

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Krill oil supplementation lowers serum triglycerides without increasing low-density lipoprotein cholesterol in adults with borderline high or high triglyceride levels

Kjetil Berge^{a,*}, Kathy Musa-Veloso^b, Melody Harwood^a, Nils Hoem^a, Lena Burri^a

^a Aker BioMarine ASA, Fjordalléen 16, 0115 Oslo, Norway

^b Intertek Cantox, Mississauga, Ontario, Canada L5N 2X7

ARTICLE INFO

Article history:

Received 14 August 2013

Revised 11 December 2013

Accepted 12 December 2013

Keywords:

Cardiovascular disease risk
clinical trial

Docosahexaenoic acid

Eicosapentaenoic acid

Krill oil

Low-density lipoprotein cholesterol

Omega-3 index

Phospholipids

Triglycerides

ABSTRACT

The aim of the study was to explore the effects of 12 weeks daily krill oil supplementation on fasting serum triglyceride (TG) and lipoprotein particle levels in subjects whose habitual fish intake is low and who have borderline high or high fasting serum TG levels (150–499 mg/dL). We hypothesized that Krill oil lowers serum TG levels in subjects with borderline high or high fasting TG levels. To test our hypothesis 300 male and female subjects were included in a double-blind, randomized, multi-center, placebo-controlled study with five treatment groups: placebo (olive oil) or 0.5, 1, 2, or 4 g/day of krill oil. Serum lipids were measured after an overnight fast at baseline, 6 and 12 weeks. Due to a high intra-individual variability in TG levels, data from all subjects in the four krill oil groups were pooled to increase statistical power, and a general time- and dose-independent one-way analysis of variance was performed to assess efficacy. Relative to subjects in the placebo group, those administered krill oil had a statistically significant calculated reduction in serum TG levels of 10.2%. Moreover, LDL-C levels were not increased in the krill oil groups relative to the placebo group. The outcome of the pooled analysis suggests that krill oil is effective in reducing a cardiovascular risk factor. However, owing to the individual fluctuations of TG concentrations measured, a study with more individual measurements per treatment group is needed to increase the confidence of these findings.

© 2014 The Authors. Published by Elsevier Inc. Open access under [CC BY-NC-ND license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Dyslipidemia is a lipoprotein metabolism disorder of epidemic proportions that is associated with increased risk for cardiovascular disease (CVD) [1,2]. It can include elevated blood

triglyceride (TG), total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels and/or low high-density lipoprotein cholesterol (HDL-C) concentrations. TG-rich lipoproteins have some atherogenic properties, but their inverse association with HDL-C and direct association with smaller and denser athero-

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EFSA, European Food Safety Authority; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NDA Panel, Panel on Dietetic Products, Nutrition and Allergies; NHPD, Natural Health Products Directorate; n-3 LCPUFAs, omega-3 long-chain polyunsaturated fatty acids; PL, phospholipid; SD, standard deviation; TG, triglyceride; U.S, United States.

* Corresponding author. Aker BioMarine ASA, Fjordalléen 16, NO-0115 Oslo, Norway. Tel.: +47 24130000; fax: +47 24130110.

E-mail address: kjetil.berge@akerbiomarine.com (K. Berge).

genic LDL particles is the likely cause of the increased risk for CVD in these patients [3]. One option to tackle high TG levels and potentially decrease CVD risk is by dietary supplementation with the long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFAs) that are known to decrease TG production and increase TG clearance [4].

In the Canadian Natural Health Products Directorate (NHPD) Fish Oil Monograph, the dose of n-3 LCPUFAs required for TG reductions is 1 to 3 g/day. In Europe, the Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) of the European Food Safety Authority (EFSA) recently concluded that the n-3 LCPUFAs effectively reduce TG levels when consumed at intakes of 2 to 4 g/day. There is some indication from dose–response assessments that the n-3 LCPUFAs may be efficacious in reducing fasting TG levels when consumed at doses even lower than these recommended doses. In a recent meta-analysis of randomized controlled trials, it was demonstrated that TG levels are dose-dependently reduced by the n-3 LCPUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [5]. Even though there were only a limited number of data points in the dose–response assessment at EPA and DHA intakes of less than 1 g/day, there was some suggestion that even modest intakes of the n-3 LCPUFAs could be beneficial with regards to reducing fasting serum TG levels. Likewise, in a dose–response assessment restricted to algal sources of DHA, Ryan et al. demonstrated a dose–response relationship between dose of DHA and the reduction in fasting TG level [6]. Although this latter dose–response assessment was restricted to studies conducted with algal DHA, it has been reported that EPA and DHA have similar TG-reducing effects when administered individually [7–9].

Krill oil is processed from Antarctic krill (*Euphausia superba*), small shrimp-like animals of the crustacean superorder Eucarida found in the Southern Ocean. Krill oil is a unique source of EPA and DHA because unlike most other oils of marine origin, the major part of EPA and DHA in krill oil occurs naturally in phospholipid (PL) and not in TG form [10,11]. There are indications that, compared to the delivery of EPA and DHA in the TG form, the delivery of EPA and DHA in the PL form results in higher tissue levels of EPA and DHA [12–15]. Krill oil is characterized by a higher amount of EPA compared to DHA, with a ratio of 2 to 1. While there is consensus in the scientific literature that the dietary intake of both EPA and DHA (either individually or in combination) can reduce elevated TG levels, DHA (but not EPA) has been suggested to be responsible for a simultaneous elevation in LDL-C seen particularly in patients with very high (>500 mg/dL) TG levels [8,9,16].

