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Randomized Placebo-Controlled Pilot Trial of Omega 3 Fatty Acids for Prevention of Aromatase Inhibitor-Induced Musculoskeletal Pain

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Abstract

Purpose—Aromatase inhibitor (AI) induced joint symptoms negatively impact drug adherence and quality of life in breast cancer survivors. Mechanisms underlying symptoms may include inflammation. It is hypothesized that n-3 polyunsaturated fatty acids (PUFAs) have anti-inflammatory properties and may reduce symptoms.

Methods—We conducted a randomized, double-blind, placebo-controlled study comparing 4.3 g/day n-3 PUFA supplements vs placebo for 24 weeks in postmenopausal breast cancer patients starting adjuvant AIs. Primary endpoints were adherence and tolerability; secondary outcomes included inflammatory cytokines and symptoms assessed by the Brief Pain Inventory short form (BPI-SF) and Functional Assessment of Cancer Treatment-Endocrine Symptoms (FACT-ES) at 0, 12 and 24 weeks.

Results—Forty-four women were randomized; 35 completed the study. Adherence was 88% based on these 35 patients with pills counts as well as change in red blood cell (RBC) n-3 PUFAs. Common toxicities included grade 1 flatulence (55% of both groups) and belching (45% of n-3 group). Mean pain severity scores (BPI-SF) did not change significantly by time or treatment arm.

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Compliance with Ethical Standards

Conflict of Interest: All authors declare he/she has no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Quality of life, based on FACT-ES scores, significantly decreased within placebo ($p=0.04$), but not the n-3 group ($p=0.58$), with a trend toward between group differences ($p=0.06$) at 12 weeks; but no significant differences at 24 weeks. RBC n-3 levels were strongly, positively correlated with FACT-ES at 12 weeks, but attenuated at 24 weeks.

Conclusion—High dose n-3 PUFA supplementation is feasible and well tolerated when administered with AIs. Additional studies are needed to evaluate efficacy in prevention of joint symptoms.

Keywords

aromatase inhibitors; arthralgias; joint symptoms; omega-3 fatty acids; breast cancer survivors

Introduction

Aromatase inhibitors (AIs) significantly reduce local and distant recurrences in postmenopausal women with estrogen receptor positive breast cancer.¹ Approximately 20–47% of women develop musculoskeletal symptoms during AI therapy including joint stiffness and pain.^{2,3,4} Onset of symptoms can occur at any time point with many reporting symptoms at 1–3 months after initiation of therapy.⁵

The biologic mechanisms for AI induced musculoskeletal symptoms are poorly understood. Intra-articular tenosynovial inflammatory changes have been described on MRI imaging of the hand and wrist in women on AIs.^{6,7} Therapeutic options for AI induced joint symptoms remain limited. Non-adherence rates leading to early termination have been estimated at 50% of women taking AIs.⁸ Worse patient outcomes are associated with non-adherence/non-compliance with therapy.⁹

Fish and fish oil supplements contain the long chain n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A meta-analysis showed reduced joint pain intensity, morning stiffness, and analgesic use in patients with rheumatoid arthritis supplemented with long chain n-3 PUFAs.¹⁰ Painful symptoms may be related to stimulation of transient receptor potential ankyrin 1 (TRPA1) resulting in activation of neurogenic inflammation.¹¹ EPA and DHA have been shown to inhibit TRPA1 and inflammatory pain in mouse models¹², and may exert beneficial effects in women on AI therapy via the modulation of inflammatory processes. A randomized placebo-controlled study investigated the role of n-3 PUFA for the treatment of AI-induced musculoskeletal symptoms and reported substantial improvement in joint pain overall, but no differences in symptoms between the n-3 or placebo groups.¹ However, the clinical effects may be different in the prevention setting.

This is the first placebo-controlled randomized study to evaluate the feasibility of preventing AI induced musculoskeletal symptoms with n-3 PUFA. We hypothesized that patients randomized to n-3 PUFA would tolerate supplementation and develop fewer symptoms of AI-induced arthralgias.

