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High-Dose Eicosapentaenoic Acid and Docosahexaenoic Acid Supplementation Reduces Bone Resorption in Postmenopausal Breast Cancer Survivors on Aromatase Inhibitors: A Pilot Study

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Postmenopausal breast cancer survivors are living longer; however, a common class of drugs, aromatase inhibitors (AI), depletes estrogen levels, promotes bone loss, and heightens fracture risk. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may offset AI effects to bone because of the known effects on cellular processes of bone turnover. Therefore, we hypothesized that 4 g of EPA and DHA daily for 3 mo would decrease bone turnover in postmenopausal breast cancer survivors on AI therapy in a randomized, double-blind, placebo controlled pilot study that included 38 women. At baseline and 3 mo, serum fatty acids, bone turnover, and inflammatory markers were analyzed. Serum EPA and DHA, total and long-chain (LC) omega (n)-3 polyunsaturated fatty acids (PUFA) increased, whereas arachidonic acid, total and LC n-6 PUFA, and the LC n-6:n-3 PUFA ratio decreased compared to placebo (all $P < .05$). Bone resorption was inhibited in the fish oil responders compared to placebo ($P < .05$). Inflammatory markers were not altered. This short-term, high-dose fish oil supplementation study's findings demonstrate that fish oil can reduce bone resorption; however, longer-term studies are needed to assess bone density preservation and to explore mechanistic pathways in this population at high risk for bone loss.

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INTRODUCTION

Long-term relative survival rates for postmenopausal women with breast cancer have increased upwards of 24% in the last 30 yr (1). The positive survival rates are a result of advanced and targeted maintenance drug therapies. One such class of drugs is the aromatase inhibitors (AI); this class of drug inhibits the aromatase enzyme complex and depletes whole body estrogen levels. AI, used either as a monotherapy or after 2 to 3 yr of tamoxifen treatment for estrogen receptor positive breast cancer, can significantly lower recurrence rates in postmenopausal women compared to the use of tamoxifen alone (2,3). Although AI treatment can increase survival in women with estrogen positive breast cancer, this treatment has a negative effect on bone with increased bone resorption and amplified bone mineral density (BMD) losses; one of the most noteworthy risk factors is a higher risk of fracture in women on AI therapy compared to

women without breast cancer at an equal age (3). Conversely, tamoxifen can increase bone density and reduce incidence of fractures in postmenopausal breast cancer patients (4). Drug treatment of bisphosphonates, specifically zoledronic acid, to offset AI's detrimental effects on bone is not without side effects including osteonecrosis of the jaw (5), arthritis, myalgias, cramps, atypical femur fractures, and hypocalcaemia (6). Alternatives to slow or reduce bone loss are therefore warranted. One such potential alternative therapy is omega (n)-3 polyunstaurated fatty acids (PUFA).

The long chain (LC) n-3 PUFA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have demonstrated benefit to bone in animal models via reduced inflammatory cytokines, increased calcium absorption, and enhanced skeletal calcium levels (7–9). EPA and/or DHA treatment in *in vitro* and *in vivo* models show inhibition of osteoclastogenesis (10–14) and protection of osteoblastogenesis (15–18). An animal model of aging mice demonstrated preservation of BMD, reduced tumor necrosis factor (TNF)α and reduced bone resorption in fish oil fed mice compared to those given corn oil as the lipid source (10).

The results from studies of older adults are varied. Most cross-sectional studies show a benefit of LC n-3 PUFA or fatty fish on BMD (19–22), without effect on fracture (23–25). A recent nested case-control study from the Women's Health Initiative found that higher concentrations of red blood cell (RBC) α-linolenic acid and EPA was associated with lower risk of hip fracture; conversely, a higher RBC n-6/n-3 PUFA ratio was associated with higher hip fracture risk using stratified COX proportional hazard models (26). There are few intervention studies in adults and they have yielded mixed results. Supplementation of 1.2 g EPA and DHA per day for 3 mo to mildly depressed adults found no effect on bone resorption (27); others showed decreased bone resorption after 6 wk of consumption of a high α-linolenic acid diet in middle aged adults (28). Studies supplementing with both LC n-3 and n-6 PUFA in the form of EPA or a fish oil and gamma linolenic acid or evening primrose oil show no benefit on bone density or turnover (29,30).

