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NO EFFECT OF CALANUS OIL ON MAXIMAL OXYGEN UPTAKE IN HEALTHY PARTICIPANTS: A RANDOMIZED CONTROLLED STUDY

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ABSTRACT

We aimed to investigate the long-term effect of daily Calanus oil supplementation on maximal oxygen uptake (VO_{2max}) in healthy 30–50-year-old participants. The study was motivated by preclinical studies reporting increased VO_{2max} and metabolic health with omega-3 rich Calanus oil. In a double-blinded study, 71 participants were randomized to receive two $g \cdot day^{-1}$ of Calanus or placebo supplementation for a total of six months. The participants underwent exercise testing and clinical investigations at baseline, three months, and six months. Main study endpoint was change in VO_{2max} from baseline to six months. Fifty-eight participants completed the 6-month test and were included in the final data analysis [Age: Calanus, 39.7 (38.0-41.4) and placebo, 38.8 (36.8-40.9) years; BMI: Calanus, 24.8 (24.0-25.6) and placebo, 24.8 (23.7-25.8) $kg \cdot m^2$; VO_{2max}: Calanus, 50.4 (47.1-53.8) and placebo 50.2 (47.2-53.1) $ml \cdot kg^{-1} \cdot min^{-1}$]. There were no between-group differences at baseline, nor were there any between-group differences in absolute [Calanus, 3.74 (3.44-4.04) and placebo, 3.79 (3.44-4.14) $L \cdot min^{-1}$] or relative VO_{2max} [Calanus, 49.7 (46.2-53.2) and placebo, 49.5 (46.0-53.1) $ml \cdot kg^{-1} \cdot min^{-1}$] at six months (mean (95% CI)). There were no between groups change in clinical measures from baseline to three and six months. In conclusion, VO_{2max} was unaffected by six months of daily Calanus oil supplementation in healthy, physically fit, normal to overweight men and women between 30 and 50 years old.

Abstract word count: 217

INTRODUCTION

Maximal oxygen uptake (VO_{2max}) a robust measure of human endurance and metabolic capacity, defined as the highest oxygen uptake utilized during maximal intensity exercise with large muscle mass (Keren et al., 1980). VO_{2max} is documented to be the single best predictor of longevity and cardiovascular disease mortality, and systematic endurance exercise training increasing VO_{2max} has beneficial health effects (Blair et al., 1995; Blair et al., 1989; Gulati et al., 2003; Lee et al., 2011; Myers et al., 2015; Myers et al., 2002). The associations between dietary supplementation with omega-3 polysaturated fatty acids, exercise performance and VO_{2max} have been studied, but the results are conflicting (Da Boit et al., 2017; Macaluso et al., 2013; Zebrowska et al., 2015).

Calanus oil, which is extracted from the marine copepod *Calanus finmarchicus* (Melle et al., 2004), has a unique chemical composition consisting of phytosterol, antioxidants, and monounsaturated and polyunsaturated fatty acids. The fatty acids are bound to aliphatic long-chain monounsaturated fatty alcohols in the form of wax esters (Gasmi et al., 2020; Hoper et al., 2013; Pedersen et al., 2014), and conversion of the fatty alcohols to their corresponding monounsaturated fatty acids could boost the uptake of these specific fatty acids. In healthy adults, plasma EPA and DHA were increased 72 hours after 4 g of Calanus ingestion (Cook et al., 2016). Twelve weeks of dietary Calanus supplementation in combination with exercise training increased the omega-3-index from 6.07% to 7.37% and the level of EPA and DHA increased by 44% and 17% respectively, with no changes detected in the non-exercising control group also on Calanus supplementation (Wasserfurth, Nebl, Bosslau, et al., 2020).

In a preclinical study with high fat-fed mice, dietary supplementation for 27 weeks with wax esters from Calanus oil increased both VO_{2max} and attenuated glucose intolerance compared to that of obese control mice (Hoper et al., 2014). Otherwise, the study documented similar

