Marine Fatty Acid Intake Is Associated with Breast Cancer Prognosis1,2

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Abstract

EPA and DHA, long-chain (n-3) PUFA largely obtained from fish, inhibit the proliferation of breast cancer cells in vitro and reduce the initiation and progression of breast tumors in laboratory animals. Our purpose in this analysis was to examine whether intake of these marine fatty acids (EPA and DHA) were associated with prognosis in a cohort of women who had been diagnosed and treated for early stage breast cancer (n = 3,081). Median follow-up was 7.3 y. Dietary intake was assessed using 24-h recalls (~4 recalls per dietary assessment obtained at 7 time points over 6 y). Survival models with time-dependent covariates were used to examine the association of repeated measures of dietary intake of EPA and DHA from food (i.e., marine sources) and supplements with disease-free survival and overall survival. Women with higher intakes of EPA and DHA from food had an approximate 25% reduced risk of additional breast cancer events [tertile 2: HR = 0.74 (95% CI = 0.58–0.94); tertile 3: HR = 0.72 (95% CI = 0.57–0.90)] compared with the lowest tertile of intake. Women with higher intakes of EPA and DHA from food had a dose-dependent reduced risk of all-cause mortality [tertile 2: HR = 0.75 (95% CI = 0.65–1.04); tertile 3: HR = 0.59 (95% CI = 0.43–0.82)]. EPA and DHA intake from fish oil supplements was not associated with breast cancer outcomes. The investigation indicates that marine fatty acids from food are associated with reduced risk of additional breast cancer events and all-cause mortality. J. Nutr. 141: 201–206, 2011.

Introduction

The long-chain (n-3) PUFA EPA and DHA inhibit the proliferation of breast cancer cells in vitro and reduce the initiation and progression of these tumors in laboratory animals (1,2). Dietary EPA and DHA are largely obtained from fatty fish and therefore are often referred to as marine fatty acids. Despite the consistency of the experimental and animal data, it is unclear whether high consumption of marine fatty acids is associated with a reduction in risk of breast cancer in human populations.

The association of fish, total (n-3) PUFA, EPA, and DHA intake with incident breast cancer has been examined in several cohort studies (3–11) and, generally, no association was seen (12). However, results of a prospective study of women in Singapore, where fish intake is much higher than that of the US, showed an inverse association between dietary (n-3) PUFA intake from marine sources and breast cancer risk [RR 0.72 (95% CI = 0.53–0.98)] (8). In contrast to studies of self-reported intake, Saadatian-Elahi et al. (13) conducted a meta-analysis of studies that analyzed blood biomarkers of fatty acids in association with breast cancer risk. They found inverse associations for total (n-3) PUFA [RR = 0.61 (95% CI = 0.40–0.93)] as well as for EPA [RR = 0.69 (95% CI = 0.43–1.05)] and DHA [RR 0.68 (95% CI = 0.44–1.04)]. A recent analysis of the Vitamins and Lifestyle Cohort study found that among 35,016 postmenopausal women, use of fish oil supplements was associated with reduced risk of breast cancer [HR = 0.68 (95% CI = 0.50–0.92)] (14).

In comparison to incident breast cancer, there are little published data on intakes of fish or marine fatty acids (from food or supplements) and breast cancer outcomes. Two ecologic or cross-national studies found that fish intake was protective for breast cancer mortality (15,16). A study conducted in Norway found that wives of fishermen experienced a 30% reduction in breast cancer mortality compared with wives of unskilled workers (17). However, Caygill et al. (18) found no association between fish consumption and breast cancer mortality in a study conducted in 24 European countries. Holmes et al. (19) examined (n-3) fatty acid consumption (assessed using a FFQ) and all-cause mortality among 2981 women with breast cancer and found an approximate 20% nonsignificant decrease in risk for intakes greater than the lowest quartile (P = 0.10).

