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Clinical safety evaluation of marine oil derived from *Calanus finmarchicus*

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ABSTRACT

Marine oils are rich in polyunsaturated fatty acids (PUFAs), including docosahexaenoic and eicosapentaenoic acid. These PUFAs are associated with health benefits and additional sustainable sources of marine oils are desirable. One of the source organisms is *Calanus finmarchicus*, a copepod endemic to the North Atlantic. PUFAs in the lipid fraction of this organism are largely in the form of wax esters. To assess the safety of these wax esters as a source of PUFAs, a randomized, double-blinded, placebo-controlled clinical trial was conducted whereby 64 subjects consumed 2 g Calanus oil in capsule form daily for a period of one year. A group of 53 subjects consumed placebo capsules. At baseline, 6-, and 12-months, series of evaluations were conducted, including: vital signs, clinical chemistry and hematological evaluations, and adverse event reporting. Food intake and physical exercise were controlled by means of a questionnaire. There were no effects on Calanus oil treatment on any of the safety parameters measured. A slight increase in the incidence of eczema was reported in the Calanus oil group, but the response was minor in nature, not statistically significant after controlling for multiple comparisons, and could not be attributed to treatment.

1. Introduction

Over the past several decades, a number of scientific studies have documented the favorable nutritional and health benefits associated with the consumption of fish and other marine-derived oils. Marine oils are rich in polyunsaturated fatty acids (PUFAs), and particularly the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These fatty acids are thought to contribute substantively to the health benefits of seafood consumption (Deckelbaum et al., 2008). Heath benefits of DHA/EPA consumption include reduction of serum triglycerides in hypertriglycemic subjects (Bunea et al., 2004; McKenney and Sica, 2007; Berge et al., 2014; Roth, 2015), decreased mortality due to coronary heart disease/dysfunction (EFSA, 2010; Mozaffarian and Wu, 2011; Kimmig and Karalis, 2013; Pase et al., 2015), and reduction in chronic inflammation (Banni et al., 2011; Ulven et al., 2011; Ma et al., 2012; Kwantes and Grundmann, 2015; Mocellin et al., 2016).

The PUFAs, including DHA and EPA, are present in the lipids of marine oils in several forms. In fish oils, EPA and DHA are generally present as triglycerides whereby the fatty acids are attached to a glycerol backbone. These triglycerides are produced by algae on which fish feed. DHA and EPA found in krill oil, krill being small crustaceans that live in the Antarctic Ocean (Kwantes and Grundmann, 2015), is incorporated into phospholipids, especially phosphatidylcholine (Schuchardt et al., 2011; Araujo et al., 2013). The third form in which DHA and EPA are incorporated into natural marine oils is wax esters. Wax esters are formed through the condensation of a fatty acid (e.g., EPA or DHA) with a fatty alcohol. Wax esters are found in herbivorous copepods that inhabit colder waters. These substances are thought to be formed in response to the need for long-term energy storage during the winter season (Sargent et al., 1977; Hagen and Auel, 2001). Wax esters form the largest lipid class present in most species of subarctic zooplankton (Hagen and Auel, 2001; Dalsgaard et al., 2003).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APO, apolipoprotein; AST, aspartate aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; EFSA, European Food Safety Authority; EPA, eicosapentaenoic acid; ESR, erythrocyte sedimentation rate; GGT, γ-glutamyl transferase; HACCP, hazard analysis and critical control point; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HS-CRP, high sensitivity C-reactive protein; PTH, parathyroid hormone; PUFAs, polyunsaturated fatty acids; T4, free thyroxine; TSH, thyroid stimulating hormone; WH, waist-hip.

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Given concerns about sustainable harvesting of marine organisms for oil production, and potential environmental toxin contamination of long-lived oily fish species, commercial interest in smaller organisms containing PUFAs in wax ester form has been increasing. One of these source organisms is *Calanus finmarchicus*, a copepod, or small shrimp-like creature, endemic to the North Atlantic. It has been reported to be the zooplankton species with the most biomass present in Nordic Seas (Bergvik et al., 2012) and North Atlantic in general (Bergvik et al., 2012). Calanus AS of Norway has developed a sustainable harvesting and oil production method that can produce marine oil from *Calanus finmarchicus* (Calanus® Oil) which is composed of approximately 90% wax ester fatty acids on a lipid basis. Of the total fatty acid content, 21.2% are omega-3 fatty acids, including ~6 and ~5% EPA and DHA, respectively. Marine oil from *Calanus finmarchicus* is intended as an alternative source of PUFAs, including DHA and EPA, to traditional sources from fish such as cod, herring, sardine, salmon, *etc*. It is intended for commercial development both as an ingredient to be added to food as well as a dietary supplement. Currently, Calanus oil from *Calanus finmarchicus* is indirectly consumed in the diet as it is a major contributor to the lipid stores in the muscles and fat deposits of many marine organisms, such as red fish (e.g. *Sebastes* sp., Atlantic herring and salmon), from which marine oils are traditionally derived (Yusuf and Webster, 2008; FAO, 2011).