Patients and Methods

Patient Characteristics

Postmenopausal women with stage I to III estrogen receptor or progesterone receptor positive breast cancer, receiving AI (anastrozole, exemestane, letrozole) for less than 21 days prior to study enrollment were eligible. Exclusion criteria included: metastatic disease; rheumatoid arthritis or other types of autoimmune and inflammatory joint disease (patients with osteoarthritis were eligible); poorly controlled diabetes mellitus, history of stroke or transient ischemic attacks; use of warfarin and anticoagulants, or use of > 360 mg per day of EPA+DHA within six months of study initiation.

Study Design

This was a randomized, double-blind, placebo-controlled study, approved by the local Institutional Review Board. Enrollment opened at The Ohio State University Wexner Medical Center on 11/09/2011 and closed on 10/29/2013. The trial was conducted in accordance with institutional guidelines; informed consent was obtained.

Block randomization was utilized; only the pharmacist who dispensed the study agent was aware of randomization assignment. Women were advised to take 6 capsules per day in divided doses for 24 weeks to provide either 4.3 g/d of EPA+DHA or a placebo containing a mixture of fats and oils formulated to mirror the ratio of fatty acids typical of the American diet.¹³ Both types of capsules were produced by Marine Ingredients, Mt. Bethel, PA, and were matched in appearance and lemon flavoring. Each active capsule contained approximately 430 mg EPA + 230 mg DHA in triglyceride form. Fatty acid composition of capsules is provided in Supplemental Table 1.

Red blood cell (RBC) and serum samples for fatty acid and inflammatory cytokine analyses, respectively, were collected at baseline, 12 and 24 weeks. Fasting lipid levels were obtained at baseline and at the end of study.

Outcome measures

The primary endpoints were adherence and tolerability of supplements when administered with AIs. Adherence was measured using pill counts and diaries, and confirmed by change in RBC levels of n-6 and n-3 PUFA. Toxicity was evaluated monthly.

Secondary outcome was the preliminary efficacy of n-3 PUFAs in preventing AI-induced arthralgia. We used several validated instruments^{2,3,4} to give a more comprehensive picture of general pain and functional outcomes impacting quality of life (QOL). We chose the Brief Pain Inventory short form (BPI-SF) to evaluate general self-reported pain; this instrument assesses pain on a scale of 0–10 and has been used to evaluate AI-induced arthralgias.¹ Functional Assessment of Cancer Treatment-Endocrine Symptoms (FACT-ES), and Stanford's Health Assessment and Disability Questionnaire (SHAQ) are instruments used to evaluate joint symptoms, functional capacity, health and disability status^{1–4}. The FACT-ES (version 4) is a composite of the FACT-General (G) scale, the Breast Cancer Symptom Scale (BCS), and the Endocrine Symptom Scale (ESS). Higher scores reflect less symptom burden

and better QOL.³ The “short” SHAQ was designed to capture the long-term influence of chronic illness on QOL. Lower scores indicate less disability and less pain.⁵ All instruments were administered at baseline, at 12 and 24 weeks.

Fatty acids were extracted from RBC samples¹⁴, then converted to fatty acid methyl esters (FAMES) and analyzed by a gas chromatograph (Shimadzu Scientific Instruments, Addison IL, USA) using a 3:1 split and standard conditions.¹⁵ Results are reported as % fatty acid/total detected fatty acids. RBC PUFAs are indicative of usual dietary intake over approximately 3–6 months.^{16,17}

Inflammatory cytokines (IL-6, TNFR-2, IL-17) were measured in human serum using ultra-sensitive kits (Meso Scale Discovery, Rockville, MD, USA) and a Meso Scale Discovery Sector Imager 2400, at the Ohio State University Clinical Research Center. The lower limit of detection was 0.7 pg/mL for IL-6, and 0.2 pg/mL for IL-17 and TNFR-2. These cytokines were selected based on data suggesting a role in mediating joint symptoms in models of arthritis treated with n-3 PUFAs.^{19, 20}

Statistical analysis

The primary goal of this study was to evaluate the feasibility of a randomized trial for the evaluation of joint symptoms in women on AI therapy randomized to n-3 PUFA or placebo and to collect preliminary information on treatment efficacy. The sample size of 40 was chosen to provide a 95% confidence interval width of about 0.2 for an overall (both groups combined) compliance rate of 0.90. Considering attrition of 10%, forty four patients were enrolled. Compliance rates and toxicities were summarized with frequencies and percentages, and Fisher’s exact test was used to compare toxicities across treatment groups. As an additional measure of compliance, linear mixed effects models¹⁸ were used to model changes in RBC PUFAs over time and test for differences between treatment groups. Linear mixed effects models were also used to model changes in secondary and exploratory outcomes and to test for group differences. The Kenward-Roger adjustment to the degrees of freedom was used to control type I error.¹⁹ The distribution of IL-6 and IL-17 were right-skewed, and were natural log-transformed for all models to better approximate normality of residuals. All analyses were conducted in SAS version 9.4 (Cary, North Carolina).