The purpose of this secondary data analysis was to assess whether dietary intake of EPA and DHA was associated with risk of additional breast cancer events or all-cause mortality among a sample of women diagnosed and treated for early stage...
breast cancer. We also examined whether DHA and EPA intakes from food (i.e., marine sources) compared with dietary supplements were differentially associated with outcomes.

Participants and Methods

Study design and sample. The sample is composed of breast cancer survivors who participated in the Women’s Healthy Eating and Living (WHEL) Study. The purpose of the WHEL Study was to assess whether a major increase in vegetable, fruit, and fiber intake and a decrease in dietary fat intake reduces the risk of recurrent and new primary breast cancer and all-cause mortality among women with previously treated early-stage breast cancer. Details of eligibility criteria, data collection, and assessment of outcomes in the WHEL trial have been reported (20,21). Briefly, 3088 trial participants were enrolled at 7 study sites between 1995 and 2000. Major eligibility criteria included: diagnosis within the past 4 y of primary operable invasive stage I (≥1 cm), II, or IIIA breast carcinoma categorized using American Joint Committee on Cancer (edition IV); age 18–70 y at the time of diagnosis; no current or planned chemotherapy; no evidence of recurrent disease or new breast cancer since completion of initial treatment; and no other cancer in the past 10 y. WHEL Study participants received by mail a series of questionnaires for completion before or at their baseline clinic visit. At the baseline clinic visit, height and weight were measured and BMI [weight (kg)/height (m²)] was calculated. The internal review board of the baseline clinic visit, age 18–70 y at the time of diagnosis; no current or planned chemotherapy; no evidence of recurrent disease or new breast cancer since completion of initial treatment; and no other cancer in the past 10 y. WHEL Study participants received by mail a series of questionnaires for completion before or at their baseline clinic visit. At the baseline clinic visit, height and weight were measured and BMI [weight (kg)/height (m²)] was calculated. The internal review board of the University of California, San Diego, and all participating institutions approved the study procedures.

As reported in 2004 and 2007, among survivors of early-stage breast cancer, adoption of a diet that was high in vegetables, fruit, and fiber and low in fat did not reduce additional breast cancer events or mortality during a 7.3-y follow-up period (20,22,23).

Assessment of dietary intake. At each assessment time point, dietary intake was assessed using repeated 24-h recalls. Overall, 87% of recall sets contained 4 recalls/dietary assessment time point, 12.4% had 3 recalls/time point, and 0.6% contained only 2 recalls/time point. The repeated 24-h dietary recall assessments were conducted at baseline, 1 y, 4 y, 6 y, and on a randomly selected 50% sample at 6, 24, and 36 mo (21). The half-sample measures (at 6, 24, and 36 mo) were added to ascertain the trajectory of the initial change and to characterize the pattern of dietary adherence over time. The baseline 24-h recalls were collected before the initial clinic visit, and subsequent 24-h recalls were collected during a 2- to 3-wk period within 3 mo following a clinic visit.

Trained dietary assessors at the coordinating center who were unaware of the dietary assignment collected the dietary recalls on randomly selected prescheduled days stratified for weekend compared with weekdays over a 2-wk period (24). The assessors administered the structured interview of the Nutrition Data System software (University of Minnesota, Minneapolis, MN) to collect total dietary intake during the previous 24-h period (NDS-R Software). In addition to obtaining detailed data on dietary intake, the assessors also probed for the consumption of dietary supplements. The product name, manufacturer, usual dosage, and frequency of use during the previous day were recorded for each product (25).

Other assessments. Standardized questionnaires were administered at baseline to ascertain demographic, medical history, race/ethnicity, and lifestyle habits. Physical activity was assessed using a 9-item measure of physical activity from the Personal Habits Questionnaire adopted from the Women’s Health Initiative (26). Responses were converted to metabolic equivalent tasks in min/wk. This scale was validated in the WHEL Study against a standard physical activity recall and accelerometer reading (27).