The biochemical processes in which n-6 PUFA act as proinflammatory precursors and n-3 PUFA as antiinflammatory are possible mechanisms for inflammatory associated bone turnover (11–14, 31, 32) and disease states (20, 33–35). Therefore, we hypothesized that a 2:1 EPA:DHA supplementation of 4 g daily for 3 mo would, via antiinflammatory actions, decrease bone turnover and decrease cytokines associated with bone metabolism, in postmenopausal breast cancer survivors receiving aromatase inhibitors as maintenance treatment for breast cancer.

EXPERIMENTAL METHODS

Study Design and Subjects

This is a randomized, double-blind, placebo-controlled pilot study to test the effect of 4 g of EPA + DHA supplementation on bone turnover in postmenopausal women receiving AI for the treatment of estrogen-positive breast cancer for at least 6 mo with plans to continue treatment for at least a year. This study was conducted according to the Declaration of Helsinki guidelines and all procedures involving human subjects were approved by the Institutional Review Board at the University of Connecticut Health Center. Written informed consent was obtained from all subjects. Women were recruited from the Neag Cancer Center and local oncology practices, employee announcements, or advertisements. Exclusion criteria included any other disease that may affect bone metabolism (Paget's disease, primary hyperparathyroidism); other cancers of any kind, except basal or squamous cell of skin, in the past 5 yr; use of calcitonin, calcitriol, heparin, phenytoin, phenobarbital, and estrogen/testosterone/tamoxifen in the past 6 mo; ever use of bisphosphonates, long-term corticosteroids (>6 mo), methotrexate, or fluoride; current use of coumadin or clopidogrel; estimated creatinine clearance <40 ml/min; history of chronic liver disease or evidence of liver disease on screening; history of hip fracture or known vertebral fracture within the past year; or history of allergy to fish or fish oil.

Treatment

Women were randomized (1:1 ratio via randomization.com) to receive either 7 capsules/day containing 4 g EPA + DHA (2520 mg EPA, 1680 mg DHA) or 7 capsules/day of placebo containing safflower oil (9% linoleic acid, 83% oleic acid). All participants received calcium carbonate (1000 mg/day) and cholecalciferol (800 IU/day).

Measures

Serum Fatty Acid Extraction and Analysis

Fatty acid composition was determined in the total lipid and the polar lipid fraction of serum samples. For the fatty acid determination in total lipids, 100 μl of serum sample was subjected to solvent extraction using chloroform/methanol (2:1, vol/vol). The resulting lipids were converted to fatty acid methyl esters (FAME) after being treated with 0.5 N NaOH in methanol followed by boron trifluoride (BF3) in methanol (10% w/w, Supelco Inc., Bellefonte, PA). The fatty acid analysis of polar lipids was performed using 140 μl of serum. The polar lipids were isolated using solid phase extraction by eluting the polar lipid fraction with methanol in a silica cartridge (300 mg filling, Alltech) (36). Polar lipids were then transmethylated to FAME directly with 10% BF3 in methanol. The resulting FAME from total and polar lipids were analyzed by gas chromatography (GC) (HP 7890A series, autosampler 7693, GC ChemStation Rev.B.04.03, Agilent Technologies, Palo Alto, CA) using a DB-225 column (30 m, 0.25 mm i.d., 0.15 mm film thickness, Agilent Technologies, Palo Alto, CA) and flame ionization detection (37). An area percentage report was generated from total areas of FAME peaks using the ChemStation software. The FAME peaks were identified by comparing retention times to that of authentic standard mixtures of fatty acids (Nu-Chek-Prep, Elysian, MN).

Bone and Bone Metabolism Measurements

Serum C-Terminal Telopeptide (sCTX) and 25-OH vitamin D analyses were conducted using ELISA techniques with Immunodiagnostic Systems Limited Kits following manufacturer instructions. The intra assay variability was 2.2% and 5.9%; while the inter assay Coefficient of Variation (CV) was 7.7% and 6.6%, respectively. Deoxypyridinoline (DPD) was measured by ELISA (Metra Biosystems, Inc., Mountain View, CA) with inter assay variability <10%. Procollagen type 1 N-terminal propeptide (P1NP) was analyzed by radioimmunoassay from an Orion Diagnostica kit with 8.3% inter assay CV and 7.8% intra assay CV. Bone specific alkaline phosphatase (BAP) was measured by ELISA (Metra Biosystems Inc., Palo Alto, CA). Average intra assay variability was <5%. Parathyroid hormone (PTH) was analyzed by solid-phase chemiluminescent immunometric assay using a kit from Siemens Healthcare Diagnostics with 3.4% intra assay CV and 5.8% inter assay CV. BMD of the proximal femur and lumbar spine were obtained at baseline via dual energy x-ray absorptiometry (Lunar Prodigy, Madison, WI). The CVs of BMD measurement at the proximal femur and spine were <1% and 1.5%, respectively.

