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

Kurt S. Tande a, *, Trung D. Vo b, Barry S. Lynch b

a Calanus AS, Stakkevollveien 65, N-9010, Tromsø, Norway
b Intertek Scientific and Regulatory Consultancy, 2233 Argentia Road, Suite 201, Mississauga, Ontario, L5N 2X7, Canada

**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.

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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 hypertriglyceremic 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).
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; Høper et al., 2013, 2014). These studies assessed the effect of Calanus oil supplementation on metabolic disorders in diet induced obese mice (Høper et al., 2013, 2014) and on atherosclerosis in apolipoprotein 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; Høper et al., 2013) or 1.5% in the diet over a period of 27 weeks (Høper 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 oil1 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 Sverdrup 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/m². 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.
2.2. Study products

Calanus oil and olive oil were provided by Ayanda AS (Norway) as blister packs of 60 capsules each. The test materials were handled, prepared, and processed under a competent hazard analysis and critical control point (HACCP) and sanitary program consistently implemented and in accordance with 21 CFR 123. The Calanus oil contained approximately 85% wax ester with a sum of neutral lipids>90%. Subjects of the intervention group were instructed to consume two capsules of Calanus oil (500 mg per capsule) twice daily to provide a daily dose of 2 g. Olive oil was used as the placebo control. Compositional analysis indicated that the fatty acid content of the olive oil was primarily oleic acid (76.9%), palmitic acid (10.2%), and linoleic acid (7.7%). The placebo was colored with a blend of food-grade extracts of paprika and chlorophyll in an 8:1 ratio (Sensient Food Colors Germany GmbH). Subjects of the placebo group received identical capsules at similar daily doses as the intervention group.

2.3. Outcome measures

Compliance was assessed at the 6- and 12-month visits through the return of unused capsules. Compliance was calculated as the ratio between number of capsules actually used and the amount that should have been consumed.

Subjects were assessed at baseline (0), 6 and 12 months for anthropometric parameters such as BMI and waist-hip (WH) ratio. Blood pressure and pulse rate were also measured at each clinic visit. Adverse events were monitored by the study nurses at 6- and 12-month visits, and by phone contact at 3 and 9 months. Each incidence of an adverse event was tracked in a report prepared by the study nurse. All participants were asked to complete a questionnaire regarding gastrointestinal and joint-related events after 6 and 12 months. Blood samples were collected from fasted subjects at the initial visit and at 6 and 12 months. The following hematology and clinical chemistry parameters were analyzed: hemoglobin, erythrocyte sedimentation rate (ESR), C-peptide, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), creatinine, high sensitivity C-reactive protein (HS-CRP), thyroid stimulating hormone (TSH), free thyroxine (T4), parathyroid hormone (PTH), phosphate, magnesium, calcium, total cholesterol, fatty acids, MCP-1, LDL and high-density lipoprotein (HDL)-cholesterol, triglycerides, apolipoprotein (APO)-A1, APO-B, adiponectin, and hemoglobin A1c (HbA1c). Serum analyses were performed according to the methods as described by Grimmnes et al. (2011, 2012). Plasma MCP-1 and adiponectin were analyzed with the Bio-Plex™ Human Cytokine Assay. In addition to routine hematology and clinical chemistry parameters, a blood fatty acid profile, including levels of DHA and EPA, was performed on each subject at the 6- and 12-month visit. The fatty acids were analyzed by Vitas AS of Norway. At the 12-month visit, a glucose tolerance test was performed to measure glucose and insulin levels after a fast and 2 h after a 75 g glucose load.

2.4. Statistical analyses

Student’s t-test was performed to determine whether the measured clinical and laboratory parameters differed significantly between baseline and at 6 and 12 months for the continuous parametric variables (i.e., change in blood pressure, pulse, WH ratio, hemoglobin, ALP, phosphate, magnesium, calcium, free T4, PTH, total cholesterol, LDL-cholesterol, HDL-cholesterol, APO-A1, and HbA1c). The Mann-Whitney U test was used to determine changes in continuous non-parametric variables (i.e., change in BMI, ESR, triglycerides, aspartate aminotransferase, alanine transaminase, GGT, HS-CRP, APO-B, and TSH). The Chi-Square test was used to compare adverse events and clinical symptoms between the two study groups. Results were considered statistically significant if p < 0.05. The data are presented as mean ± SD.