In rodents, krill oil supplementation has been shown to suppress lipid synthesis by up-regulating genes involved in lipid oxidation and down-regulating those that are involved in lipogenesis [17,18]. Blood TG and cholesterol levels were significantly reduced after the administration of krill oil, both in normolipidemic rats [19] and in rats with diet-induced hyperlipidemia [20]. Pre-clinical experiments also suggest that the endocannabinoid system plays a major role in the action of krill oil on fat distribution in obese rats [12,21].

Thus, the objective of the clinical study described herein was to test our hypothesis that krill oil can lower serum TG levels in humans with borderline-high or high fasting serum TG levels (i.e., 150–499 mg/dL). Further, the study aimed to

evaluate whether reductions in TG levels are achievable with intakes of EPA and DHA from krill oil that are lower than those currently recommended in the NHPD Fish Oil Monograph and in the EFSA NDA Panel's Scientific Opinion for TG lowering.

2. Methods and materials

2.1. Study population and design

A randomized, double-blind, placebo-controlled, multi-center study was performed by former Cetero Research at two United States (U.S.) clinical research sites – one in Fargo, North Dakota and the other in St. Charles, Missouri. To be included, subjects had to be between 21 and 79 years of age, have a low habitual fatty fish and seafood intake (defined as the intake of fatty fish and seafood at a frequency not to exceed twice per month), and have borderline high or high fasting serum TG levels (defined as a fasting TG level of 150–499 mg/dL at Screening visit, inclusive). Subjects were not eligible for study participation if they tested positive for drug or alcohol screens, tested positive for pregnancy (for women of child-bearing potential), were on lipid lowering medications or omega-3 supplementation, had a body mass index (BMI) ≥ 35 kg/m², had CVD or other co-morbidities, bleeding disorders, hypertension, familial hypercholesterolemia, coronary, peripheral or cerebral vascular disease, or allergy to fish or crustaceans. The primary objective of the study was to assess the effects on fasting serum TG levels during 12 weeks of daily supplementation with four different daily doses of Superba™ krill oil (0.5, 1.0, 2.0 and 4.0 g). Qualifying subjects were randomly and evenly allocated into 5 study groups. Randomization was stratified by gender.

Subjects were instructed to avoid fish and seafood meals 36 hours before each clinic visit and to avoid consuming alcohol in the 24 hours before each scheduled visit. A total of 5 visits were included: one for screening, one for randomization and collection of baseline information, one at day 7 to ensure the test products were being taken appropriately, and two efficacy visits (6 and 12 weeks) when blood was drawn.

2.2. Study products

Krill oil capsules were provided by Aker BioMarine ASA (Oslo, Norway) and olive oil (placebo) was obtained from Ruiz-Canela e Hijos (Sevilla, Spain). The fatty acid and lipid profiles of the study products are presented in Table 1. All subjects were required to consume 8×500 mg capsules daily for the 12-week intervention period; 4 capsules in the morning with water before breakfast, and 4 capsules in the evening with water before dinner. Subjects allocated to the placebo group consumed 8 placebo capsules daily whereas subjects allocated to krill oil took 1, 2, 4 or 8 krill oil capsules and the remainder as placebo. The group that was assigned 1 krill oil capsule per day took it with the morning meal, otherwise the krill oil and placebo capsules were distributed evenly amongst the morning and evening doses. The varying doses of krill oil (i.e., 0, 0.5, 1, 2, and 4 g/day) corresponded to daily intakes of EPA + DHA of 0, 100, 200, 400, and 800 mg/day, respectively. All subjects filled out compliance records daily, which included questions on capsules count and time of intake.

Table 1 – Fatty acid and lipid composition of the products administered

Fatty acids	Placebo	Krill oil
16:0	10.3	13.4
18:1 n-9	61.1	5.9
18:2 n-6	8.9	1.2
18:4 n-3	0	2.2
20:5 n-3	0	13.1
22:6 n-3	0	6.5
Totals and lipids	7.9	23.4
Saturated fatty acids	10.3	21.9
n-6 PUFA	8.9	1.8
n-3 PUFA	0.7	24.5
Total fatty acids	88.2	65.7
Triglycerides	86	30
Free fatty acids	0.2	3.8
Phospholipids	0	45
Total lipids	86.8	82.6

PUFA = polyunsaturated fatty acids.

Krill oil has a distinctive odor, taste, and color. It was therefore imperative to blind the subjects to the identity of the capsules. Thus, to mask the different colors of the krill and placebo oils, the gelatin capsules were black. To make the krill oil and placebo capsules taste similar, a vanillin extract was added to all of the capsules. To make the krill and placebo oil capsules smell similar, the placebo capsules were rubbed with negligible amounts of krill oil. All capsules were provided to subjects in 7×4 AM and PM blister packs; each set of AM and PM blister packs provided a subject with a week's worth of dosing. The blister packs were coded in a manner that maintained the blinding of the study. Study subjects and personnel were blinded to the study.

2.3. Ethics

The study was conducted with approval of an Institutional Review Board and in accordance with Good Clinical Practices and the World Medical Association Declaration of Helsinki. All subjects received appropriate oral and written information on the background of the study and potential risks and benefits of taking krill oil supplements. After comprehensive information and time for questions, written informed consent was asked from all subjects who wanted to enroll in the study. It was made clear that at any time the subjects could withdraw their consent. The study was registered at www.clinicaltrials.gov (NCT01415388).

