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

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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.

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.

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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^{TM} 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.