Data on the original tumor characteristics and treatment were abstracted from medical records. Specific variables include tumor grade (well, moderately, or poorly differentiated), estrogen receptor status, adjuvant drug therapy, and cancer stage.

Outcome ascertainment. The outcomes of interest were disease-free survival and overall survival. Disease-free survival is the time from WHEL Study enrollment (1995–2000) to development of an additional breast cancer event, death, or the end of follow-up. An additional breast cancer event was defined as a recurrence from the original cancer or developing a new invasive breast cancer. Follow-up time was censored at minimum of the time to an additional breast cancer event, death, the last documented staff contact date, or study completion (June 1, 2006). Overall survival was the time from enrollment to reported/confirmed death from all causes (i.e., mortality). Follow-up time was censored at the minimum time to death, the last documented staff contact date, or study completion (June 1, 2006). New breast cancer events or death were confirmed by medical record review.

Statistical analyses. The WHEL Study intervention was designed to promote a reduction in total fat intake. However, fish intake was not a targeted dietary behavior and was neither explicitly encouraged nor discouraged. We verified that change in intake of marine fatty acids did not vary by intervention group (P > 0.05; data not shown) and therefore used a cohort analysis for this report.

We selected a priori potential confounders, including known and suspected risk factors for breast cancer or prognosis. Specifically, univariate associations with intake of marine fatty acids were examined for the following variables known to affect disease-free and overall survival: patient age, BMI, physical activity, tumor grade, stage, and years from diagnosis to study enrollment.

Time-dependent covariate Cox models were used to determine the association of dietary intake of EPA and DHA from food and supplements with disease-free survival and overall survival, adjusting for cancer stage and grade. Unlike static risk factors (such as race), dietary intake can change markedly over time and therefore a single baseline measure of intake may not adequately characterize an individual’s eating pattern. This is especially true for the intake of a specific nutrient where day-to-day variation is likely to be quite high (28). As indicated above, dietary intake was measured repeatedly during the WHEL Study, which allows us to compute a more precise estimate of each woman’s intake of marine fatty acids over time. The use of repeated measures of an exposure can be an effective method of decreasing measurement error compared with the use of a single measurement (29) and incorporate information about changes in dietary intake over time, which provides a rigorous evaluation of the relationship of this dietary factor to disease development.

We modeled dietary intake as a continuous variable and a categorical variable to test for nonlinear trends. For the categorical variables, we first identified tertiles of baseline dietary EPA and DHA intake from food sources and used the tertile cutpoints to categorize intake in each outcome model. Fish oil supplement categories were defined as none (95.8% of the cohort) and 2 categories divided at the median baseline supplemental intake among those with any fish oil supplement use at baseline. As noted above, our dietary assessment intervals were of unequal length; some assessments were 6 mo apart, some 12 mo apart, some 24 mo apart, and some longer due to missing data. For each participant at each time interval, we computed a weighted average intake of EPA and DHA for each time interval (e.g., 6–12 mo) using the dietary data collected during that time frame. If a participant did not provide dietary data during that time interval, we used the average intake of EPA and DHA calculated from all the previous time intervals (e.g., baseline to 6 mo). Therefore, all participants were treated as though they had equal numbers of dietary assessments.

Adjustment to the multivariable models was made for age at randomization, obesity (BMI > 30 kg/m²), physical activity, intervention group, and entry cohort. For each outcome, 4 time-dependent Cox proportional hazards basic models were run: a model for marine fatty acid intake from food sources, 1 from dietary supplements, 1 from both food and supplements as separate terms, and 1 for total intake (defined as the sum of food and supplemental EPA and DHA). The HR and the associated 95% CI were the measure of association. Residual plots were used to test the proportional hazards assumption.

All analyses were conducted in SAS version 9.2. Values in the text are means ± SD unless otherwise noted.