2.4. Serum lipids, omega-3 index and safety assessments

Body weight and BMI were measured for all subjects during each visit [screening, baseline, week 6 and week 12 (± 3 days)]. Blood from venipuncture for the assessment of serum lipids was obtained after an overnight fast (≥ 12 h) at screening and all visits. After sitting for 30 min at room temperature, serum was separated in silica gel tubes (BD Vacutainer) by centrifugation at 1,300 × g for 12 min at room temperature. Serum analysis of total cholesterol, LDL-C, HDL-C, and TG was performed using standard enzymatic methods on an Olympus AU 5400 or AU 5431 analyzer.

Blood for the assessment of the omega-3 index was collected at baseline, 6 and 12 weeks after an overnight fast. It was analyzed as described previously at Omegametrix GmbH (Mar-

tinsried, Germany) [22,23]. In short, fatty acid methyl esters from red blood cells were analyzed by gas chromatography (GC2010, Shimadzu, Duisburg, Germany) equipped with a SP2560 100-m column (Supelco, Bellefonte, PA, USA), using hydrogen gas as a carrier. Omega-3 index was given as EPA + DHA in red blood cells expressed as a percentage of the total identified fatty acids.

Safety assessments included measurements of blood pressure, pulse rate, body temperature, and the collection of information on unsolicited adverse events at all visits, as well as 12-lead echocardiogram (ECG), physical checkup, urinalysis, hematology and clinical chemistry at the screening and end-of-study visits.

2.5. Statistical analyses

In order to detect a difference of 15% between each of the dosing groups and the placebo group and assuming a two-tailed alpha-level of 0.05, a standard deviation (SD) in percent change from baseline in fasting serum triglycerides of 25%, and 80% power, it was estimated that 60 subjects would be required per group, and 300 subjects would be required, in total. However, a large degree of intra-individual variation was observed in the TG measurements, which were not accounted for in the power calculation. Thus, in addition to present the TG level changes after 6 and 12 weeks, the mean changes from baseline at 6 and 12 weeks in fasting TG in the four krill oil groups were pooled in a time- and dose-independent manner for comparison to the placebo group. By doing so, the statistical power was increased and the relative (%) changes from baseline in fasting TGs were compared using a Student's t-test. However, the pooling approach can only be seen as explorative data analysis. The other lipid parameters (total cholesterol, LDL-C and HDL-C) were not associated with large intra-individual variability and were therefore compared to the corresponding measures in the placebo group using an analysis of variance (ANOVA), without pooling the data points across the krill oil groups. The TG data presented in Table 4 was analyzed using ANOVA. The statistical analyses were performed in JMP 10.0.2 (SAS Institute, Cary, NC). Changes were considered statistically significant at $p < 0.05$. All data are presented as means \pm SD, unless otherwise specified.

3. Results

3.1. Baseline subject characteristics

A total of 300 subjects were randomized into five groups and were supplemented with either placebo (olive oil) or one of four krill oil doses (0.5, 1, 2 or 4 g/day) (Fig. 1). Altogether, data for 33 subjects were not included in the efficacy analysis. The average of the Screening and Day 0 TG values was used as baseline TG values. However, twenty-four subjects had a fasting TG level within the range required for inclusion at screening (i.e., between 150 and 499 mg/dL, inclusive); and not at baseline, where the fasting TG levels were normal (i.e., <150 mg/dL). Data for these 24 subjects were excluded from the analysis. Of the other 9 subjects whose data were not included in the efficacy analysis, 1 subject was determined from the dietary records to consume fatty fish more than twice per month, 1 subject had adverse events (hypertension; not

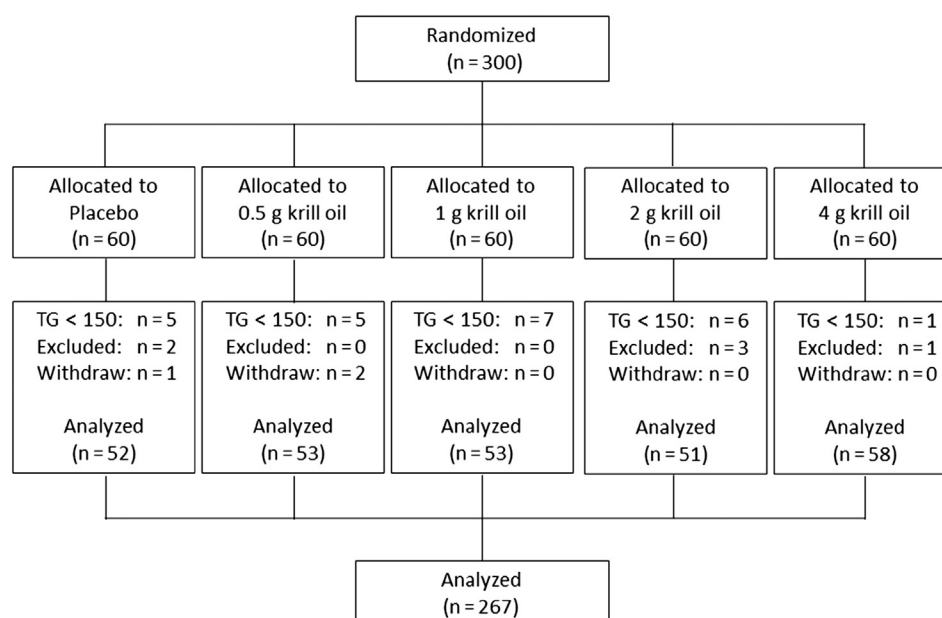


Fig. 1 – Study design and disposition of participating study subjects. “TG < 150” states the number of subjects that had triglyceride levels below 150 at baseline and were excluded from final study analysis. “Excluded” includes subjects that failed on inclusion/exclusion criteria, experienced adverse events or had major protocol deviations.

related to study product intake), 3 subjects withdrew from the study (two because of scheduling conflicts and one for personal reasons) and 4 subjects had major protocol deviations (all four were not fasted at blood sampling).

