Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women

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Abstract

Antarctic krill, also known as Euphausia superba, is a marine crustacean rich in both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We tested the hypothesis that krill oil would increase plasma concentrations of EPA and DHA without adversely affecting indicators of safety, tolerability, or selected metabolic parameters. In this randomized, double-blind parallel arm trial, overweight and obese men and women (N = 76) were randomly assigned to receive double-blind capsules containing 2 g/d of krill oil, menhaden oil, or control (olive) oil for 4 weeks. Results showed that plasma EPA and DHA concentrations increased significantly more (P < .001) in the krill oil (178.4 ± 38.7 and 90.2 ± 40.3 μmol/L, respectively) and menhaden oil (131.8 ± 28.0 and 149.9 ± 30.4 μmol/L, respectively) groups than in the control group (2.9 ± 13.8 and −1.1 ± 32.4 μmol/L, respectively). Systolic blood pressure declined significantly more (P < .05) in the menhaden oil (−2.2 ± 2.0 mm Hg) group than in the control group (3.3 ± 1.5 mm Hg), and the response in the krill oil group (−0.8 ± 1.4 mm Hg) did not differ from the other 2 treatments. Blood urea nitrogen declined in the krill oil group as compared with the menhaden oil group (P < .006). No significant differences for other safety variables were noted, including adverse events. In conclusion, 4 weeks of krill oil supplementation increased plasma EPA and DHA and was well tolerated, with no indication of adverse effects on safety parameters.

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Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SEM, standard error of mean; TC, total cholesterol; MITT, modified intent-to-treat; TG, triglyceride.

1. Introduction

Consumption of fish and fish oil, rich in the long-chain omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been associated with reduced cardiovascular disease risk in both cohort studies and randomized clinical trials [1-4]. One of the largest intervention trials, the Gruppo Italiano per lo Studio
della Sopravvivenza nell'Infarto miocardico—Prevenzione trial, reported a risk ratio of 0.83 (95% confidence interval, 0.71–0.97) for cardiovascular death and a risk ratio of 0.74 (95% confidence interval, 0.58–0.93) for sudden death compared to usual care for patients receiving an omega-3 fatty acid supplement containing 840 mg/d of EPA plus DHA as ethyl esters for an average of 3.5 years after a recent myocardial infarction [5]. The reported cardioprotective effects of omega-3 fatty acids may be mediated by the effects of EPA and DHA to reduce susceptibility to cardiac arrhythmia, alter lipoprotein lipids, and lower blood pressure, platelet aggregation, and inflammation [6–9].

Antarctic krill, also known as *Euphausia superba*, is a marine crustacean rich in both EPA and DHA. Krill oil differs from other dietary sources of omega-3 in that it contains a relatively high amount of omega-3 fatty acids from phospholipids. Fish oil supplements typically contain omega-3 fatty acids in the triglyceride form or as fatty acid ethyl esters [10]. The purpose of the present investigation, therefore, was to examine the effects of a krill oil supplement on plasma EPA and DHA concentrations and other indicators of safety, tolerability, and selected metabolic parameters. We hypothesized that krill oil would increase plasma concentrations of EPA and DHA at least as well as similar doses of EPA and DHA from fish (menhaden) oil.

2. Methods and materials

2.1. Study design

This was a 4-week, randomized, double-blind, controlled, parallel clinical trial conducted at 2 clinical research sites in the United States (Provident Clinical Research, Bloomington, Ind, and Meriden Research, St. Petersburg, Fla). The study included 3 visits: 2 screening/baseline visits (weeks −1 and 0) and 1 end-of-treatment visit (week 4). An independent institutional review board, Quorum Review, Inc (Seattle, Wash), approved the protocol before initiation of the study, and written informed consent was obtained from all subjects before protocol-specific procedures were performed.

2.2. Subjects

Subjects included generally healthy men and women, 35 to 64 years of age, with waist circumference of 102 cm or greater (men) or 88 cm or greater (women). Pregnant (or those planning to become pregnant during the study period) and lactating women were excluded. Volunteers who consumed fish more than 3 times in the month before screening were not eligible for enrollment, and consumption of fish and seafood products was prohibited during the study.

Individuals with a self-reported history of diabetes, inflammatory bowel disease, pancreatitis, and gallbladder or biliary disease in the 12 months before the screening visit were excluded from the study. In addition, those with a history of cancer (except for nonmelanoma skin cancer) in the 2 years before screening or any major trauma or surgical event within 3 months before screening were not enrolled. Volunteers were also excluded if they had serum triglycerides (TG) ≥500 mg/dL, total cholesterol (TC) ≥300 mg/dL, or uncontrolled hypertension (systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥100 mm Hg) at screening.