Inflammatory Markers

Interleukin-6 (IL-6), interleukin-1 β, high sensitivity C-reactive protein (hs-CRP), migratory inhibitory factor, and TNFα were measured by immunoassay (Diagnostic Products Corporation, Immulite 1000, Los Angeles, CA) with an intra assay CV of <5% for each assay.

Evaluations

At the baseline visit a health history questionnaire was completed and height (cm) and weight (kg) for each participant were measured to calculate body mass index (BMI, kg/m^2). Three-day diet records were recorded by participants at baseline to determine nutrient intake. Records were reviewed with the study dietitian and analyzed using Nutritionist Pro (ESHA version 10.1).

Statistical Analysis

Data are presented as means ± SD. Baseline characteristics were analyzed using a *t*-test for continuous variables and chi squared test for categorical variables. Paired *t*-tests were used for within-group analysis from baseline to 3 mo. Difference scores were generated by subtracting baseline scores from posttest scores. Then, to examine whether there were differential changes in dependent variables (bone turnover, regulatory and inflammatory markers, absolute change in serum fatty acids) between fish oil and placebo group, the difference scores were subjected to an independent samples *t*-test with treatment (fish vs. placebo oil) as the independent variable. Pearson's correlation was used to test for associations between changes in bone turnover markers with serum fatty acids.

Upon fatty acid assessment, four individuals in the fish oil supplement group had no change in DHA or EPA (% change in EPA score range from −0.72 to 4%). We, therefore, performed post-hoc analyses using only those who responded to fish oil (fish oil responders with positive changes in EPA and DHA of at least 15%) to assess changes in outcome measures. The analyses mirrored those conducted for the entire group.

RESULTS

Participants

Thirty-eight women were randomized ($n = 20$ fish oil, $n = 18$ placebo) from recruitment efforts that screened 127 women and found 48 eligible for participation, although 10 did not consent to the study (Fig. 1). After randomization, 4 women dropped from the study. Three dropped due to scheduling conflicts (2 from fish oil, 1 from placebo) and one in the fish oil group was unwilling to be in a study with 50% chance of non-treatment. Women were predominately Caucasian (89.5%, $n = 34$). The subjects' average age was 62 (range 48–84 years) with 29% overweight [body mass index (BMI) between 25 and 29.9) and 29% obese (BMI > 30). There were no between-treatment group differences at baseline in age, BMI, ethnicity, education, comorbidities, prescribed drug intake, smoking status, reported dietary or alcohol intake, or serum bone marker values (Table 1). Baseline total femoral and trochanter BMD was greater in the placebo group compared to fish oil group. All women had normal femoral neck BMD (*t* score > −1.0) at baseline.

Dietary Intake

Women consumed an average of 1700 ± 326 kcals, 74 ± 19 g protein, 66 ± 20 g total fat, 22.5 ± 8.3 g saturated fat, 760 ± 508 mg omega-3 PUFA, 5930 ± 3595 mg omega-6

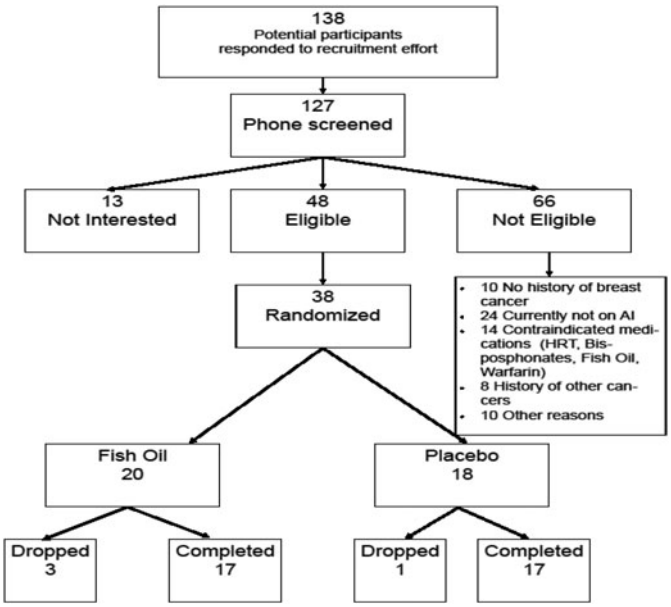

FIG. 1. Flow chart of study participant selection.