Daily EPA and DHA doses are depicted in Table 2, as are the numbers of subjects that could be used for the efficacy assessments. More males (69%) than females participated in the study. Most subject characteristics at baseline were not significantly different between the groups. In particular mean fasting serum TG values at baseline, which were approximately 232 mg/dL, were similar between the groups. Fasting serum TG concentrations of <150 mg/dL (<1.7 mmol/L) are considered to be normal, while values between 150–199 mg/dL (1.7–2.25 mmol/L) define borderline hypertriglyceridemia, 200–499 mg/dL (2.25–5.65 mmol/L) define high TGs, and >500 mg/dL

(>5.65 mmol/L) define very high TGs. Also serum LDL-C concentrations were similar across the groups, with a mean value of 127 mg/dL.

Some significant differences between the treatment and placebo groups at baseline were observed for HDL-C, BMI and the omega-3 index (Table 2); nonetheless, overall, the subjects had a low omega-3 index (between 3.5–4%) and BMI was around 30 kg/m².

3.2. Adverse events, body weight and blood pressure

Only three participants withdrew from the study (Fig. 1). Overall, krill oil supplementation was well tolerated in all groups and no serious adverse events related to study product occurred during the study. There were two subjects with

Table 2 – Baseline parameters and blood measurements of subjects

Characteristics	Overall	Placebo	0.5 g krill oil	1 g krill oil	2 g krill oil	4 g krill oil	Pooled data for krill oil groups
EPA dose (mg/day)		0	67	134	268	536	257.7
DHA dose (mg/day)		0	33	66	132	264	126.9
Number of subjects	267	52	53	53	51	58	215
Weight (kg)	89.6 ± 13.4	90.5 ± 14.1	90.1 ± 13.4	87.6 ± 14.7	91.7 ± 12.5	88.2 ± 12.1	89.3 ± 13.2
Height (cm)	1.74 ± 0.1	1.73 ± 0.11	1.73 ± 0.1	1.73 ± 0.09	1.75 ± 0.09	1.73 ± 0.09	1.74 ± 0.1
BMI (kg/m ²)	29.6 ± 3	30.2 ± 3	29.9 ± 3.3	29 ± 3.3*	29.7 ± 2.6	29.3 ± 2.9	29.5 ± 3
Age (y)	44.1 ± 12.3	43.5 ± 11	46.4 ± 12.7	40.5 ± 13.1	44.3 ± 12.1	45.5 ± 11.9	44.2 ± 12.6
Men (%)	69	65	66	64	73	67	67
White (%)	94	98	85	98	94	95	93
Omega-3 index (%)	3.68 ± 0.88	3.54 ± 1.05	3.66 ± 0.9	3.56 ± 0.81	4 ± 0.88*	3.65 ± 0.7	3.71 ± 0.84
Triglycerides (mg/dL)	232.3 ± 71.5	236.5 ± 80.9	230.1 ± 71.4	238.9 ± 69.8	237.4 ± 73.1	220.2 ± 63	231.3 ± 69.2
Total cholesterol (mg/dL)	215.2 ± 35.7	207.9 ± 32.4	221.1 ± 37.1	209.4 ± 40.3	221.4 ± 36.1	216.2 ± 31.2	216 ± 36.3
HDL cholesterol (mg/dL)	43 ± 10.2	39.5 ± 9.3	44.5 ± 10.8*	42.1 ± 10.6	42.1 ± 9.2	46.2 ± 10*	43.8 ± 10.3
LDL cholesterol (mg/dL)	127 ± 33.1	122.8 ± 4.7	132.7 ± 4.6	120.8 ± 4.6	133.1 ± 4.7	125.8 ± 4.4	128 ± 33.8

Table 3 – Omega-3 index levels of subjects in the placebo and krill oil supplementation after 6 and 12 weeks

	Placebo	0.5 g krill oil	1 g krill oil	2 g krill oil	4 g krill oil
Baseline	3.54 ± 1.05	3.66 ± 0.9	3.56 ± 0.82	4 ± 0.88*	3.65 ± 0.7
6 weeks	3.44 ± 0.88	3.83 ± 0.75*	4 ± 0.75*	4.76 ± 0.86*	5.54 ± 0.87*
12 weeks	3.43 ± 0.77	3.97 ± 0.8*	4.19 ± 0.79*	5.17 ± 0.96*	6.3 ± 0.99*

unrelated serious adverse events, including asthma and cellulitis. Other incidences of non-serious adverse events that could possibly be related to study product intake were: hypertension (1), soft stool (2), flatulence (1), upset stomach (3), gastrointestinal discomfort (1), decreased appetite (1), headache (1), taste change (1), diarrhea (4), fishy burps (1), heartburn (1) and intermittent belching (1). Body weight and blood pressure remained unchanged during the 12-week study compared to baseline values in all five groups.

3.3. Omega-3 index

Compliance was confirmed by measuring the omega-3 index (Table 3). The omega-3 index levels increased significantly in all treatment groups after both 6 and 12 weeks of krill oil supplementation, whereas the placebo group remained unchanged. The omega-3 index changed by –3, 5, 12, 19 and 52% from baseline in the placebo, 0.5, 1, 2 or 4 g krill oil groups, respectively, after 6 weeks of supplementation. The corresponding changes after 12 weeks were –3, 8, 18, 29, and 73%.