The use of lipid-altering medications or supplements, non–study-related omega-3 fatty acid supplements (eg, flaxseed, fish, or algal oils) or omega-3 fatty acid–enriched foods, and anticoagulants was prohibited within 2 weeks of screening and throughout the study.

2.3. Study procedures

At baseline, eligible subjects were randomly assigned to 1 of 3 groups: 2 g/d of either krill oil (Superba krill oil, Aker BioMarine ASA, Oslo, Norway), menhaden oil (Omega-Pure, Houston, Tex), or menhaden oil (control). Subjects were instructed to consume four 500 mg capsules per day, preferably 2 capsules with each of 2 meals, for 4 weeks. Four capsules of the krill oil supplement provided 216 mg/d EPA and 90 mg/d DHA, and the menhaden oil supplement provided 212 mg/d EPA and 178 mg/d DHA.

2.4. Measurements

Consumption of fish and fish oil supplements was evaluated at each clinic visit using a Fish Intake Questionnaire [11]. The screening assessment (week −1) assessed fish and fish oil intake over the prior year. At visit 2 (week 0) and visit 3 (week 4), subjects completed a gastrointestinal (GI) tolerability questionnaire [12], which assessed the presence and severity (on a scale of 0 to 5) of GI symptoms such as gas, bloating, nausea, flatulence, diarrhea, constipation, and cramping over the prior 7 days. Subjects completed a symptom checklist at visit 3, which assessed the incidence of or changes in a variety of symptoms (eg, irritability, nervousness, mood, blurred vision, drowsiness, mental sharpness, and hair and skin changes) in the previous 4 weeks on a scale of 1 (a lot less) to 5 (a lot more).

Adverse events were assessed from the time subjects signed the informed consent form at screening (week −1) and continued through the end of the study. Compliance with consumption of the study product was evaluated by subject interview. In addition, subjects were required to return all unused study capsules at the final visit, at which time the capsules were counted and the percentage of scheduled doses consumed was determined.

Body weight was measured at each clinic visit; height was measured only at screening (week −1). Standardized blood pressure measurements were taken after 5 minutes of seated rest at each visit. Subjects were required to refrain from smoking cigarettes or ingesting caffeine during the 30 minutes preceding the measurement. Systolic and diastolic blood pressures were measured using an automated blood pressure measurement device (Welch Allyn, Model 3500, Skaneateles Falls, NY), with the appropriate-sized cuff.
calculated according to the Friedewald equation [13] as follows: LDL-C = TC − HDL-C − TG/5. Because this equation is not valid when the TG concentration is greater than 400 mg/dL, LDL-C values were not calculated under these circumstances. Insulin resistance was assessed by the homeostasis model assessment (HOMA-IR) method using the following equation: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405 [14]. Fatty acid composition of the study capsules was analyzed using the American Oil Chemist Society Official Method Ce 1b-89 (Marine Oil Fatty Acid Composition by gas-liquid chromatography [GLC]) using C23:0 fatty acid methyl ester as an internal standard [15]. Plasma fatty acids (EPA and DHA) were measured at Oy Jurilab LTD (Kuopio, Finland) using the method of Nyyssönen et al [16]. Total lipids were extracted from an aliquot of plasma with methanol/chloroform (1:2), and fatty acids were identified using gas chromatography. F2-isoprostanes in urine were analyzed at Oy Jurilab LTD. Briefly, pentafluorobenzyl esters of F2-isoprostanes were prepared and purified by high-performance liquid chromatography, silylated, and then analyzed by gas chromatography with negative chemical ionization mass spectroscopy according to the method described by Walters et al [17].

2.5. Statistical analyses

Statistical analyses were conducted using SAS version 9.1.3 (SAS Institute, Cary, NC). All tests of significance were performed at \( \alpha = .05 \), 2-sided. The Shapiro-Wilk test was used to test the normality of residuals before running the final models for continuous outcome variables. If it was determined that the distribution could not be approximated by a normal curve, then the values were ranked in ascending order, with tied values given a mean rank, before running the final statistical models.

The analysis of the outcome parameters was completed on a modified intent-to-treat (MITT) population, which included all subjects who were randomized, consumed at least 1 dose of study product, and provided at least 1 postrandomization blood sample. The safety population included all subjects who were randomized into the study and received at least 1 dose of study product.