TABLE 1
Baseline characteristics

	Placebo Mean ± SD	Fish oil Mean ± SD	*P* value*
Age (yr)	63.6 ± 8.6	60.9 ± 10.5	0.402
Height (cm)	161.5 ± 6.3	162.0 ± 6.1	0.797
Weight (kg)	72.9 ± 14.0	72.5 ± 18.2	0.943
BMI (kg/m^2)	28.3 ± 5.8	27.63 ± 5.9	0.740
Normal (18.5–24.9) %(n)	18.4 (7)	23.7 (9)	0.703
Overweight (25–29.9) %(n)	15.8 (6)	13.2 (5)	0.572
Obese (30 +) %(n)	13.2 (5)	15.8 (6)	0.880
Bone density			0.315
Osteoporotic %	0	0	
Low bone density %(n)	16.2 (6)	29.7 (11)	0.134
Normal bone density %(n)	32.4 (12)	21.6 (8)	0.134
Smoker % (current)**	1	0	0.579
Drinks alcohol %	37.8 (14)	43.2 (16)	0.618
AI drug brand %#			0.245
Letrozole	12 (4)	12 (4)	0.931
Anastrozole	29 (10)	35 (12)	0.638
Exemestane	3 (1)	9 (3)	0.316
Drug intake %			
NSAIDS	5.4 (2)	10.8 (4)	0.412
Aspirin	10.8 (4)	13.5 (5)	0.772
Comorbidity %			
CHD	0	5.4 (2)	0.157
Diabetes	5.4 (2)	2.7 (1)	0.515
Hypertension	10.8 (4)	13.5 (5)	0.772
Depression	2.7 (1)	8.1 (3)	0.316
Osteoarthritis	13.5 (5)	10.8 (4)	0.634

BMI = body mass index; AI = aromatase inhibitor; NSAIDS = nonsteroidal antiinflammatory drugs; CHD = coronary heart disease.

*Statistical analyses conducted were t-tests for continuous variables and chi-squared for categorical variables.

**Chi-squared test between current, previous, and never smokers.

#Letrozole, Novartis Oncology, East Hanover, NJ; Anastrozole, AstraZeneca, Wilmington, DE; Exemestane, Pfizer Pharmacia & Upjohn Company, New York, NY.

PUFA, 729 ± 324 mg calcium, 107 ± 101 IU vitamin D, 94 ± 45 mg vitamin C, and 56 ± 65 mcg vitamin K from their diet at baseline, there were no significant differences between groups.

Fatty Acids

The fatty acid profile of total and polar lipid fractions followed similar trends. More 20 and 22 carbon fatty acids were identified in the polar fraction compared to total lipids. As the 20 and 22 carbon fatty acids (EPA and DHA) were concentrated in the treatment, only polar lipid fraction results are presented in Table 2. Oleic acid (18:1n9) concentration was greater in the placebo group compared to the fish oil group at baseline ($P = .01$); no other fatty acids were different at baseline between groups. All fatty acid levels were consistent, no significant differences, from baseline to 3 mo in the placebo group. After 3 mo of supplementation, women in the fish oil group had greater concentrations of total and LC n-3 PUFA with decreased total and LC n-6 PUFA levels compared to placebo. The fish oil group as a whole is seen with increased EPA and DHA levels as expected with supplementation of 2.52 g EPA and 1.68 g DHA per day, resulting in an average of 402% increase in EPA [177: 628%; 95% confidence interval (CI)] and 99% (63: 135%; 95% CI) increase in DHA. Evaluation of the percent change in EPA and DHA showed some noncompliance/nonresponse as there were individuals in the fish oil group with less than a 15% increase in EPA and DHA. Fig. 2 depicts the percent change in EPA plotted with percent change in DHA. The nonresponders in the fish oil group plot within the region of those in the placebo group; and they were those with less than 15% increase in EPA.