Results

Enrollment, Dropout and Evaluability

Between 11/09/2011–10/29/2013, 44 women were enrolled, 44 randomized, and 35 (80%) were evaluable at 24 weeks (Figure 1). There was no evidence of differential dropout between treatment groups ($p=0.88$). The main reasons for not continuing treatment were difficulty remembering to take supplements ($n=2$); significant arthralgias resulting in therapy switch ($n=1$); intolerance of supplements ($n=3$); and scheduling difficulty ($n=1$).

Baseline Participant Characteristics

Baseline demographic and clinical characteristics were similar between the n-3 PUFA and placebo arms (Table 1/Figure 2). The mean age was 59.5 (SD 8.1), with a range of 43–76

years. The majority of patients (n=27, 61%) had stage I breast cancer. History of medical comorbidities, including osteoarthritis, was similar between groups, as was chemotherapy exposure.

Adherence and Tolerability

Compliance was determined as the proportion of patients who reported taking at least 80% of the supplement during the 24 weeks based on the monthly self-reported data. Three participants, two assigned to n-3 PUFA, and one to placebo, were intolerant of supplements and dropped out of the study before 4 weeks. Overall, 38/44 (86%) women reported having taken 80% or more of the supplements, 19/22 (86%) in the n-3 PUFA group and 19/22 (86%) in the placebo group. Toxicities are detailed by treatment group in Table 2. Most toxicities were grade 1, with belching being the most common side effect. The n-3 PUFA arm had 1 (4.5%) grade 3 toxicity of diarrhea. The patient with grade 3 diarrhea had grade 2 elevated non fasting triglycerides that went up to grade 3 at week 20.

RBC PUFAs

The mean n-3 index (EPA+DHA in RBCs) was 4.0% in the placebo group and 4.7% in the n-3 group at baseline (Table 3). In the n-3 group, RBC EPA and DHA increased significantly ($p < 0.001$) by 12 weeks and continued to rise through the 24 week study visit, while total n-6 PUFAs decreased significantly ($p < 0.001$), resulting in a higher n-3 index. Neither the n-3 index, nor any other PUFAs, changed significantly in the placebo group.

Pain outcomes

Many women reported “no pain today” on the BPI-SF lead-in question and did not complete the remainder of the form (45% of all completed BPI-SF forms). For these responses, scores on the remaining questions were set to zero (lowest score; no pain); research assistants confirmed that those who skipped the rest of the questionnaire did not have any recent pain. The mean BPI-SF worst pain score at baseline was 1.8 (+/- 2.3) for women in the n-3 PUFA arm and 2.4 (+/- 2.6) for placebo, reflecting very low “worst” pain at baseline (question ranges from 0, “no pain” to 10, “pain as bad as you can imagine”). Overall BPI-SF severity and interference scores and changes over time are presented in Table 4. Mean pain severity scores did not change significantly in the n-3 group from baseline to 12 weeks ($p=0.68$) or baseline to 24 weeks ($p=0.78$). Pain severity did not change significantly over time in the placebo group, but scores trended toward declines (12 week change: -0.69 , $p=0.11$; 24 week change: -0.70 , $p=0.09$). Pain severity was not significantly different between groups at any time point. We found no significant changes in mean pain interference in either group.

In order to evaluate changes in joint pain, we analyzed differences by group in response to Question 19 from the FACT-ESS: “I have pain in my joints (scored as 0= not at all, to 4=very much). At baseline, 28 (64%) of women reported at least some joint pain. There was evidence that joint pain symptoms were increasing over the first 24 weeks of AI administration ($p=0.01$), but these changes were at the same rate for both groups ($p=0.79$) (Figure 3).