Differences between treatment groups in responses for continuous variables were assessed by analysis of covariance using the SAS general linear modeling procedure. The initial models included terms for baseline value, center, treatment group, and center-by-treatment group interaction. Models were reduced in a stepwise manner until only significant terms (\( P < .05 \)) or treatment group remained. Pairwise comparisons between groups were completed when the F ratio for the treatment group term was statistically significant (\( P < .05 \)), with Hochberg procedure employed to adjust for multiple comparisons. Frequencies of treatment-emergent adverse events (overall and by type of adverse event) and responses of 4 or more (more or much more than usual) on the GI tolerability questionnaire were compared between groups using \( \chi^2 \) tests. Fisher exact tests were used for pairwise comparisons where the overall \( \chi^2 \) test had \( P < .05 \). For pairwise comparisons, each \( P \)-value was adjusted by a factor of 2.95 to maintain a familywise error rate of 0.05 and both unadjusted and adjusted \( P \)-values are provided. The data are presented as means ± standard error of the mean unless otherwise indicated.

3. Results

Fig. 1 shows the disposition of all subjects. Of the 96 subjects screened for enrollment, 76 were randomly assigned to one of the 3 treatment groups: 25 to krill oil, 26 to menhaden oil, and 25 to control (olive) oil. Of the 76 subjects, 75 completed the study. One subject in the menhaden oil group discontinued before completion due to adverse events: hand swelling and generalized itching.

Baseline demographic and anthropometric characteristics by treatment group are shown in Table 1. No significant differences were observed among the 3 groups at baseline. Most of the subjects were women (83%) of non-Hispanic white ethnicity (99%) with a mean age of approximately 49 years. The mean body mass index of the study sample was approximately 33 kg/m².

Mean compliance with the study product was 96% for the krill oil group and 99% for both the menhaden oil and placebo groups at week 4 (\( P = .313 \)). Overall, 96% of subjects in the krill oil group, 100% of subjects in the menhaden group, and 96% of subjects in the control group were at least 80% compliant with consumption of the study product (\( P = .586 \)). No differences between treatment groups were present for fish intake over the prior year or during the study (data not shown).

(bladder within the cuff encircled \( \geq 80\% \) of the arm). Two measurements were taken, separated by at least 2 minutes, and the average of both was recorded unless they differed by more than 5 mm Hg. In that case, an additional reading was obtained, and the average of all 3 readings was recorded.

Subjects collected all of their urine for 12 hours before the visits at weeks 0 and 4 for F2-isoprostane analysis. A spot sample of morning urine was collected if a 12-hour urine collection was not available at these visits.

Clinical laboratory measurements (plasma chemistry, hematology, urine, and lipids) were conducted by Elmhurst Memorial Hospital Laboratory (Elmhurst, Ill), unless otherwise stated, using instruments by Beckman, Inc (Fullerton, Calif). All samples were collected under fasting conditions. Samples for the plasma chemistry analysis were collected at each visit and for the serum hematology analysis at screening (week −1) and week 4.

Fasting plasma lipid profiles were performed at each visit. Cholesterol and TG levels were measured using the Beckman Coulter’s LX20 PRO. Low-density lipoprotein cholesterol (LDL-C) concentration in milligram per deciliter was obtained, and the average of all 3 readings was recorded. Differences between treatment groups in responses for continuous variables were assessed by analysis of covariance using the SAS general linear modeling procedure. The initial models included terms for baseline value, center, treatment group, and center-by-treatment group interaction. Models were reduced in a stepwise manner until only significant terms (\( P < .05 \)) or treatment group remained. Pairwise comparisons between groups were completed when the F ratio for the treatment group term was statistically significant (\( P < .05 \)), with Hochberg procedure employed to adjust for multiple comparisons. Frequencies of treatment-emergent adverse events (overall and by type of adverse event) and responses of 4 or more (more or much more than usual) on the GI tolerability questionnaire were compared between groups using \( \chi^2 \) tests. Fisher exact tests were used for pairwise comparisons where the overall \( \chi^2 \) test had \( P < .05 \). For pairwise comparisons, each \( P \)-value was adjusted by a factor of 2.95 to maintain a familywise error rate of 0.05 and both unadjusted and adjusted \( P \)-values are provided. The data are presented as means ± standard error of the mean unless otherwise indicated.
Body weight was stable in all groups throughout the study. No significant differences were observed among groups in the changes from baseline to week 4 in body weight ($P = .417$) or diastolic blood pressure ($P = .481$). Systolic blood pressure declined modestly in both the krill oil ($-0.8 \pm 1.4 \text{ mm Hg}$) and menhaden oil ($-2.2 \pm 2.0 \text{ mm Hg}$) groups, whereas it increased somewhat in the control group ($3.3 \pm 1.5 \text{ mm Hg}$). The systolic blood pressure response in the control group differed significantly from that in the menhaden group ($P = .032$), but no significant differences were present for the other pairwise comparisons.