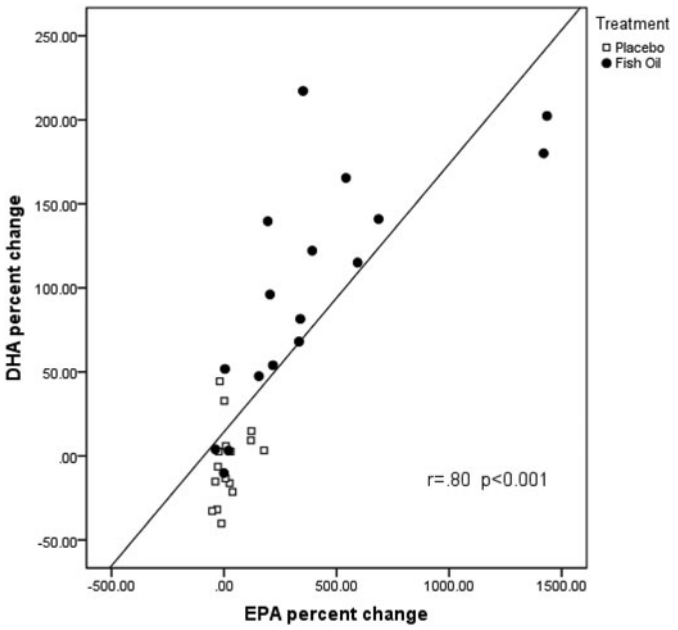

FIG. 2. Scatterplot of percent change eicosapentaenoic acid (EPA) and of percent change docosahexaenoic acid (DHA). Noncompliers/nonresponders are identified as those in the fish oil group (filled circle) who plot with individuals in the region of placebo treatment group (open square).

Bone and Bone Metabolism

Bone formation (BAP and P1NP) and resorption (sCTX and DPD) marker levels were similar between groups at baseline (all $P > .10$) (Table 3). Baseline 25(OH)D and PTH were also comparable (Table 3). Within the fish oil group, DPD, P1NP, and BAP all significantly decreased from baseline to 3 mo; sCTX, DPD, and P1NP, within the fish oil responders, were also

TABLE 2
Fatty acid composition of serum fatty acid methyl esters (FAME) of postmenopausal breast cancer survivors on aromatase inhibitors

	Polar FAME				
	Placebo		Fish oil		
Fatty acid	Baseline	3 mo	Baseline	3 mo	*P* value[#]
14:0	0.68 ± 0.35	0.66 ± 0.40	0.88 ± 0.39	1.02 ± 0.49	.43
16:0	22.14 ± 4.04	22.29 ± 3.83	23.48 ± 3.53	22.68 ± 4.32*	.63
16:1t	0.26 ± 0.07	0.28 ± 0.09	0.25 ± 0.08	0.23 ± 0.06	.28
16:1n7	0.89 ± 0.37	0.88 ± 0.35	0.90 ± 0.44	0.73 ± 0.39*	.27
17:0	0.37 ± 0.07	0.37 ± 0.07	0.35 ± 0.07	0.35 ± 0.06	.57
18:0	15.44 ± 1.39	15.27 ± 2.04	15.15 ± 1.77	15.58 ± 1.58	.38
18:1n9	13.99 ± 1.91	14.79 ± 3.08	13.67 ± 1.57	12.68 ± 1.93*	.01
18:1n7	1.77 ± 0.29	1.72 ± 0.26	1.58 ± 0.18	1.57 ± 0.20	.75
18:2n6	19.92 ± 2.60	19.68 ± 2.95	20.50 ± 2.96	19.31 ± 3.48	.15
18:3n6	0.27 ± 0.08	0.30 ± .10	0.24 ± 0.07	0.21 ± 0.08	.47
18:3n3	0.51 ± 0.12	0.49 ± 0.12	0.51 ± 0.15	0.53 ± 0.14	.43
20:2n6	0.36 ± 0.07	0.34 ± 0.06	0.32 ± 0.07	0.32 ± 0.05	.38
20:3n6	2.66 ± 0.85	2.62 ± 0.82	2.64 ± 0.69	1.95 ± 0.73*	<.001
20:4n6	12.28 ± 2.27	11.84 ± 1.96	11.19 ± 1.84	9.28 ± 1.81*	<.001
20:5n3	1.04 ± 0.47	1.14 ± 0.72	1.02 ± 0.54	3.86 ± 2.14*	<.001
22:4n6	0.39 ± 0.10	0.38 ± 0.10	0.42 ± 0.12	0.25 ± 0.11*	<.001
22:5n6	0.29 ± 0.08	0.27 ± 0.07	0.33 ± 0.11	0.20 ± 0.08*	<.001
22:5n3	1.05 ± 0.28	0.97 ± 0.23	0.93 ± 0.16	1.41 ± 0.41*	<.001
22:6n3	3.51 ± 0.90	3.32 ± 0.82	3.12 ± 0.86	5.71 ± 1.52*	<.001
Total SAT	38.58 ± 3.72	38.66 ± 3.91	39.78 ± 2.85	39.45 ± 3.98	.84
Total MONO	17.15 ± 2.37	17.87 ± 3.54	16.63 ± 1.94	15.50 ± 2.25*	.02
Total PUFA	41.99 ± 3.18	41.25 ± 2.52	41.19 ± 3.36	42.90 ± 3.44*	.03
Total n-3	6.10 ± 1.48	5.92 ± 1.24	5.58 ± 1.24	11.51 ± 3.79*	<.001
Total n-6	35.89 ± 2.88	35.33 ± 2.50	35.61 ± 3.02	31.40 ± 4.88*	<.001
n-6:n-3	6.19 ± 1.57	6.21 ± 1.35	6.67 ± 1.49	3.15 ± 1.49*	<.001
LC n-3	5.60 ± 1.44	5.43 ± 1.27	5.08 ± 1.24	10.98 ± 3.79*	<.001
LC n-6	15.79 ± 2.40	15.44 ± 2.08	14.90 ± 2.27	11.95 ± 2.48*	<.001
LC n-6:n-3	2.97 ± 0.76	2.98 ± 0.71	3.08 ± 0.73	1.28 ± 0.69*	<.001