QOL outcomes

Health-related QOL was assessed by the FACT-ES scales using mixed effects models (Table 5). Based on the overall FACT-ES score, the placebo group experienced a significant drop in QOL from baseline to week 12 (-6.4 ± 3.0 ; $p=0.04$), and the n-3 group did not (1.6 ± 2.9 ; $p=0.58$), resulting in a trend toward significant difference in QOL by treatment group at week 12 ($p=0.06$). However, mean FACT-ES scores were not significantly different at week 24 ($p=0.95$) (Figure 4). Women in the n-3 arm had significantly better mean scores on the Functional Well-Being and Social Well-Being subscales at 12 weeks than women in the placebo arm, but no significant group differences were seen in any FACT scales by 24 weeks. Similar to the analysis of treatment group effects, Pearson correlations between RBC PUFAs and FACT scores at each study visit showed strong, positive relationships between FACT-ES and RBC n-3 PUFA levels, particularly the Functional Well-Being subscale ($r = 0.51$; $p<0.05$) at 12 weeks. However, few significant correlations were seen at 24 weeks (Supplemental Table 2). There were no significant changes in disability over 24 weeks by the SHAQ (data not shown).

Inflammatory Cytokines

At baseline, mean levels of inflammatory cytokines in all participants were: IL-6 $1.8 (+/-1.2)$ pg/mL, TNFR-2 $6374 (+/-1507)$ pg/mL, and IL-17 $0.52 (+/-0.45)$ pg/mL. There were significant decreases in individual cytokines within treatment groups, but these changes were not significantly different between the groups (Table 6).

Baseline levels of IL-6 were significantly higher in women who had undergone previous chemotherapy compared to those who had not [$2.4 (+/-1.6)$ vs. $1.5 (+/-0.88)$], respectively $p = 0.02$]. Women who had chemotherapy and were in the n-3 group had a sharp drop in IL-6 levels over 24 weeks (data not shown); however, we did not have sufficient sample size to test for differences by chemotherapy status.

Discussion

In concert with the finding that the women in our study were compliant with n-3 PUFA supplementation and tolerated a dose of 4.3 g EPA+DHA well, similar high compliance was noted in a recent study by Hershman et al. of n-3 PUFA supplementation in breast cancer patients with established AI-induced arthralgias.¹ Additionally, previous research in women with high risk of breast cancer suggests that doses up to 7.6 g/day of EPA+DHA are well tolerated with excellent compliance¹.

Using 4.3 g EPA+DHA, women achieved an n-3 index well above the 8% that is recommended for reducing systemic inflammation associated with heart disease.³⁰ However, this dose of supplementation had a modest effect, if at all, on reducing IL-6 and no effect on TNFR-2. Because we did not have a comparative group not on AI, it is unclear if this lack of effect on markers of inflammation is due to AI effect or another factor that may block an anti-inflammatory effect of EPA+DHA.

Overall pain severity and pain interference did not significantly differ between treatment groups, but there was a trend toward less pain severity in the placebo group. The placebo

may have contained beneficial components (e.g. bioactive fatty acids) resulting in pain improvement or masking differences between treatment groups. Participation in a clinical trial or suggestive effects of potential benefit could also have impacted participants' perception of pain (i.e. the "placebo effect").²⁰ Our results are similar to results from Hershman et al. who found no significant differences in change in pain between n-3 and placebo groups in breast cancer patients with established AI-induced arthralgias.¹

The high dose of n-3 in our study (4.3 g EPA+DHA) and in Hershman et al's study (3.3 g EPA+DHA) may have influenced outcomes. We tested a higher dose of fish oil than Hershman et al. (4.3 g vs 3.3 g EPA+DHA/d). In a randomized clinical trial of patients with osteoarthritis and knee pain, low dose fish oil (0.45 g EPA+DHA combined with high oleic acid sunflower oil) was more effective in improving knee pain scores [as measured by the Western Ontario and McMaster Universities Arthritis Index (WOMAC)] and functional measurements than a higher dose of EPA+DHA (4.5 g/d) after 2 years of supplementation.²¹ While we chose a dose of EPA+DHA based on improvement in joint symptoms in individuals with rheumatoid arthritis,^{22, 23} it is possible that increasing n-3 PUFAs above a specific threshold may not provide additional benefit. Additionally, response to n-3 supplementation may differ based on genetic variations²⁴ or baseline inflammatory status.²⁵

Based on FACT-ES scores in our study, QOL significantly decreased within placebo, but not the n-3 group, with a trend toward between group differences at 12 weeks; but no significant differences at 24 weeks. This could reflect beneficial effects of n-3 PUFAs early in AI treatment, but the inability of n-3 supplementation to affect QOL as AI side effects progress.