Results

The final sample for this analysis was composed of all women who provided dietary recall data prior to study entry (n = 3081).
Characteristics of the study sample have been published (20). Briefly, at baseline, age was 52.7 ± 9.0 y, BMI was 27.3 ± 6.1, and 85.3% were non-Hispanic white. With regard to the original tumor, 38.6% were Stage I and 5% IIIA, 35.9% were poorly and 40.2% were moderately differentiated, and 74.2% were estrogen receptor-positive. Overall, 59.6% of women used tamoxifen. At the end of the trial (June 01, 2006), there were 517 (16.8%) additional invasive breast cancer events and 314 (10.2%) deaths from all causes. Among the deaths, 261 (83.1%) were caused by breast cancer, 27 (8.6%) were caused by other cancers, 7 (2.2%) were caused by heart disease, and 19 (6.1%) were the result of other causes.

Over the WHEL Study duration (1995–2006), information concerning the intake of marine fatty acids from foods and supplements was collected (Fig. 1). Self-reported intakes of marine fatty acids from food were a mean ± SEM of 186 ± 5 mg/d at baseline and 237 ± 7 mg/d at y 6. Approximately 4.3% of women reported using fish oil supplements at baseline compared with 10.4% at y 6. In the total WHEL sample, intake of marine fatty acids from foods and from supplements increased over time (P-trend < 0.0001). Among users of fish oil supplements, the median intake was 360 mg/d at baseline. Mean ± SD intake of marine fatty acids by case status as well as the number of dietary assessment sets (i.e. repeated 24 h recalls) used in the models was assessed (Table 1). Among women who did not experience additional breast cancer events, total intake of marine fatty acids from foods and supplements increased (P-trend < 0.0001). However, among women who experienced additional events, there was no increase in intake.

Multivariable-adjusted associations between intake of marine fatty acids from foods (as a continuous variable) and additional breast cancer events were calculated using a time-dependent covariate survival model that included 14,095 dietary assessment sets totaling 54,457 dietary recalls. The HR for this model was 0.78 (95% CI = 0.56–1.07). Multivariable-adjusted associations between categorical measures of marine fatty acids and additional breast cancer events were also calculated (Table 2). Compared with the lowest tertile of intake of marine fatty acids from food (EPA and DHA), there was an ~25% reduction in risk for additional breast cancer events (P < 0.0001) in women in whose intake was reported to be within the upper 2 tertiles of intake, although the trend was not significant (P = 0.06). After adjusting for EPA and DHA intake from fish oil supplements, the reduction in risk persisted (P-trend < 0.05). When marine fatty acids from foods were combined with dietary supplement sources of these fatty acids, the point estimates remained significant, but the trend became nonsignificant (P = 0.16). Further, dietary supplement sources of EPA and DHA were not associated with additional breast cancer events.

The multivariable-adjusted association between marine fatty acids from food (as a continuous variable) and mortality using a time-dependent covariate survival model was 0.78 (95% CI = 0.52–1.15). Multivariable-adjusted associations between categorical measures of marine fatty acids and all-cause mortality events were computed (Table 3). Compared with women in the lowest tertile of intake, those in the highest tertile had a 40% reduction in risk of overall mortality (P-trend = 0.006). This association remained strong whether adjusting for marine fatty acid supplement use or combining food and supplement sources.

**TABLE 1** Intake of marine fatty acids from food by case status in a cohort of women with a history of breast cancer and the proportion that consumed EPA + DHA supplements

<table>
<thead>
<tr>
<th>Time point</th>
<th>No additional breast cancer events</th>
<th>Additional breast cancer events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/d EPA and DHA</td>
<td>n</td>
</tr>
<tr>
<td>Food sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2564</td>
<td>190 ± 283</td>
</tr>
<tr>
<td>6 mo</td>
<td>1214</td>
<td>199 ± 328</td>
</tr>
<tr>
<td>12 mo</td>
<td>2331</td>
<td>200 ± 288</td>
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<tr>
<td>24 mo</td>
<td>1129</td>
<td>211 ± 341</td>
</tr>
<tr>
<td>36 mo</td>
<td>1111</td>
<td>215 ± 296</td>
</tr>
<tr>
<td>48 mo</td>
<td>2189</td>
<td>241 ± 346</td>
</tr>
<tr>
<td>72 mo</td>
<td>2085</td>
<td>240 ± 332</td>
</tr>
</tbody>
</table>

1 Values are means ± SD or %. P-trend over time (food and supplements) for no additional breast cancer events < 0.0001; P-trend over time (food and supplements) for additional breast cancer events = 0.29.