Data are presented as mean ± SD. Calculations for the total fatty acids are as follows: long-chain (LC) n-3: 20:5n3, 22:5n3, 22:6n3; LC n-6: 20:2n6, 20:3n6, 20:4n6, 22:4n6, 22:5n6. Within the fish oil group, an asterisk depicts a significant difference between baseline and 3 mo. SAT = saturated fatty acids; MONO = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

# denotes the *P* value for the differences in change from baseline to 3 mo in placebo vs. fish oil group.

significantly decreased (Table 3). When comparing the change from baseline to 3 mo between groups, one of the resorption markers, DPD, was reduced in the fish oil group ($P = .042$). As noted above, not all participants in the fish oil group responded to treatment. Therefore, we analyzed the data with the nonresponders removed ($n = 4$ from the fish oil group). The fish oil responders compared to placebo differed for bone resorption. sCTX levels significantly decreased in fish oil responders, whereas the DPD trend remained, it was no longer significant, compared to placebo (Table 3). No significant bone formation or metabolism differences were observed between placebo and fish oil responders at 3 mo.

Fatty Acid and Bone Marker Associations

The large range of serum EPA and DHA response to fish oil supplementation led us to hypothesize that a greater change in serum EPA and DHA would be more favorable for bone turnover, particularly resorption based upon the data presented herein. Therefore, exploratory correlational analyses were conducted. The bone resorption marker, sCTX's change from baseline to 3 mo was inversely associated with changes in EPA and DHA ($r = -.45$, $P = .01$; $r = -.44$, $P = .01$; respectively). The change in arachidonic acid ($r = .36$, $P = .04$) and the LC n-6/n-3 ratio ($r = .455$, $P \leq .01$) were positively associated with change in sCTX. No other significant associations were noted.

TABLE 3
Bone turnover and metabolism markers: No significant differences between groups at baseline or 3 mo

	Placebo		Fish oil		
Markers	Baseline	3 mo	Baseline	3 mo	*P* value[#]
sCTX (ng/mL)	0.68 ± .25	0.66 ± .30	0.84 ± .39	0.70 ± .30	0.090
FO responders			0.76 ± 0.33	0.66 ± 0.30*	0.038
DPD (nM/mM)	7.18 ± 2.44	7.02 ± 2.00	7.93 ± 1.99	6.48 ± 1.90*	0.043
FO responders			7.39 ± 1.78	6.62 ± 1.86*	0.074
P1NP (ug/L)	66.2 ± 26.0	56.7 ± 20.5	71.6 ± 26.4	59.7 ± 21.8*	0.495
FO responders			66.1 ± 27.1	57.2 ± 22.8*	0.402
BAP (UL)	32.5 ± 9.06	29.9 ± 8.4	36.8 ± 10.5	32.0 ± 9.4*	0.218
FO responders			34.0 ± 9.1	31.1 ± 9.1†	0.257
25(OH) vit D(nmol)	76.5 ± 17.2	75.4 ± 17.4	71.3 ± 24.9	75.6 ± 19.3	0.700
FO responders			75.5 ± 16.6	74.6 ± 13.0	0.697
PTH (pg mol)	25.4 ± 14.4	26.2 ± 11.8	32.1 ± 12.7	26.5 ± 10.5	0.700
FO responders			29.25 ± 9.86	24.76 ± 9.50†	0.251