The primary limitation of this study was the small sample size, but despite this limitation, we were able to demonstrate adherence, tolerability and preliminary data suggesting benefit of n-3 supplementation on certain functional outcomes. The relatively short length of the study was also a limitation because the musculoskeletal effects of AIs may not occur until later. Also, symptom questionnaires were not specific to AI patients and additional instruments such as the WOMAC may have been helpful. Future studies will also need to evaluate as a secondary measure endocrine therapy compliance which ultimately has the greatest impact on breast cancer outcomes.

Conclusions

This study showed that n-3 PUFA supplementation to prevent AI-induced joint symptoms is feasible and well tolerated. However, we found no evidence of improvement in joint symptoms with high dose n-3 PUFA supplementation. Despite that, our data suggest potential short-term benefit in QOL outcomes. A larger randomized study with longer duration would be needed to establish whether this intervention may help prevent the burden of AI-induced arthralgias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
CONSORT diagram of participant flow

Figure 2. n3:n6 ratio by treatment group

Comparison of red blood cell n-3:n-6 ratio between n-3 PUFA group and placebo over treatment period. Ratio of n-3:n-6 was significantly higher in the n-3 PUFA group ($p < 0.001$) while there was no change in the ratio in the placebo group.

Figure 3. Joint pain by treatment group

Comparison of joint pain, based on question #19 of the FACT-ES, between n-3 PUFA group and placebo over treatment period. With 64% of women reporting at least some pain at baseline, joint pain increased at the same rate in n-3 PUFA and placebo group ($p = 0.79$).

Figure 4. FACT-ES scores by treatment group

Comparison of QOL, assessed by FACT-ES scores, between n-3 PUFA group and placebo over treatment period. QOL significantly decreased in the placebo group from baseline to week 12 ($p = 0.04$), while the n-3 PUFA group experienced no change ($p = 0.58$).

Table 1

Baseline characteristics of women enrolled in randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

	Overall (n=44)	Placebo (n=22)	n-3 (n=22)
Age, years			
Mean (SD)	59.5 (8.1)	57.8 (9.1)	61.2 (6.6)
Range	43–76	43–76	49–74
Body Mass Index			
Mean (SD)	29.8 (6.2)	29.2 (6.5)	30.3 (5.9)
Range	21–46	21–46	22–44
Race			
White	43 (98%)	21 (95%)	22 (100%)
Black	1 (2%)	1 (5%)	0 (0%)
Performance Status			
0	35 (80%)	18 (82%)	17 (77%)
1	7 (16%)	2 (9%)	5 (23%)
Unknown	2 (5%)	2 (9%)	0 (0%)
Prior Chronic Conditions			
Osteoarthritis	5 (11%)	4 (18%)	1 (5%)
Fibromyalgia	1 (2%)	1 (5%)	0 (0%)
Hyperlipidemia	5 (11%)	1 (5%)	4 (18%)
Hypertension	14 (32%)	5 (23%)	9 (41%)
Stage			
DCIS ^a	1 (2%)	0 (0%)	1 (5%)
I	27 (61%)	13 (59%)	14 (64%)
II	10 (23%)	6 (27%)	4 (18%)
III	6 (14%)	3 (14%)	3 (14%)
ER ^b status			
Positive	43 (98%)	22 (100%)	21 (95%)
Negative	1 (2%)	0 (0%)	1 (5%)
PR ^c status			
Positive	39 (89%)	18 (82%)	21 (95%)
Negative	5 (11%)	4 (18%)	1 (5%)
HER2 ^d status			
Positive	2 (5%)	1 (5%)	1 (5%)
Negative	34 (77%)	18 (82%)	16 (73%)
Unknown	8 (18%)	3 (14%)	5 (23%)
Prior Treatment(s)			
Chemotherapy (Taxanes)	15 (34%)	7 (32%)	8 (36%)
Radiation	26 (59%)	14 (64%)	12 (55%)
First AI ^e			