The changes from baseline to week 4 in blood urea nitrogen were significantly different among the treatment groups ($P = .008$). Pairwise comparisons revealed significant differences in mean blood urea nitrogen responses between the krill oil ($-1.0 \pm 0.5 \text{ mg/dL}$) and the menhaden oil ($1.4 \pm 0.6 \text{ mg/dL}$) groups ($P = .006$). The change from baseline in the control group ($0.6 \pm 0.6 \text{ mg/dL}$) did not differ significantly from that of the other 2 treatment arms.

Changes or percent changes from baseline to week 4 in plasma EPA and DHA, fasting lipoprotein lipids, measurements of glucose homeostasis, high-sensitivity C-reactive protein (hs-CRP), and F2-isoprostanes are summarized by treatment group in Table 2.

Table 1
Baseline demographic and anthropometric characteristics of subjects in the MITT sample by treatment group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Krill oil (n = 25)</th>
<th>Menhaden oil (n = 26)</th>
<th>Control (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>3 (12.0)</td>
<td>5 (19.2)</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>22 (88.0)</td>
<td>21 (80.8)</td>
<td>20 (80.0)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white, n (%)</td>
<td>24 (96.0)</td>
<td>26 (100)</td>
<td>25 (100)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>1 (4.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Age, y, mean ± SEM</td>
<td>49.4 ± 1.7</td>
<td>49.6 ± 1.4</td>
<td>47.4 ± 1.6</td>
</tr>
<tr>
<td>Weight, kg, mean ± SEM</td>
<td>89.7 ± 4.5</td>
<td>90.5 ± 3.7</td>
<td>95.5 ± 6.2</td>
</tr>
<tr>
<td>Height, cm, mean ± SEM</td>
<td>165.6 ± 1.3</td>
<td>167.6 ± 1.4</td>
<td>168.9 ± 2.1</td>
</tr>
<tr>
<td>Body mass index, kg/m², mean ± SEM</td>
<td>32.6 ± 1.5</td>
<td>32.1 ± 1.1</td>
<td>33.3 ± 1.7</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg, mean ± SEM</td>
<td>118.6 ± 2.1</td>
<td>119.8 ± 2.6</td>
<td>119.6 ± 2.3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg, mean ± SEM</td>
<td>74.9 ± 2.0</td>
<td>76.2 ± 2.0</td>
<td>78.0 ± 1.7</td>
</tr>
</tbody>
</table>

SEM indicates standard error of the mean.

* There were no significant differences between groups for any variable (analysis of variance).
which the investigator judged to be probably related to the study product. The most common adverse events were hyperglycemia, which occurred in 3 (12.0%) krill oil subjects, 2 (7.7%) menhaden oil subjects, and 1 (4.0%) control subject, and flatulence, which occurred in 2 (8.0%) krill oil subjects, none of the menhaden oil subjects, and 3 (12.0%) control subjects.

Results from the GI tolerability questionnaire are summarized in Table 3. There were no significant differences among treatment groups at baseline in the number of subjects with a score of 4 or higher (more or much more than usual) in any of the measures of GI tolerability, which included gas/bloating, nausea, flatulence, diarrhea or loose stools, constipation, or GI cramping. At week 4, significant differences were observed among the treatment groups in the number of subjects with scores of 4 or higher for gas or bloating (P = .050) and flatulence (P = .034). The number of subjects with gas or bloating increased from 2 (8%) at baseline to 5 (20%) at week 4 in the krill oil group and from 1 (4.0%) at baseline to 5 (20.0%) in the control group. No subjects in the menhaden oil group experienced gas or bloating at either time point. Similarly, the number of subjects with flatulence increased from 2 (8%) at baseline to 5 (20.0%) in the krill oil group and from 1 (4.0%) at baseline to 5 (20.0%) in the control group. No significant differences were observed among the treatment groups in the frequencies of any symptoms assessed with the symptom checklist (data not shown).

4. Discussion

The objective of this study was to evaluate the safety, tolerability, and selected metabolic effects of krill oil supplements (2 g/d) in overweight and obese men and women, as compared with menhaden oil and a control (olive) oil. The results indicate that krill oil was generally well tolerated and did not show evidence of any adverse influence on safety parameters. Significant increases from baseline in plasma levels of EPA and DHA were observed with krill oil supplementation.
The observed increases in plasma EPA and DHA were similar to those observed with the menhaden oil supplement and are consistent with results reported after several weeks of supplementation with other sources of EPA and DHA when provided at similar dosages [18-20].