3.4. Serum triglycerides

After 6 weeks, subjects in the 1, 2 and 4 g/day krill oil groups revealed a 18.6 to 19.9 mg/dL decrease in fasting serum TG levels, whereas the 0.5 g/day group showed a 13.1 mg/dL decrease, when compared to baseline (Table 4). However, a significant change in TG levels was lost at 12 weeks in all groups. Still, after 12 weeks of supplementation, subjects receiving krill oil demonstrated a 10.2% decrease in fasting serum TG values, when assessed by a pooled group- and time-independent approach that included all the measurements after 6 and 12 weeks in the 0.5, 1, 2 and 4 g/day krill oil groups compared to placebo (Fig. 2). The changes (%) from baseline in TG levels amongst subjects supplemented with krill oil were significant relative to the (%) change from baseline in TGs in the placebo group ($p = 0.0389$). The change from baseline in fasting TGs was 3.9% in the placebo group and –6.3% in the krill oil group.

3.5. Serum lipoproteins

Total cholesterol (Fig. 3), LDL-C (Fig. 4), and HDL-C (Fig. 5) at 6 weeks and at 12 weeks remained unchanged relative to baseline in the placebo and krill oil groups.

4. Discussion

In order to accurately assess the efficacy of krill oil supplementation in lowering fasting levels of TGs, the TG levels have to be reliable. Studies wherein the primary outcome variable is fasting TG level are challenging for several reasons. Serum TG levels are known to show day-to-day biological variations within individuals that can be as high as 25% in healthy fasted subjects when measured 2.5 months apart [24]. Hypertriglyceridemic individuals can have even greater fluctuations in fasting TG levels. Other reasons for intra-individual variability in TG measures can be associated with the preparation, processing, storage, and analysis of blood samples. Despite attempts to minimize variability during sample collection, storage, shipment, and measurement, the individual biological fluctuations in fasting TGs were large, thereby resulting in a much higher intra-individual variation than accounted for in the power calculation (Supplementary Fig. 1). Multiple TG measurements at the individual visits, higher subject numbers or less dose groups should be considered in future studies. In order to circumvent these limitations, an explorative data analysis approach was chosen to increase the statistical power of the study. Hence, the mean of 6 and 12 weeks treatment TG measurements of the four krill oil groups were pooled in a group- and time-independent manner.

Across the 4 krill oil groups, the mean intake of krill oil was 1.875 g/day, and the associated intake of EPA and DHA was calculated to be 385 mg/day. This theoretical intake of EPA and DHA resulted in a 6.3% reduction from baseline in fasting TGs and a 10.2% placebo-adjusted reduction from baseline in fasting TGs. The efficacy of krill oil in reducing fasting serum TG levels has been reported in other studies; however, the doses of krill oil administered were larger than what was administered in the current study. Ulven et al. demonstrated that a daily dose of 2 g krill oil lowered fasting TGs in participants with borderline high and high TG levels over a 7-week period [25]. Krill oil has also been found to be effective in hyperlipidemic patients without exclusion of lipid-lowering medication by significantly reducing total cholesterol, LDL-C, and TG, and by increasing HDL-C levels after 3 months of supplementation; moreover, krill oil appeared more effective than fish oil in reducing glucose, TG, and LDL-C levels [26]. The

Table 4 – Triglyceride levels (mg/dL) and change from baseline of subjects in the placebo and krill oil supplementation after 6 and 12 weeks

	Placebo	0.5 g krill oil	1 g krill oil	2 g krill oil	4 g krill oil
Baseline	236.5 ± 80.9	230.1 ± 71.4	238.9 ± 69.8	237.5 ± 73.1	220.2 ± 63
Change after 6 weeks	12.0 ± 10.7	–13.1 ± 10.8	–19.9 ± 10.8*	–19.9 ± 10.8*	–18.6 ± 10.3*
Change after 12 weeks	6.4 ± 12.7	–23.0 ± 12.7	–9.1 ± 12.8	–16.1 ± 12.7	2.1 ± 12.1

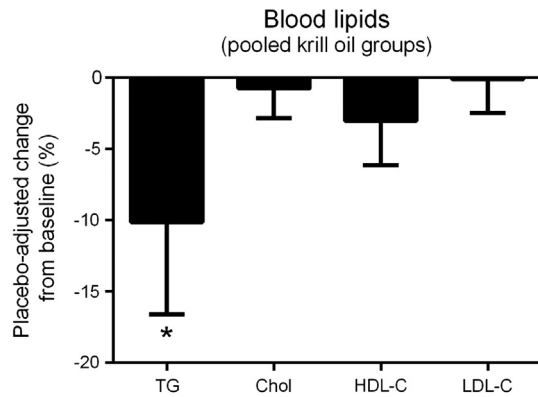


Fig. 2 – Placebo-adjusted changes (%) from baseline of fasted triglyceride (TG), total cholesterol (Chol), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations after 12 weeks of krill oil supplementation. All four krill oil group measurements were pooled and the mean compared to the mean of the placebo group. Significant changes are highlighted by (*), $p < 0.05$.

study, however, lacked information about the nature of the placebo and, more importantly, information about the baseline characteristics of the groups, particularly with respect to medication use (i.e. lipid-lowering drugs). Very recently, a pilot study demonstrated that daily supplementation of 4 g krill powder (containing 60% krill oil) over 24 weeks showed a significant TG-lowering effect in obese subjects [27]. The study product, as well as the length of the study, is not directly comparable with the current study, but the findings support the hypothesis that krill oil has beneficial effects in subjects with elevated levels of serum TGs.