2 Number of women who provided dietary assessments (four 24-h recalls/assessment) at each time point.

FIGURE 1 Intake of marine fatty acids from food (A) by women with a history of breast cancer and the proportion that consumed EPA + DHA supplements (B). In A, values are means ± SEM, n = 3081, 1409, 1244, 1211, 2333, and 2145 in y 1–6, respectively. Intake of marine fatty acids from foods and supplements increased over time, P-trend < 0.0001 (mixed models).
of these fatty acids. Marine fatty acids from fish oil supplements were not significantly associated with mortality.

**Discussion**

To our knowledge, this is the first study conducted in breast cancer survivors to indicate that consumption of marine fatty acids is associated with improved breast cancer prognosis. Specifically, in this cohort of 3081 breast cancer survivors, higher intakes of EPA plus DHA from food were associated with a reduction in additional breast cancer events and all-cause mortality. It is notable that the models using marine fatty acids as a continuous variable were not significant, indicating that there was not a linear relationship between this exposure and breast cancer outcome. Specifically, for additional breast cancer events, there appears to be a threshold effect with intakes above 73 mg/d associated with a 25% reduction in risk ($P < 0.05$).

For all-cause mortality, there was a modest suggestion of a dose-response relationship, with median intake of 73 mg/d associated with a 25% reduction in risk and median intake of 365 mg/d associated with a 40% reduction in risk ($P$-trend < 0.05).

In this analysis, we evaluated both food and dietary supplement sources of EPA and DHA given the mounting evidence in diet-cancer epidemiological studies that differential associations with risk can be demonstrated. Fish oil supplements, which provided substantially higher amounts of the marine fatty acids, were not associated with either additional breast cancer events or mortality. However, use of fish oil supplements in this cohort of breast cancer survivors was low (generally <5%) and therefore we were not well powered to examine this exposure. Alternatively, the observation that use of fish oil supplements did not contribute to improved outcomes in this cohort may suggest

<table>
<thead>
<tr>
<th>Cutpoints, mg/d</th>
<th>Median, mg/d</th>
<th>Food only</th>
<th>Food adjusted for supplements</th>
<th>Food plus supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA and DHA</td>
<td>n</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Tertile 1</td>
<td>36.7</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>37–152.9</td>
<td>0.74* (0.58–0.94)</td>
<td>0.74* (0.59–0.94)</td>
<td>0.75* (0.60–0.94)</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>≥153</td>
<td>0.72* (0.57–0.90)</td>
<td>0.71* (0.56–0.90)</td>
<td>0.76* (0.61–0.95)</td>
</tr>
</tbody>
</table>


1 Time-dependent covariate Cox models using weighted average of all measurements. All models adjusted for tumor stage, grade, and time between diagnosis and study entry. When models are rerun with additional adjustments for obesity, age, physical activity, intervention group, and entry cohort; all significant results remain so (data not shown). *Different from tertile 1, $P < 0.05$.

2 Food includes only marine sources of EPA and DHA (i.e. fish and seafood).

3 Cutpoints (median) for tertiles of food plus supplements: ≤73.5 (118); 73.5–165 (80); ≥166 (410).

4 Tertile and level cutpoints based on baseline intake.
that higher intakes of these fatty acids from foods are indicative of other dietary factors, lifestyle characteristics, or an overall dietary pattern that may be the true protective factor. Although adjustments in analysis attempt to isolate an independent effect, subtle differences or unmeasured variables cannot be completely examined in an observational study of this type.