	Overall (n=44)	Placebo (n=22)	n-3 (n=22)
Anastrozole	31 (70%)	14 (64%)	17 (77%)
Exemestane	1 (2%)	1 (5%)	0 (0%)
Letrozole	12 (27%)	7 (32%)	5 (23%)
Total omega-3 consumption (g/week)			
Mean (SD)	1.43 (0.86)	1.33 (0.85)	1.52 (0.88)
Range	0.28–4.13	0.28–4.13	0.32–3.62
Total EPA ^f consumption (g/week)			
Mean (SD)	0.046 (0.051)	0.042 (0.042)	0.050 (0.060)
Range	0.002–0.29	0.002–0.14	0.005–0.29
Total DHA ^g consumption (g/week)			
Mean (SD)	0.10 (0.11)	0.09 (0.09)	0.11 (0.13)
Range	0.005–0.62	0.005–0.30	0.011–0.62
Serum Vitamin D (ng/mL)			
Mean (SD)	29.1 (9.7)	29.1 (7.8)	29.2 (11.7)
Range	14.2–59.4	17.6–49.7	14.2–59.4

^aDCIS-ductal carcinoma in situ

^bER-estrogen receptor

^cPR-progesterone receptor

^dHER2-human epidermal growth factor receptor 2

^eAI-aromatase inhibitor

^fEPA-eicosapentaenoic acid

^gDHA-docosahexaenoic acid

Table 2

Toxicities reported during 24 week randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

	n-3 (n=22)				Placebo (n=22)				P value ^a
	All Grades	Grade 1	Grade 2	Grade 3	All Grades	Grade 1	Grade 2	Grade 3	
Abdominal pain	3 (14%)	2 (9%)	1 (5%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	0.61
Alanine aminotransferase increased	1 (5%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	1.00
Aspartate aminotransferase increased	1 (5%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Bloating	2 (9%)	1 (5%)	1 (5%)	0 (0%)	3 (14%)	3 (14%)	0 (0%)	0 (0%)	0.61
Bruising	7 (32%)	6 (27%)	1 (5%)	0 (0%)	4 (18%)	4 (18%)	0 (0%)	0 (0%)	0.49
Diarrhea	10 (45%)	9 (41%)	0 (0%)	1 (5%)	10 (45%)	8 (36%)	2 (9%)	0 (0%)	0.59
Dyspepsia	3 (14%)	3 (14%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)	0.23
Epistaxis	3 (14%)	3 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.23
Flatulence	12 (55%)	12 (55%)	0 (0%)	0 (0%)	12 (55%)	12 (55%)	0 (0%)	0 (0%)	1.00
GI disorder, other ^b	1 (5%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	1.00
Headache	9 (41%)	8 (36%)	1 (5%)	0 (0%)	10 (45%)	7 (32%)	3 (14%)	0 (0%)	0.81
Nausea	7 (32%)	6 (27%)	1 (5%)	0 (0%)	9 (41%)	6 (27%)	3 (14%)	0 (0%)	0.73
Pain in extremity	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	1.00
Vaginal discharge	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)	1.00
Vomiting	5 (23%)	4 (18%)	1 (5%)	0 (0%)	3 (14%)	1 (5%)	2 (9%)	0 (0%)	0.50
Lipid-related adverse event	1 (5%)	1 (5%)	0 (0%)	0 (0%)	3 (14%)	3 (14%)	0 (0%)	0 (0%)	0.61
Belching	10 (45%)	10 (45%)	0 (0%)	0 (0%)	8 (36%)	8 (36%)	0 (0%)	0 (0%)	0.76
Fishy aftertaste	6 (27%)	6 (27%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	0.09

^aP-values from Fisher's exact test comparing toxicities of all grades combined for placebo vs. n-3 groups

^bTaste alteration (n=1, n3 group); bleeding with bowel movement (n=1, placebo group)