The results of our study lend support to the suggestion made by Musa-Veloso and colleagues that reductions in fasting TG levels are possible with EPA and DHA intakes that are less than the 2 g/day dose suggested by the EFSA NDA panel. According to the equation of the first-order elimination function presented by Musa-Veloso and colleagues, an intake

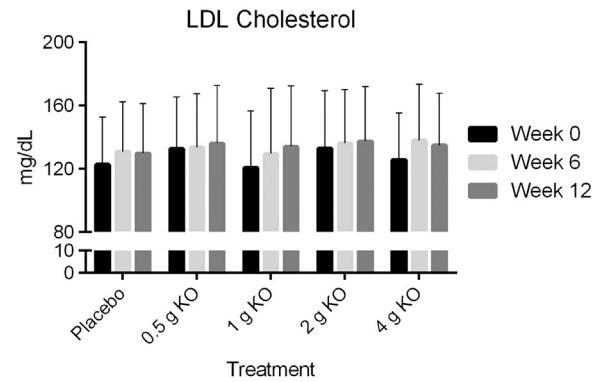


Fig. 4 – Comparison of mean serum values of LDL-C (low-density lipoprotein cholesterol) in the placebo or 0.5, 1, 2 and 4 g krill oil treatment groups after 0, 6 and 12 weeks of supplementation. Bars represent means \pm SD. No significant changes ($p < 0.05$) were detected.

of 385 mg/day of EPA and DHA is estimated to result in a placebo-adjusted reduction from baseline in fasting TGs of approximately 5.2% (Fig. 6). This estimated reduction underestimates the theoretical pooled TG reduction in our study of 10.2%. The reason for the higher-than-predicted reduction in fasting TGs in our study is not clear. It may be that the first-order elimination function used by Musa-Veloso et al. underestimates reductions in TGs at lower intakes of EPA and DHA; indeed, if the dose-response equation by Ryan et al. [6] is used, which was linear as opposed to non-linear, but which did not correct for changes in TGs observed in the placebo group, the predicted reduction in fasting serum TGs at an EPA and DHA intake of 385 mg/day is 12.4%. Although the dose-response assessment undertaken by Ryan et al. included only studies in which algal sources of DHA were administered, EPA and DHA are generally similarly efficacious in reducing fasting serum TGs, although DHA (but not EPA) tends to cause slight increases in LDL-C [7,8]. Qualitatively, it appears from the data points presented in Fig. 1 of the study by Ryan et al. [6] that the

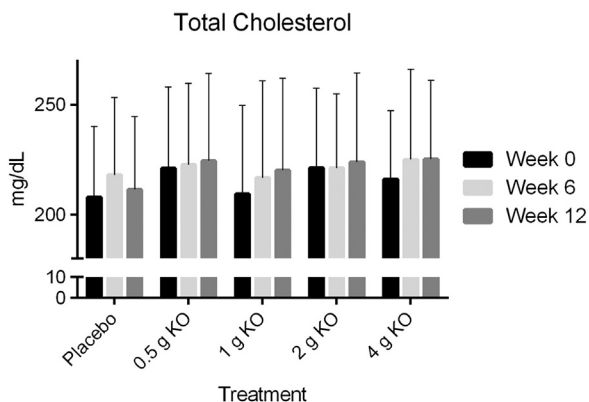


Fig. 3 – Comparison of serum total cholesterol in the placebo or 0.5, 1, 2 and 4 g krill oil treatment groups after 0, 6 and 12 weeks of supplementation. Bars represent means \pm SD. No significant changes ($p < 0.05$) were detected.

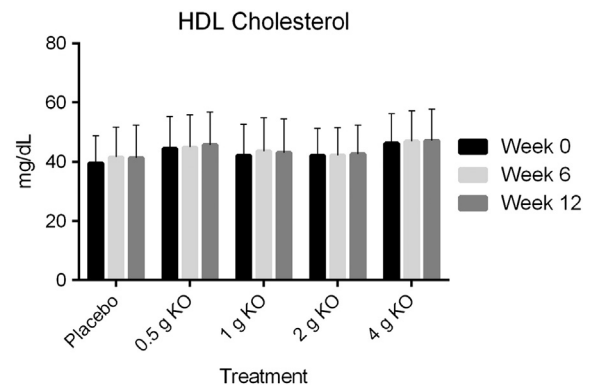


Fig. 5 – Comparison of mean serum values of HDL-C (high-density lipoprotein cholesterol) in placebo, 0.5, 1, 2 and 4 g krill oil treatment groups. Bars represent means \pm SD. No significant changes ($p < 0.05$) were detected after 0, 6 and 12 weeks of supplementation.

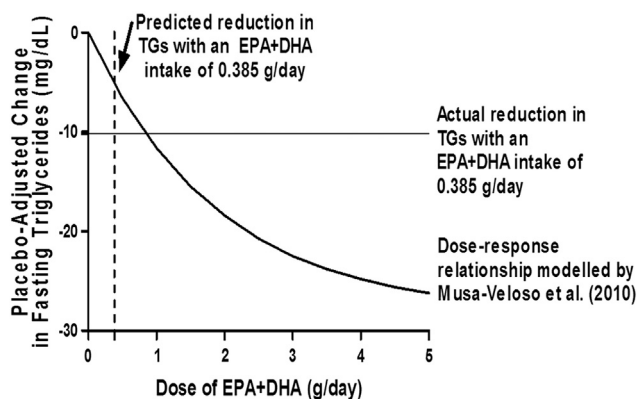


Fig. 6 – TG reductions predicted by the first-order elimination function modeled by Musa-Veloso et al. The placebo-adjusted reduction in fasting TG with the consumption of 0.385 g/day of EPA and DHA from krill oil was 10.2%, a value less than the 5.2% reduction predicted by the first-order elimination function modeled by Musa-Veloso et al. [5].

dose–response relationship is non-linear as opposed to linear. This observation is supported by the y-intercept of the equation of the line, which is -11.3% . Likely, the predicted reduction from baseline in fasting TGs is underestimated by the model generated by Musa-Veloso et al. but overestimated by the model generated by Ryan et al. [6].