3. Results

3.1. Evaluation of study subjects

A total of 127 subjects were enrolled in the study; 64 subjects (27 males and 37 females; age 50.7 ± 7.7 years) were randomized into the intervention group and 63 subjects (27 males and 36 females; age 49.0 ± 9.4 years) were included in the placebo group. At the 6-month visit, 52 subjects (31 females and 21 males) from the intervention group and 54 subjects from the placebo group were evaluated. Fifty (50) subjects from both groups attended the 12-month visit.

Twenty-seven subjects (14 treated and 13 controls) were not included at the 12-month evaluation. Of these, 10 subjects were excluded based on analysis of the initial blood sample collection, 1 subject was unable to be contacted by the hospital, and 16 subjects left the study due to lack of compliance, adverse effects, or no given explanation. In those subjects not completing the study, there were no differences between the treated and control groups with respect to clinical measures or adverse effects.

There were no statistically significant differences in the Calanus oil and control groups with respect to differences in food (calorie) intake or levels of physical activity.

3.2. Adverse events, clinical measures, and compliance

Overall, the compliance rate reported for both intervention and placebo groups was good (86–88%). After 6 months, four subjects (two in each of the Calanus oil and placebo groups) had a compliance ratio of less than 65%. After 12 months, one subject in the placebo and two subjects in the Calanus oil groups had compliance ratios of less than 65%.

No significant differences in BMI, systolic or diastolic blood pressure, pulse, or WH ratio were noted between the intervention and placebo groups at baseline or at the 6- or 12-month visit (Table 1). A total of 258 adverse events were reported, of which 130 events occurred in the intervention group and 128 events in the placebo group (Table 2). No significant differences were noted with respect to the number of adverse events between the two groups with the exception of skin-related events (i.e., eczema). A significant (p ≤ 0.05) difference was observed in the number of skin-related adverse events between the intervention and placebo groups; seven subjects in the treated group versus one subject in the placebo group. However, this significance was not observed when corrected for multiple testing. Moreover, in all cases the severity of the eczema-like condition was considered minimal.

3.3. Results of questionnaires

Study subjects were asked to complete a questionnaire at the 6- and 12-month visit regarding gastrointestinal and joint-related events (Table 3). There were no significant differences between the intervention group and placebo group regarding the results of the questionnaire.

3.4. Hematology and clinical chemistry parameters

Administration of Calanus oil at a dose of 2 g/day for 12 months did not result in changes in hematology or clinical chemistry parameters.
parameters that were significantly different from those observed in the placebo control group at any time point (Table 4). Likewise, there was no effect of treatment on the inflammatory marker MCP-1 (data not shown).

The levels of DHA and EPA in the blood were generally higher in the Calanus oil group over baseline values relative to the placebo controls. While there was no clear effect on blood EPA and DHA concentrations other fatty acids, such as gondoic acid and cetolecic acid, tended to be higher in the Calanus oil treated groups compared to the placebo controls.

3.5. Glucose tolerance test

The results of the glucose tolerance test are presented in Table 5. Following an oral glucose load (75 g), there were no statistically significant differences in the responses of the Calanus oil versus the placebo control group. Likewise, there was no statistical significant effect of treatment blood levels adiponectin, a hormone involved in glucose homeostasis and fatty acid metabolism (data not shown).

4. Discussion

Due to the increasing demand for long-chain PUFA-rich oils as dietary supplements and the need to identify alternative sources of marine oils as a result of current unsustainable practices, the use of Calanus oil is attractive from an ecological and commercial perspective. Comparatively, Calanus oil contains DHA and EPA in the wax ester form rather than the triglyceride, ethyl ester, or phospholipid form in traditional marine oils. Therefore, given the perspective. Comparatively, Calanus oil contains DHA and EPA in the wax ester form rather than the triglyceride, ethyl ester, or phospholipid form in traditional marine oils. Therefore, given the different ester form of PUFAs in Calanus oil, it was considered prudent to conduct a safety assessment of this oil in humans to address any potential adverse effects upon consumption of long-chain PUFAs from wax esters to ethyl esters.