Table 3

Changes in red blood cell n-3 and n-6 fatty acids during 24 week randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a
EPA									
Placebo	0.77 ± 0.22	–0.086 ± 0.35	0.81			<.0001	–0.077 ± 0.33	0.82	<.0001
n-3	1.1 ± 0.22	2.9 ± 0.35	<.0001				3.3 ± 0.33	<.0001	
DHA									
Placebo	3.2 ± 0.20	–0.026 ± 0.25	0.92			<.0001	–0.060 ± 0.24	0.80	<.0001
n-3	3.6 ± 0.20	2.3 ± 0.25	<.0001				2.9 ± 0.23	<.0001	
ALA									
Placebo	0.69 ± 0.11	–0.054 ± 0.11	0.64			0.76	–0.086 ± 0.11	0.43	0.37
n-3	0.59 ± 0.11	–0.004 ± 0.11	0.97				0.053 ± 0.11	0.62	
LA									
Placebo	14.8 ± 0.46	0.17 ± 0.54	0.75			0.001	–0.39 ± 0.51	0.45	0.01
n-3	14.2 ± 0.46	–2.4 ± 0.54	<.0001				–2.3 ± 0.50	<.0001	
AA									
Placebo	14.6 ± 0.34	0.47 ± 0.39	0.24			<.0001	0.45 ± 0.37	0.24	<.0001
n-3	14.3 ± 0.34	–2.3 ± 0.39	<.0001				–3.0 ± 0.37	<.0001	
n-3 Index									
Placebo	4.0 ± 0.39	–0.12 ± 0.56	0.83			<.0001	–0.12 ± 0.54	0.83	<.0001
n-3	4.7 ± 0.39	5.2 ± 0.56	<.0001				6.2 ± 0.53	<.0001	
Total n-3									
Placebo	6.6 ± 0.48	–0.19 ± 0.68	0.79			<.0001	–0.08 ± 0.65	0.91	<.0001
n-3	7.3 ± 0.48	6.1 ± 0.68	<.0001				7.6 ± 0.64	<.0001	
Total n-6									
Placebo	35 ± 0.63	0.56 ± 0.85	0.51			<.0001	0.10 ± 0.81	0.90	<.0001
n-3	34 ± 0.63	–6.8 ± 0.85	<.0001				–7.8 ± 0.79	<.0001	
n-3:n-6 ratio									
Placebo	0.19 ± 0.021	–0.0091 ± 0.031	0.77			<.0001	–0.0032 ± 0.030	0.91	<.0001

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a
n-3	0.22 ± 0.021	0.29 ± 0.031	<.0001	0.36 ± 0.029		<.0001			

^a All values are estimates from mixed effects models. Tests are from orthogonal contrasts in the models

^b EPA-eicosapentaenoic acid,

^c DHA-docosahexaenoic acid,

^d ALA-alpha linolenic acid,

^e LA-linoleic acid,

^f AA-arachidonic acid,

^g n-3 index-EPA+DHA in red blood cells

Table 4

Changes in Brief Pain Inventory (BPI) during 24 week randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a
BPI – Severity ^b						0.38			0.16
Placebo	1.6 ± 0.33			–0.69 ± 0.42		0.11	–0.70 ± 0.41		0.09
n-3	1.1 ± 0.33			–0.17 ± 0.41		0.68	0.11 ± 0.40		0.78
BPI – Interference ^c						0.76			0.31
Placebo	1.0 ± 0.32			–0.32 ± 0.36		0.38	–0.56 ± 0.36		0.13
n-3	0.93 ± 0.32			–0.48 ± 0.36		0.18	–0.05 ± 0.35		0.89

^a All values are estimates from mixed effects models, including subjects and completers (tests are from orthogonal contrasts in the models).

^b Scores range from 1 to 10 with high values indicating worse pain; 4 item scale

^c Scores range from 1 to 10 with high values indicating worse interference; 7 item scale is sum of two other interference scales