Laboratory and animal data have identified numerous mechanisms as to how marine fatty acids may inhibit carcinogenesis, including anti-inflammatory, proapoptotic, antiproliferative, and angiogenic effects; downregulation of estrogen synthesis; and modulation of insulin sensitivity (1,30–32). EPA and DHA are thought to reduce inflammation through the inhibition of NF-κB (33), which acts as a transcription factor for targets associated with inflammation, including IL-6 and cyclooxygenase-2 (34). Because EPA and DHA are incorporated into cell phospholipids at the expense of arachidonic acid [(n-6) PUFA], they reduce the reservoir of arachidonic acid for COX-2 to synthesize prostaglandin E2 (33). Experimental studies in rodents have shown a reduction in PGE2 levels and mammary tumor incidence with diets high in (n-3) PUFA found in fish oil (35–37). In humans, dietary intake of (n-3) PUFA or fish has been inversely associated with blood concentrations of inflammatory markers such as C-reactive protein, TNFα, and IL-6 (38,39).

Many previous studies were limited by the use of a single variable for servings of total fish intake, which does not distinguish between good and poor sources of EPA and DHA. Concentrations of these fatty acids vary considerably among species of fish. Higher amounts are found in fattier fish that are native to cold waters, such as Pacific herring, Greenland halibut, king mackerel, and Chinook salmon. For example, a 100-g serving of king mackerel is estimated to provide 1000 mg EPA and 1200 mg DHA (30). In the United States, the average estimated daily intake of EPA plus DHA is 130 mg (40). An additional 2 servings/wk of foods rich in EPA and DHA would increase the intake to over 250 mg/d. The protective effect of EPA and DHA from food sources observed in this analysis was seen at a median of 73 mg/d, suggesting that it would be feasible to obtain sufficient intake of these fatty acids from fish consumption.

This analysis is limited by the use of self-reported dietary intake via 24-h recalls. It is notable that a study using unobtrusive observation of food intake found that of 25 foods and food groups, fish was one of the least likely foods to be omitted or erroneously recalled among 140 adults who completed 24-h recalls (41). Furthermore, a recent review of dietary assessment methods on (n-3) fatty acid intake concluded that compared with biomarkers such as adipose tissue and phospholipids, food records produced good estimates of EPA intake \( r = 0.69 \) and DHA intake \( r = 0.47 \) (42) However, a number of studies have indicated that dietary recalls (or food records) provide better estimates of usual dietary intake than FFQ (43–45). Furthermore, the NCC Food and Nutrient Database is quite complete (46) such that EPA and DHA values were available for 100% of the core foods in the database (47). Nonetheless, we may not have completely captured EPA from specially supplemented foods, such as certain brands of orange juice or eggs. An additional strength of this study was that fish oil supplement use was assessed via an inventory with transcription of the exact dose contained in the supplement. In addition, the use of repeated measures of dietary intake allows for a more precise estimate of a participant’s true long-term intake than a single measurement (29). However, this was a post hoc analysis and we were somewhat limited given that the WHEL dietary intervention reduced intake of total fat, including (n-6) PUFA. Therefore, in this cohort analysis, we were unable to investigate the hypothesis that the balance between intakes of (n-6) and (n-3) fatty acids plays a role in breast cancer (48). Finally, we were unable to examine the effects of EPA separately from DHA, because these 2 compounds were highly correlated \( r = 0.87 \) in food and \( r = 0.78 \) in supplements.

Our investigation indicates that marine fatty acids from foods are associated with reduced risk of additional breast cancer events and all-cause mortality. Additional studies are warranted examining the relationship between breast cancer outcomes and intakes of marine fatty acids, using precise estimates of intake or dietary biomarkers.

**Acknowledgments**

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**Literature Cited**


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