		n = 48		n = 46	
Group 2 Ral 60 mg	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)
	n = 53		n = 41		n = 38	
Group 3 Ral 30 mg	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)
	n = 53		n = 41		n = 37	
Group 4 Lovaza 4 gm	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)
	n = 54		n = 50		n = 48	
Group 5 Lovaza 4 gm + Ral 30 mg	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)
	n = 53		n=45		n = 44	

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 60mg					
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*	68.88 ± 14.06	113.2 ± 55.08
24 months	38	196.6 ± 30.64	104.7 ± 28.13+	68.63 ± 15.04	116.9 ± 50.17*
Group 3 Raloxifene 30mg					
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 4gm					
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*
Group 5 Lovaza 4gm plus Raloxifene 30mg					
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+	96.58 ± 26.37+	76.11 ± 18.61*	83.71 ± 31.08+
24 months	44	192.6 ± 30.02	99.48 ± 25.2*	75.77 ± 17.8*	86.43 ± 35*

^a: data represents mean ± SD

*p<0.05 versus control

+p<0.01 versus control

Figure 1

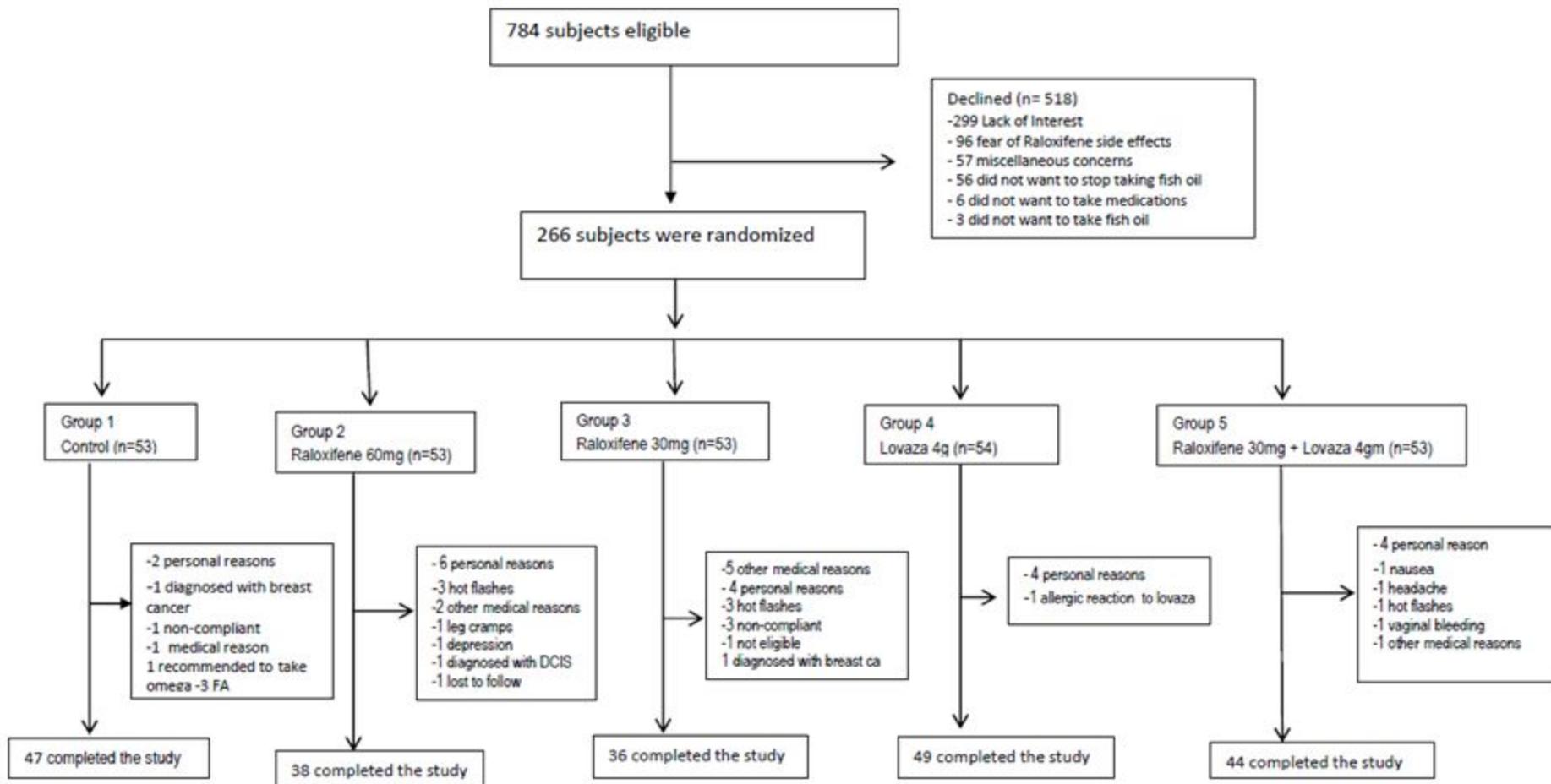


Figure 2

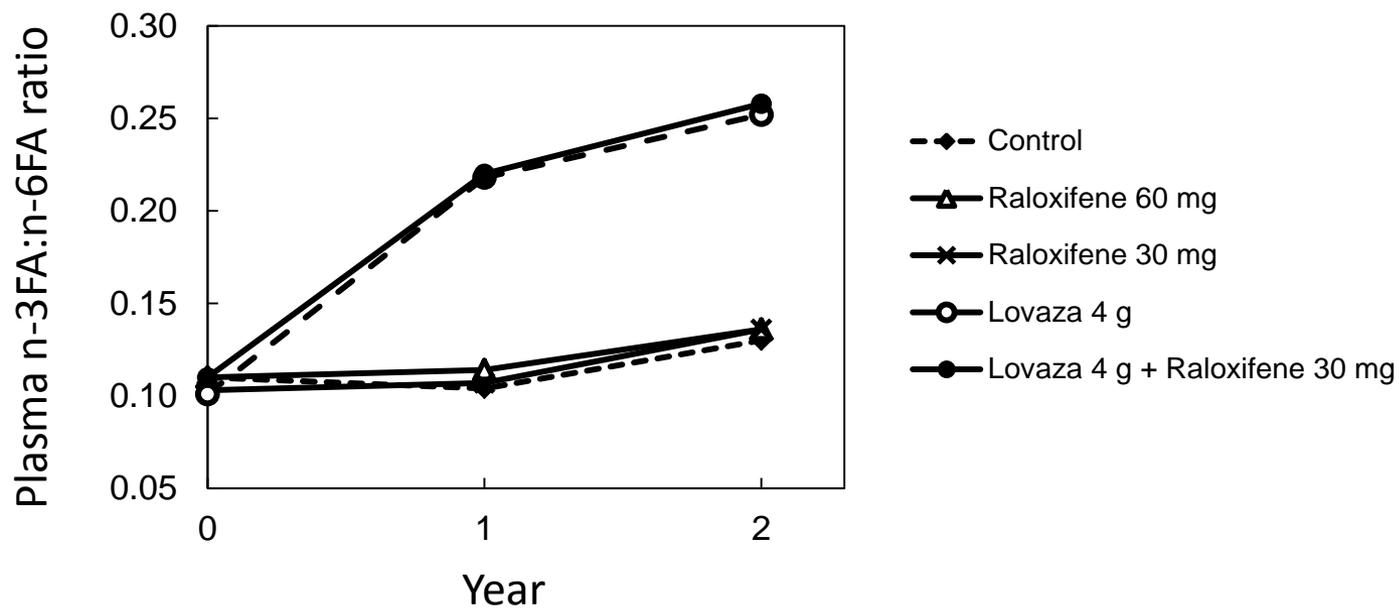
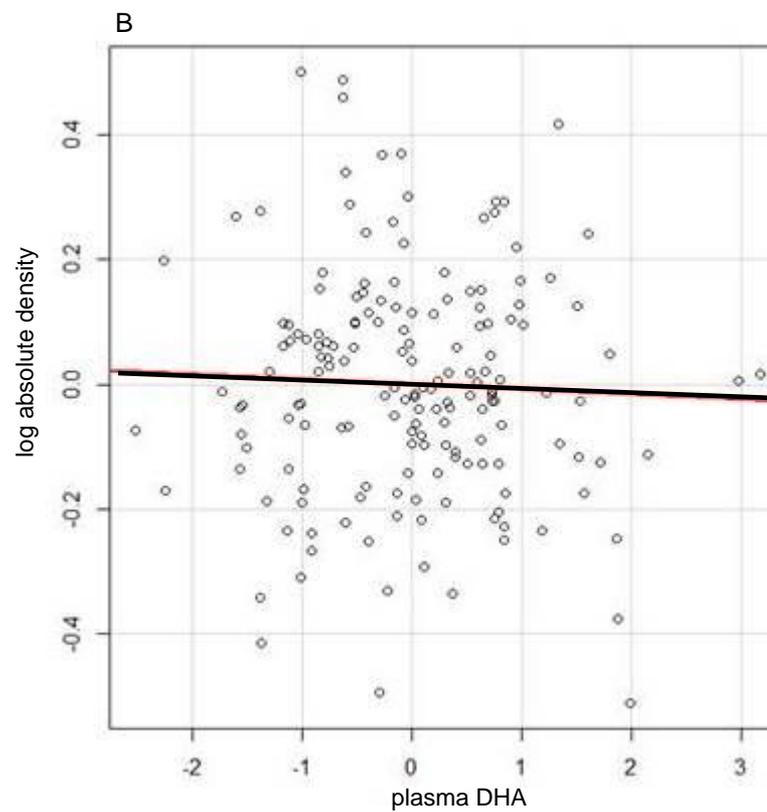
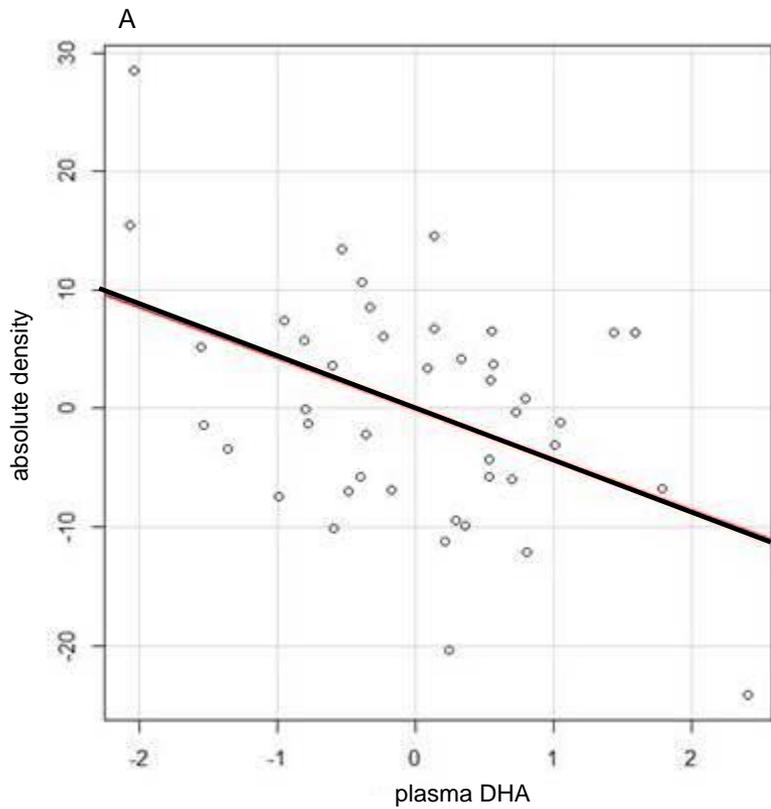


Figure 3



Cancer Prevention Research

Influence of obesity on breast density reduction by omega-3 fatty acids: Evidence from a randomized clinical trial

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

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