Data presented as mean ± SD. Intent-to-treat (ITT) analysis: placebo, $n = 18$, $n = 16$; fish oil (FO), $n = 20$, $n = 17$, baseline and 3 mo, respectively. FO responders only: $n = 16$, $n = 13$, baseline and 3 mo, respectively. sCTX = serum C-terminal telopeptide; DPD = Deoxypyridinoline; P1NP = procollagen type 1 N-terminal propeptide; BAP = bone-specific alkaline phosphastase; 25(OH) vitamin D = 25 hydroxyl vitamin D; PTH = parathyroid hormone.

Within the FO group, * depicts a significant difference, $P < .05$, between baseline and 3 mo; † denotes a trend, $P = .06$–$.08$; and # denotes the *P* value for the differences in change from baseline to 3 mo in placebo vs. FO group.

Inflammatory Markers

Inflammatory markers were tested as a potential mechanism of action for the decreased bone formation found in fish oil responders. Baseline hs-CRP values were comparable to other studies with similar populations (38). The wide range of BMI in our small sample also reflected what is a common occurrence, higher BMI correlates with hs-CRP ($r = .469$, $P = .003$). After 3 mo of fish oil consumption, hs-CRP was noted to increase (2.86 ± 3.29 to 4.79 ± 6.59, $P = .027$); however, 4 participants were outliers with excessive increases in hs-CRP levels. Secondary analysis with outliers removed continued to show a significant difference in hs-CRP change (7.78 ± 8.38 and 2.38 ± 3.25, $P = .022$, fish oil and placebo group, respectively). No other inflammatory markers differed with or without the outliers (data not shown).

DISCUSSION

Breast cancer survivors on AI therapy experience accelerated bone resorption and losses (3). At the initiation of our study, women were included if they followed an AI regimen for at least 6 mo and planned to continue the regimen for up to a year. Prior studies that demonstrated a detrimental effect of AI treatment on bone found an approximately 10% increase in bone turnover markers during the first 6 mo of treatment (39). Findings from the Anastrozole, Tamoxifen Alone or in Combination (ATAC) Trial demonstrated up to 26% increased bone resorption over 1 yr; however, most of the changes were observed from 3 to 6 mo with the 6–12 mo time frame maintaining the higher level (40). We, therefore, expected to observe a high but steady state of bone turnover from baseline to Month 3 in the placebo group. However, the bone turnover markers remained stable in the placebo group. The most likely explanation for the stability of bone turnover markers in the placebo group is that the women in our study have already reached a higher bone turnover maintenance level. Alternative explanations include the possibilities that our choice for placebo was not a true placebo or the calcium and vitamin D provided to all participants attributed to the stabilization of bone turnover makers.

The identified bone turnover increase with earlier treatment (0–6 mo) and higher maintenance level with increased duration on AI treatment suggests that the time frame of our study may have missed the crucial period of up to 6 mo. The lack of flux in bone resorption or formation in our study from 0 to 3 mo supports the idea that women on AI therapy have reached a new baseline for their bone turnover status. The women were still experiencing high rates of bone turnover, albeit at a higher maintenance level. The high-dose fish oil was able to retard bone resorption with the expected small decline in bone formation that follows bone resorption.

An alternative explanation for the maintenance of bone turnover from 0 to 3 mo in the placebo group is the choice for placebo, a high oleic acid safflower oil (9% linoleic acid, 83% oleic acid). In a Greek population, monounsaturated fatty acids (MUFA) intake was positively associated with BMD (41). Data from the Women's Health Initiative suggest that diets high in MUFA and PUFA may decrease risk for fracture in postmenopausal women (24). Further, osteoblast-like cells treated

with oleic acid had similar effects as EPA on cannabinoid receptor 2 (42), this receptor appears to be an important factor to induce bone formation (43). The rationale for use of a high oleic oil as the placebo was to promote a neutral effect because a true fatty acid placebo is not achievable. LC n-6 PUFA are known to have proinflammatory effects (44) and maybe detrimental to bone by promoting osteoclast activation. Whereas, saturated fatty acids may also be detrimental to bone (45,46).

Stability of bone turnover in the placebo group during the 3-mo study could also be attributed to the supplementation of all participants with both calcium and vitamin D3. Prestwood and colleagues observed declines in bone resorption without effect on bone formation in older postmenopausal women supplemented with 1500 mg calcium and 1000 IU vitamin D3 daily for 6 wk (47).