Data from preclinical studies in mice exist to indicate potential efficacy of Calanus oil and safety in use (Eilertsen et al., 2012; Höper et al., 2013, 2014). These studies assessed the effect of Calanus oil supplementation on metabolic disorders in diet induced obese mice (Höper et al., 2013, 2014) and on atherosclerosis in apoplipoprotein E-deficient mice (Eilertsen et al., 2012). There was no indication of adverse effects of Calanus oil in these studies at doses of 1% in the diet over periods of 13–20 weeks (Eilertsen et al., 2012; Höper et al., 2013) or 1.5% in the diet over a period of 27 weeks (Höper et al., 2013).

While the investigational studies in mice do not indicate any adverse effects of Calanus oil at doses of up to 1.5% in the diet of mice (~2 g/kg body weight/day basis), concerns about the safety of high dietary exposures to components of marine oils, and of PUFAs in particular, have been raised by regulatory authorities (EFSA, 2012). Such safety concerns included increased bleeding time, platelet dysfunction, effects on glucose homeostasis, low-density lipoprotein (LDL)-cholesterol, lipid peroxidation and immune function. After a comprehensive review of the safety data, the European Food Safety Authority (EFSA, 2012) concluded that supplemental intakes of omega-3 PUFAs at up to 5 g/day do not appear to increase risk for the aforementioned endpoints. EFSA (2012) further concluded that supplemental intakes of EPA and DHA in combination at up to 5 g/day do not raise safety concerns for adults. While the EFSA (2012) opinion provides considerable assurance about the safety of Calanus oil, it was considered prudent to conduct a safety evaluation of Calanus oil in humans due to its potential use as a source of DHA and EPA. A study was previously conducted in 15 healthy subjects divided into 3 groups (5 subjects/group), each receiving 1, 2, or 4 g Calanus oil/day for 4 weeks, to determine the tolerability and safety of consumption of the marine oil. The doses used in this preliminary study are reflective of the expected intake level of the marine oil to be efficacious. Blood samples were taken at 0, 2, and 4 weeks for hematology and liver and kidney function analyses. No adverse changes in the study parameters were observed, and no adverse events were reported by any study subject. Based on the results of this study, it was determined that a long-term study be conducted using similar doses of Calanus oil. Thus, the current clinical study was conducted to evaluate the safety of consumption of Calanus oil[1] at a dose of 2 g/day over a period of 12 months in a healthy population.

2. Materials and methods

2.1. Study population and design

A randomized, double-blinded, placebo-controlled study was performed at the University Hospital of North Norway (Tromsø, Norway) between 2010 and 2013. The study protocol was approved by the Regional Ethics Committee at the University Hospital of North Norway. A signed informed consent statement was obtained from each subject at the first visit prior to any study procedures. Study subjects were recruited through advertisements in the local press and billboards at the University of Tromsø and the University Hospital of North Norway.

The number of subjects to be enrolled was based on a similar study performed by Svartberg et al. (2008), which indicated a need for 80 subjects to complete the study with an 80% chance of finding statistically significant ($p < 0.05$) changes of clinical importance. Based on previous comparable studies conducted at the University Hospital of North Norway, a drop-out rate of 30% was anticipated. Thus, when taken into account for subject dropouts, 120 subjects was determined to be an appropriate sample size.

The inclusion criteria included healthy male and female volunteers aged 20–65 years with a body mass index (BMI) between 25 and 35 kg/m^2. Individuals were excluded if they had a serious ongoing disease such as diabetes type 1 or 2, coronary infarction or stroke in the last 12 months, unstable angina, or were diagnosed with cancer in the last 5 years. Individuals were also excluded if they were pregnant, lactating or under 50 years of age without safe contraception (i.e., hormonal contraception or IUD); currently taking lipid lowering medication; participating in other clinical trials or organized fitness programs; had a fish or seafood allergy; or their systolic or diastolic blood pressure was above 170 mmHg or 105 mmHg, respectively; had a hemoglobin level below the reference range; serum creatinine level was greater than 110 μmol/L in males and 100 μmol/L in females; serum liver transaminases (AST and ALT) was greater than 3 times the upper reference range; or had abnormal blood tests. Randomization of the study subjects into the intervention group or the placebo group was performed by the University Hospital of North Norway clinical research unit and was stratified by gender.

Subjects were instructed to avoid marine oils, such as cod liver oil and omega-3 products, at the screening stage before inclusion into the study and throughout the duration of the study. Subjects were not given any other diet, lifestyle, medication, or exercise advice/instructions. Subjects continued on with their normal daily activities and routines. Confounding by diet and physical activity was controlled through use of the International Physical Activity Questionnaire and the Norwegian food frequency questionnaire (Andersen et al., 1999). Analysis of confounders was undertaken on the basis of 32 subjects in the Calanus oil treated group and 31 subjects in the control group. The first 32 subjects enrolled in each group did not fill in the questionnaire at the baseline measurement and hence were not included in the confounder analysis.

Subjects visited the study site for initial screening and collection of baseline information, and again at 6 months and 12 months. Subjects were contacted at 3 months and 9 months by telephone to check for compliance and for occurrence of any adverse events.

[1] Calanus oil refers in this study to the product Calanus® Oil produced and sold by Calanus AS.