The daily quantity of EPA provided in the krill (216 mg) and menhaden oil (212 mg) supplements in this study was comparable. However, the DHA present in the krill oil (90 mg/d) was approximately one half that provided in the menhaden oil (178 mg/d). At the end of the treatment period, the mean plasma EPA concentration was somewhat higher in the krill oil group compared with the menhaden oil group (377 vs 293 μmol/L), whereas the mean plasma DHA concentrations were comparable (476 vs 478 μmol/L). These results suggest that the EPA and DHA from krill oil are absorbed at least as well as that from menhaden oil.

The metabolic and cardiovascular effects of EPA and DHA are well known and include lowering of triglyceride and very-low-density lipoprotein cholesterol levels when provided in sufficient dosages [8,19] and reduction of blood pressure [9,21]. Additional effects that have been reported include improvement of vascular reactivity and anti-inflammatory, antithrombotic, and antiarrhythmic properties [6]. In this study, TG and other lipoprotein lipid responses did not differ across treatments. This is not surprising given the relatively short period of intake (4 weeks) and small dosages of EPA and DHA provided (<400 mg/d). Intakes of greater than 500 mg/d of EPA + DHA have generally been required to produce significant lowering of the TG concentration [22]. Moreover, subjects in the current trial were normolipidemic, and the lipid-altering effects of DHA + EPA appear to be more pronounced in subjects with elevated baseline triglyceride concentrations [18,19].

The mean systolic blood pressure response differed significantly (P = .032) in the menhaden oil group (−2.2 mm Hg) compared with the control group (3.3 mm Hg). EPA + DHA consumption has previously been reported to modestly reduce blood pressure [3,9,21]. Thus, the reduction could be attributable to the supplement. However, because of the lack of difference in response for diastolic blood pressure and the fact that blood pressure was not significantly reduced in the krill oil group, despite similar increases in plasma EPA and DHA concentrations, it is also possible that this is a chance finding.

The 2 g/d dose of krill oil provided in this study was generally well tolerated. The frequencies of adverse events and were similar for all groups, and no adverse changes in mean values for laboratory measures of safety were noted. Subjects in the krill oil group experienced more frequent gas/bloating and flatulence than did subjects in the menhaden oil group; however, the frequencies of these effects did not differ between the krill oil and control groups, and none of the subjects felt that the effects were sufficient to warrant discontinuation of the study product. Mild GI side effects are common with consumption of omega-3 fatty acids, and these appear to be dose dependent, with increased severity of symptoms at doses greater than 3 g/d [6].

Omega-3 fatty acids found in fish and fish oils have been shown to have anti-inflammatory properties. In vitro studies have demonstrated that long-chain omega-3 fatty acids impact inflammation directly by replacing arachidonic acid as a substrate for enzymes involved in prostaglandin production and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation [23]. They also appear to enhance activity of resolvins, a family of anti-inflammatory mediators [23]. In this study, despite relatively high levels at baseline in this group of overweight and obese subjects, there was no difference across treatment groups in the change from baseline in hs-CRP, a marker for systemic inflammation. This finding is in agreement with those from some other trials that have reported no change in hs-CRP concentration in response to EPA + DHA supplementation [24-26]. However, some studies in individuals with pro-inflammatory states have suggested that increased consumption of EPA and DHA has been associated with a reduction in hs-CRP concentration [27]. Thus, the influence of omega-3 fatty acid intake on hs-CRP concentration has not been fully characterized and will require further study.

Both EPA and DHA are highly unsaturated fatty acids and are therefore prone to oxidize more readily than less saturated fatty acids. The urinary F2-isoprostanes concentration is an indicator of lipid peroxidation and oxidative stress [28]. In this study, no differences were observed across treatment groups in the changes from baseline in levels of urinary F2-isoprostanes, suggesting that supplementation at the dosages studied herein did not produce any measurable changes in whole-body lipid peroxidation.

A limitation of this investigation is that a single dosage level of EPA + DHA from krill oil was studied over a relatively short treatment period. A longer feeding period may be needed to achieve steady-state plasma EPA and DHA concentrations [29], and additional research is warranted to assess the longer-term effects of krill oil supplementation over a range of intake levels.

In summary, 4 weeks of krill oil supplementation (2 g/d) produced significant elevations in plasma levels of EPA and DHA. Compared with both menhaden oil and olive oil, krill oil was generally well tolerated and showed no indication of adverse effects on safety parameters.

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References