A strength of the fish oil intervention is that relative compliance/intake can be measured biochemically via serum EPA and DHA concentrations. Serum EPA and DHA levels in this study demonstrated some noncompliance/nonresponse in the treatment group. Four participants in the fish oil group had less than 15% change in serum EPA levels; subanalysis with only EPA and DHA responders ($n = 13$) was then conducted and showed, even with a smaller sample size, more pronounced inhibition of bone resorption suggesting the high dose of fish oil and increase in serum EPA and DHA is needed for reversal of bone resorption in this high risk sample of breast cancer survivors.

Serum LC n-3 PUFA concentrations depicted a wide range of response in the EPA levels. The EPA concentration in the intervention was twice that of DHA, a 2:1 EPA:DHA in the oil capsule. The upper bound of the 95% CI for EPA increase was 628% with an average of 402%, whereas DHA increased to a lesser extent. This variation in serum EPA and DHA levels demonstrates that the degree of absolute compliance cannot be determined by serum levels; therefore, we can only speculate that the participants with greater increases in EPA and DHA in the serum were likely to be more compliant and/or have a greater lipid uptake than those with little to no change in serum EPA and DHA. Stark and Holub (48) noted a pronounced increase in serum phospholipid EPA in postmenopausal women not receiving hormone replacement therapy (HRT) compared to those women who were receiving HRT. The AI treatment regimen used in this study opposes that of HRT in that AIs deplete the body of estrogen by inhibiting the aromatase enzyme from biosynthesizing estrone and estradiol from adrenal androgens. There was no significant difference between the AI drug brands (Aromasin, Arimidex, or Femora) on serum fatty acid levels or bone turnover. The lack of circulating estrogen in our subjects may have enhanced the variability of serum EPA levels.

Our subjects were unique compared to other investigations of the effects of omega-3 PUFA on bone turnover in that it was 100% postmenopausal women at increased risk for fracture and we chose a high dose of EPA + DHA (4 g). Work by Appleton and colleagues found no benefit of 1.48 g EPA + DHA compared to an olive oil placebo in depressed individuals (27). Our study differs greatly in that the depressed individuals were both men and women and with a wide age range (18–67 yr). Subanalysis of women over 50 yr also showed no effect of EPA and DHA supplementation after 12 wk (27). The women over age 50 were not stratified by menopausal status neither was there any indication of bone related drug intake. The dose we provided to postmenopausal women with no circulating estrogen was twice that of the Appleton and colleagues' supplementation. Both sample and dosage differences are probable rationale for differences in findings between the 2 studies. Our study concurs with findings from a dietary intervention to increase α-linolenic acid intake compared to an average American diet (28), highlighting the importance of dietary intake as well as supplementation of n-3 PUFA.

Inflammatory markers were analyzed as a potential mechanism of action for the effects of fish oil on bone resorption. However, there was no effect of high dose fish oil on inflammatory markers in breast cancer survivors on AI therapy in the current study. Estrogen has a complex interrelationship with inflammation (49) and is compounded by AI therapy (50). Breast cancer survivors, especially those who are overweight or obese have documented high hs-CRP levels (38). In light of the lack of changes of the other inflammatory markers that were tested, we postulate that the excessive rise in hs-CRP demonstrates an acute phase response outside of what was tested or examined in this study. An alternate mechanism of action for the reduced bone resorption could be via resolvins formed from EPA and DHA (51). EPA may affect bone resorption via resolvin E1 (52) and/or DHA via the inhibition of osteoclastogenesis by resolvin D1(14).

The study and findings are limited by the small sample size, the variability in time from treatment and lack of selection of sample for history of osteoporosis. Further, the study began at least 6 mo after AI initiation; therefore, we missed any short-term changes that may have occurred and lack specific data during the 0–6 mo time frame of AI treatment.

In summary, after 3 mo of high-dose fish oil supplementation, serum LC n-3 PUFA concentrations increased and were associated with a reduction in bone resorption in women on maintenance AI therapy. The bone resorption inhibition was significant in fish oil supplement responders compared to placebo without an impact on bone formation. In the short-term of 3 mo, fish oil supplementation may slow bone loss and the associated complications in postmenopausal breast cancer survivors on AI therapy and at elevated risk for fracture. Longer term trials with a greater sample size to provide adequate statistical power are needed to assess bone density and fracture risk.

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