Alternatively, the higher-than-expected reduction in fasting TGs in our study may be due to the unique compositional qualities of krill oil over other oils of marine origin, namely the fact that krill oil is rich in PLs. This structural difference may impact tissue uptake; indeed, it has been demonstrated that PLs were a more efficient delivery form of n-3 LCPUFAs than TGs [13,15,21]. The presence of PLs in krill oil [28] might be of importance not only as a vehicle for transporting EPA and DHA to tissues, but in lowering serum and liver cholesterol and TG levels, whilst increasing HDL-C [29]. PLs might exert these benefits by affecting biliary cholesterol excretion, intestinal cholesterol absorption and gene expression for lipoprotein metabolism. Some studies have demonstrated that PLs containing n-3 PUFAs have more potent effects on liver and blood plasma lipid levels, compared to PLs without n-3 PUFAs [30,31]. A speculative explanation for the observed increase in serum TG levels after 12 weeks in comparison to 6 weeks of krill oil supplementation could be a beneficial effect of krill oil on hepatic TG levels. Release of hepatic TG might take more than 6 weeks to establish itself and could explain the observed increase in serum TG levels after 12 weeks of supplementation. This potential beneficial effect of krill oil on the liver in addition to a high variation in TG measurements could have caused the loss of significance in serum TG reduction at 12 weeks in particular in the 4 g krill oil group.

The reliability of plasma cholesterol measurements is much lower than for TG measurements [24]. It was therefore possible to compare individual treatment groups for changes in cholesterol levels at weeks 6 and 12. In our study, no significant effects of krill oil treatment on serum HDL-C and LDL-C concentration could be observed at any time point. The EPA to DHA ratio of 2:1 in krill oil might help to prevent an increase

in LDL-C that has been observed with fish oil intake or the intake of n-3 LCPUFA preparations containing predominantly DHA [32,33].

Another suggested risk factor for CVD is the omega-3 index that gives the percentage of EPA and DHA in total fatty acids in red blood cells [34]. Red blood cell omega-3 fatty acids are highly correlated with their corresponding arterial fatty acids [35].

In this study, the omega-3 index was significantly increased at both time-points with all krill oil doses given and confirmed regular study product intake. Furthermore, approximately 2/3 of the omega-3 index increase during the study period was already seen after the first 6 weeks.

Noteworthy, the omega-3 index went from 3.7 to 6.3% at 4 g daily krill oil intake. Similar changes were associated with decreased risk for sudden cardiac death in a prospective cohort study by about 80% [36] and by a 90% reduction for risk of primary cardiac arrest in a case–control study [37].

In conclusion, the hypothesis could be confirmed and the combination of n-3 PUFA and PLs in krill oil has shown to be a safe and promising intervention with regards to reducing fasting serum TG levels and increasing omega-3 index, while not increasing LDL-C or total cholesterol. Krill oil in combination with lifestyle changes that include diet and exercise may therefore significantly reduce one's risk for CVD morbidity and mortality. However, due to the individual fluctuations of TG concentrations measured, a potential biasing effect of TG release from presumably fatty liver with time or other reasons, a new study with more individual measurements per treatment group and preferentially over a longer study period would help to clarify the shortcomings of this study.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nutres.2013.12.003>.

Acknowledgments

We are very grateful for comments on the protocol from Intertek Cantox (Canada) and on the manuscript from the Aker BioMarine science board members. Thanks to Laura Stibich for editing the final manuscript. The study was partly funded with a grant from the Norwegian Research Council (Grant nr 199360) and Norwegian SkatteFUNN (Grant nr 223281).

REFERENCES

- [1] Assmann G, Schulte H. Role of triglycerides in coronary artery disease: lessons from the Prospective Cardiovascular Munster Study. *Am J Cardiol* 1992;70:10H–3H.
- [2] Assmann G, Schulte H, Cullen P. New and classical risk factors—the Munster heart study (PROCAM). *Eur J Med Res* 1997;2:237–42.
- [3] Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S, Ozawa Y, Tsubota K, et al. Inhibition of choroidal neovascularization with an anti-inflammatory carotenoid astaxanthin. *Invest Ophthalmol Vis Sci* 2008;49:1679–85.
- [4] Shearer GC, Savinova OV, Harris WS. Fish oil – how does it reduce plasma triglycerides? *Biochim Biophys Acta* 1821;2012:843–51.
- [5] Musa-Veloso K, Binns MA, Kocenas AC, Poon T, Elliot JA, Rice H, et al. Long-chain omega-3 fatty acids eicosapentaenoic