Table 5

Changes in Functional Assessment of Cancer Therapy (FACT) during 24 week randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^a
FACT-G^b						0.001			0.45
Placebo	91 ± 2.2	–5.9 ± 1.8	0.002				0.047 ± 1.8	0.98	
n-3	92 ± 2.2	2.6 ± 1.8	0.15				1.9 ± 1.7	0.27	
-Physical WB						0.15			0.97
Placebo	25 ± 0.59	–1.4 ± 0.72	0.05				0.015 ± 0.71	0.98	
n-3	25 ± 0.59	0.057 ± 0.71	0.94				–0.027 ± 0.69	0.97	
-Social WB						0.01			0.38
Placebo	25 ± 0.96	–3.2 ± 1.1	0.01				–0.96 ± 1.1	0.38	
n-3	24 ± 0.96	0.88 ± 1.1	0.42				0.39 ± 1.1	0.72	
-Emotional WB						0.25			0.65
Placebo	20 ± 0.54	–0.34 ± 0.62	0.59				0.36 ± 0.61	0.56	
n-3	20 ± 0.54	0.66 ± 0.61	0.28				0.76 ± 0.60	0.21	
-Functional WB						0.04			0.69
Placebo	21 ± 1.1	–0.83 ± 0.71	0.25				0.72 ± 0.70	0.30	
n-3	23 ± 1.1	1.2 ± 0.70	0.09				1.1 ± 0.68	0.11	
BCS^c						0.72			0.72
Placebo	26 ± 0.84	1.5 ± 0.90	0.09				0.96 ± 0.88	0.28	
n-3	27 ± 0.84	1.1 ± 0.88	0.22				0.51 ± 0.86	0.56	
FACT-B^d (G+BCS)						0.02			0.63
Placebo	117 ± 3	–4.4 ± 2.4	0.07				0.95 ± 2.3	0.68	
n-3	119 ± 2.7	3.7 ± 2.3	0.11				2.5 ± 2.3	0.27	
ESS^e						0.93			0.42
Placebo	61 ± 1.7	–1.8 ± 1.3	0.16				–1.0 ± 1.3	0.43	
n-3	66 ± 1.7	–2.0 ± 1.3	0.12				–2.4 ± 1.2	0.05	
FACT-ES^f (G+BCS+ESS)						0.06			0.95

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	P for change ^a	P for comparison of changes ^a	Mean ± SEM	P for change ^a	P for comparison of changes ^a
Placebo	179 ± 4.0	–6.4 ± 3.0	0.04	–0.22 ± 2.9	0.94		–0.22 ± 2.9	0.94	
n-3	185 ± 4.0	1.6 ± 2.9	0.58	0.027 ± 2.9	0.99		0.027 ± 2.9	0.99	

^a All values are estimates from mixed effects models; tests are from orthogonal contrasts in the models. For all scales, higher score = higher quality of life.

^b FACT-G = General: All “WB” (well-being) scales

^c BCS = Breast Cancer Symptom Scale

^d FACT-B = Breast: FACT G + BCS

^e ESS = Endocrine Symptom Scale

^f FACT-ES = Endocrine Symptoms: FACT G + BCS + ESS

Changes in serum cytokines during 24 week randomized pilot study of n-3 fatty acid supplements versus placebo for prevention of aromatase inhibitor-induced musculoskeletal symptoms

Table 6

	Baseline (week 0)			Change, week 0–12			Change, week 0–24		
	Mean ± SEM	Mean ± SEM	P for change ^a	Mean ± SEM	Mean ± SEM	P for comparison of changes ^d	Mean ± SEM	Mean ± SEM	P for comparison of changes ^d
log(IL-6) ^b						0.26			0.27
Placebo	0.44 ± 0.14	0.052 ± 0.10	0.60				-0.087 ± 0.10	0.40	
n-3	0.50 ± 0.13	-0.11 ± 0.11	0.28				-0.25 ± 0.10	0.02	
TNFR-2 ^c						0.77			0.74
Placebo	6135 ± 311	-81 ± 225	0.72				-459 ± 225	0.05	
n-3	6546 ± 316	-179 ± 237	0.45				-354 ± 225	0.12	
log(IL-17) ^d						0.15			0.65
Placebo	-0.93 ± 0.16	-0.33 ± 0.16	0.04				-0.14 ± 0.16	0.36	
n-3	-1.00 ± 0.17	0.005 ± 0.17	0.98				-0.041 ± 0.16	0.80	

^aAll values are estimates from mixed effects models; tests are from orthogonal contrasts in the models

^bValues of IL-6 (interleukin-6) were natural log transformed due to skewness

^cTNFR-2: tumor necrosis factor alpha receptor 2

^dValues of IL-17 were natural log transformed due to skewness; values are negative due to natural log transformation