Overall, consumption of Calanus oil at a dose of 2 g/day did not significantly affect any study parameter. No significant effects were observed on the measured hematological and clinical chemistry parameters. No statistically significant differences in the Calanus oil and control groups with respect to changes in food (calorie) intake or levels of physical activity were observed. However, it is acknowledged that self-reported food intake and energy expenditure by means of a questionnaire may not be reliable with respect to quantifying food intake and energy expenditure, as the actual values may be misreported (Lichtman et al., 1992). A total of 258 adverse events were reported throughout the study period. Generally, the number of adverse events did not differ between the intervention group and the placebo control group. A statistically significantly greater number of eczema-type events were reported in subjects of the intervention group compared to the subjects receiving the placebo. It should be noted that the statistical significance was not apparent when the data was corrected for multiple testing. Furthermore, the eczema was minor in nature and it is noteworthy that marine oils have been evaluated in clinical studies with respect to modulation of allergic responses (Furuhjelm et al., 2011; D’Vaz et al., 2012a; Palmer et al., 2012, 2013), including eczema (Heine and Tang, 2008; Furuhjelm et al., 2008; Bath-Hextall et al., 2012; D’Vaz et al. 2012b). Such studies have either indicated no effect or an attenuating, not exacerbating, effect of marine oil consumption. It should be noted that studies in adults that pertain to the immunomodulation effects of marine oils were not identified, and the majority of the identified studies were conducted following consumption in pregnant women during the gestation period through to lactation. While there was no clear efficacy in
these studies, there was no indication that marine oil treatment was associated with increased incidence and/or severity of allergy symptoms. As such, it can be considered that the relationship between the increase in eczema-type responses in the intervention group and treatment with Calanus oil is unknown.

Gastrointestinal-related effects, such as anal leakage, are characteristic of consumption of high doses of wax esters, specifically PUFAs EPA and DHA. No wax ester-specific digestive lipase have been identified in mammals; however, lipases and carboxyl esterases can hydrolyze wax esters at a slow rate into their fatty acid and fatty alcohol components, which are then readily absorbed into the intestinal epithelium (Hargrove et al., 2010; K€ohler et al., 2015; Yurko-Mauro et al., 2016).

It has been thought that there are significant differences in the rates of hydrolysis, and hence bioavailability, of PUFAs from tri- and diglycerides, phospholipids and ethyl esters, with higher bioavailability for triglycerides and phospholipids as compared to ethyl esters (Dyserberg et al., 2010; K€ohler et al., 2015; Yurko-Mauro et al., 2015).

While no published data exists for humans, bioavailability of PUFAs from wax esters has conventionally been thought to be low given symptoms of poor digestibility following high oral doses. However, the results of an in vitro study demonstrated that wax esters (C18:0 and C22:0) are susceptible to hydrolysis by pancreatic lipase following incubation in the presence of human pancreatic lipase and porcine pancreatic colipase (Gorreta et al., 2002). The majority of the wax ester (approximately 80%) was digested within 4 h, and completely hydrolyzed within 24 h. Studies with rats also indicate significant bioavailability of PUFAs in wax ester form
(Gorreta et al., 2002). When a single bolus dose of 5 g/kg body weight of n-3 PUFA in the form of a C22:0 wax ester preparation, fish oil (triglycerides), or ethyl esters was administered by gavage to CD-COBS rats, it was determined that the extent of absorption of wax esters was similar to the absorption of the ethyl ester (Gorreta et al., 2002). The results of this study indicate that incorporation of n-3 PUFAs from these two sources were comparable. In a subsequent study, the diets of rats were supplemented with n-3 PUFA in the form of a C22:0 wax ester preparation, fish oil (triglycerides), or ethyl esters at a concentration of 150 mg/animal/day for 4 weeks (Gorreta et al., 2002). At the end of the study period, the sum of n-3 PUFAs in plasma phospholipids, as well as the proportion of EPA, were comparable between the three groups and were significantly increased compared to the control group. As such, it was determined that n-3 fatty acids from wax esters were incorporated into plasma phospholipids to an extent comparable to ethyl esters and triglycerides in rats.

Initial research into the bioavailability of EPA and DHA from Calanus oil wax esters in humans indicates that PUFAs from the wax esters are at least as bioavailable (i.e., have similar or greater rates of hydrolysis of the ester bond to liberate the fatty acid) than the ethyl ester form of most commercially available dietary supplement products (Cook et al., in review). This finding is consistent with the lack of gastrointestinal side effects in the current study in which subjects consumed 2 g/day of Calanus oil.

In conclusion, the results of this clinical trial demonstrate that daily consumption of 2 g/day of Calanus oil for 1-year was safe in overweight, but otherwise healthy, adults, and may be a suitable source of DHA and EPA. Moreover, the results of this study are consistent with the conclusion of EFSA (2012) regarding the safety of dietary supplementation with EPA and DHA.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2016.05.030.

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