- acid and docosahexaenoic acid dose-dependently reduce fasting serum triglycerides. *Nutr Rev* 2010;68:155–67.
- [6] Ryan AS, Keske MA, Hoffman JP, Nelson EB. Clinical overview of algal-docosahexaenoic acid: effects on triglyceride levels and other cardiovascular risk factors. *Am J Ther* 2009;16:183–92.
 - [7] Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr* 2012;142:614S–25S.
 - [8] Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. *Curr Atheroscler Rep* 2011;13:474–83.
 - [9] Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* 2000;71:1085–94.
 - [10] Araujo P, Zhu H, Breivik JF, Hjelle JI, Zeng Y. Determination and Structural Elucidation of Triacylglycerols in Krill Oil by Chromatographic Techniques. *Lipids Nov* 5 2013, E-pub ahead of print.
 - [11] Schuchardt JP, Schneider I, Meyer H, Neubronner J, von Schacky C, Hahn A. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations – a comparative bioavailability study of fish oil vs. krill oil. *Lipids Health Dis* 2011;10:145.
 - [12] Batetta B, Griinari M, Carta G, Murru E, Ligresti A, Cordeddu L, et al. Endocannabinoids may mediate the ability of (n-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker rats. *J Nutr* 2009;139:1495–501.
 - [13] Graf BA, Duchateau GS, Patterson AB, Mitchell ES, van Bruggen P, Koek JH, et al. Age dependent incorporation of 14C-DHA into rat brain and body tissues after dosing various 14C-DHA-esters. *Prostaglandins Leukot Essent Fatty Acids* 2010;83:89–96.
 - [14] Lagarde M, Bernoud N, Brossard N, Lemaitre-Delaunay D, Thies F, Croset M, et al. Lysophosphatidylcholine as a preferred carrier form of docosahexaenoic acid to the brain. *J Mol Neurosci* 2001;16:201–4.
 - [15] Wijendran V, Huang MC, Diao GY, Boehm G, Nathanielsz PW, Brenna JT. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. *Pediatr Res* 2002;51:265–72.
 - [16] Grimsgaard S, Bonna KH, Hansen JB, Nordoy A. Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am J Clin Nutr* 1997;66:649–59.
 - [17] Burri L, Berge K, Wibrand K, Berge RK, Barger JL. Differential effects of krill oil and fish oil on the hepatic transcriptome in mice. *Front Nutrigenomics* 2011;2:1–8.
 - [18] Ferramosca A, Conte A, Burri L, Berge K, De Nuccio F, Giudetti AM, et al. A krill oil supplemented diet suppresses hepatic steatosis in high-fat fed rats. *PLoS one* 2012;7:e38797.
 - [19] Ferramosca A, Conte L, Zara V. A krill oil supplemented diet reduces the activities of the mitochondrial tricarboxylate carrier and of the cytosolic lipogenic enzymes in rats. *J Anim Physiol Anim Nutr* 2011;1–12.
 - [20] Zhu JJ, Shi JH, Qian WB, Cai ZZ, Li D. Effects of krill oil on serum lipids of hyperlipidemic rats and human SW480 cells. *Lipids Health Dis* 2008;7:30.
 - [21] Di Marzo V, Griinari M, Carta G, Murru E, Ligresti A, Cordeddu L, et al. Dietary krill oil increases docosahexaenoic acid and reduces 2-arachidonoylglycerol but not N-acyl ethanolamine levels in the brain of obese Zucker rats. *Int Dairy J* 2010;20:231–5.
 - [22] Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med* 2004;39:212–20.
 - [23] Neubronner J, Schuchardt JP, Kressel G, Merkel M, von Schacky C, Hahn A. Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur J Clin Nutr* 2011;65:247–54.
 - [24] Jacobs Jr DR, Barrett-Connor E. Retest reliability of plasma cholesterol and triglyceride. The Lipid Research Clinics Prevalence Study. *Am J Epidemiol* 1982;116:878–85.
 - [25] Ulven SM, Kirkhus B, Lamglait A, Basu S, Elind E, Haider T, et al. Metabolic effects of krill oil are essentially similar to those of fish oil but at lower dose of EPA and DHA, in healthy volunteers. *Lipids* 2011;46:37–46.
 - [26] Bunea R, El Farrah K, Deutsch L. Evaluation of the effects of Neptune Krill Oil on the clinical course of hyperlipidemia. *Altern Med Rev* 2004;9:420–8.
 - [27] Berge K, Piscitelli F, Hoem N, Silvestri C, Meyer I, Banni S, et al. Chronic treatment with krill powder reduces plasma triglyceride and anandamide levels in mildly obese men. *Lipids Health Dis* 2013;12:78.
 - [28] Winther B, Hoem N, Berge K, Reubsæet L. Elucidation of phosphatidylcholine composition in krill oil extracted from *Euphausia superba*. *Lipids* 2011;46:25–36.
 - [29] Sahebkar A. Fat Lowers Fat: Purified Phospholipids as Emerging Therapies for Dyslipidemia. *Biochim Biophys Acta* 1831;2013:887–93.
 - [30] Dasgupta S, Bhattacharyya DK. Dietary effect of eicosapentaenoic acid (EPA) containing soyphospholipid. *J Oleo Sci* 2007;56:563–8.
 - [31] Shirouchi B, Nagao K, Inoue N, Ohkubo T, Hibino H, Yanagita T. Effect of dietary omega-3 phosphatidylcholine on obesity-related disorders in obese Otsuka Long-Evans Tokushima fatty rats. *J Agric Food Chem* 2007;55:7170–6.
 - [32] Bernstein AM, Ding EL, Willett WC, Rimm EB. A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases HDL-cholesterol and LDL-cholesterol in persons without coronary heart disease. *J Nutr* 2012;142:99–104.
 - [33] Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J Lipid Res* 1989;30:785–807.
 - [34] Harris WS. The omega-3 index: from biomarker to risk marker to risk factor. *Curr Atheroscler Rep* 2009;11:411–7.
 - [35] Metcalf RG, Cleland LG, Gibson RA, Roberts-Thomson KC, Edwards JR, Sanders P, et al. Relation between blood and atrial fatty acids in patients undergoing cardiac bypass surgery. *Am J Clin Nutr* 2010;91:528–34.
 - [36] Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113–8.
 - [37] Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995;274:1363–7.