metabolic effects as with crude oil supplementation (Hoper et al., 2013), i.e., attenuated obesity, inflammation, and glucose intolerance in high fat diet-induced overweight mice (Hoper et al., 2013). The mechanism behind the improved VO_{2max} in obese mice in response to dietary supplementation with Calanus oil-derived wax ester is unknown but improved cardiac energy metabolism and increased voluntary exercise have been suggested (Hoper et al., 2013). The rationale behind the clinical follow up of the preclinical findings in (Hoper et al., 2014) and the improvement in VO_{2max} after dietary Calanus oil ingestion is hypothesized to be through the unique effect alcohol esters have on fat metabolism. Previous studies have shown that the long-chain alcohol octacosanol improve energy mobilization in rats (Kato et al., 1995), most likely through an acceleration of lipid metabolism in skeletal muscles during exercise, thereby increasing endurance capacity (Kabir et al., 1994; Kim et al., 2003). To our knowledge, no studies has to date investigated if Calanus supplementation increase EPA or DHA levels within skeletal muscles. In line with this notion, dietary Calanus oil was shown to improve glucose oxidation and reduce the reliance of fat oxidation in hearts from obese mice, thereby preventing the overreliance of fatty acid oxidation and accumulation of lipids in the myocardium of obese species, and at the same time protect the hearts from ischemic stress (Jansen et al., 2019).

To our knowledge, no clinical studies have investigated the effect of daily long-term Calanus oil supplementation on VO_{2max} in healthy individuals. Therefore, the primary aim of this study was to investigate the long-term effect of daily Calanus oil supplementation on VO_{2max} in healthy, normal-weight to overweight participants. Our working hypothesis was that daily Calanus supplementation would give a clinical relevant increase in VO_{2max} (~ 3.5 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}) (Hoper et al., 2014). A hypothetical positive effect of Calanus oil on human VO_{2max} could potentially serve as a non-pharmacological lifestyle disease prevention and public health strategy.

METHODS

Study design

In a double-blinded randomized controlled study, supplementation of 2 g·day^{-1} of Calanus oil or placebo vegetable oil was given for a total of six months, with exercise testing and clinical investigations at baseline, three months, and six months. The primary study outcome was a change in VO_{2max} from baseline to six months. Secondary outcomes were changes in other measures of maximal performance, body composition, blood pressure, physical activity, and selected blood biomarkers measured from baseline to three and six months.

Participants

Seventy-one eligible volunteers were randomized 1:1 (no stratification) to receive either Calanus oil (n = 36) or placebo oil (n = 35) supplementation. The study flowchart is shown in Figure 1. In short, 105 volunteers responded to the public study announcements, and 81 participants were pre-screened using a standardized phone call and clinical screening.

Inclusion criteria for participation were healthy men and women between 30 and 50 years of age with a BMI between 18.5 and 29.9 kg·m^2. Exclusion criteria were any medical condition limiting VO_{2max} (i.e., COPD or asthma), a history of cardiovascular disease, any other serious medical condition (i.e., cancer), any medication affecting VO_{2max} (i.e., beta-blockers), pregnancy, participation in other clinical studies, shellfish allergy, contraindications of physical activity, systolic blood pressure (SBP) >170 mmHg, and diastolic blood pressure (DBP) >105 mmHg. The occurrence of adverse disease (i.e., cancer, stroke, myocardial infarction, or unstable angina) or pregnancy in participants during the study were predefined for exclusion during the intervention.

Ten of the potential volunteers that were pre-screened were not randomized, five due to no time for participation with the amount of time required in the study and five did not fulfill the inclusion criteria [low BMI (n = 1), young age (n = 2), heart disease (n = 1), and breastfeeding (n = 1)]. The Calanus safety study did not include breast feeding women, thereby the one screened breast feeding subject was not included in this study (Tande et al., 2016). Randomization was made after initial screening by research personnel not involved in testing or follow-up of the participants through a web-database at the unit for clinical research at the Norwegian University of Science and Technology

Participants were encouraged to continue their normal lifestyle and to continue with their normal exercise or physical activity routines throughout the study. No other diet, lifestyle, medication, or exercise advice was given. Participants self-reporting use of omega-3- or performance-enhancing supplementation (i.e., caffeine or energy drinks) (n = 7) were instructed to end the supplementation before inclusion and for the duration of the study.

Ethical approval

The study was conducted according to the Declaration of Helsinki and were approved by the regional ethical committee for medical research (REK#2015/2303). Written informed consent was obtained from all subjects. The study is registered in the ClinicalTrials.gov database (NCT02908828).

Intervention

Participants received pre-packed boxes of supplementation after completing the initial screening and clinical and physiological tests. Boxes were identically labeled (by Calanus

AS) and the liquid-filled vials were visually identical for Calanus and the vegetable oil (sun-flower oil with artificial red color) supplementation. The supplements were from the same production batch, and were provided by Calanus AS with a dosage guarantee from the manufacturer. The participants were instructed to ingest a daily dose of four capsules for a total of six months. This corresponded to two $g \cdot day^{-1}$ of Calanus- or vegetable oil. The dose of two $g \cdot day^{-1}$ of Calanus oil was chosen based on a previous safety study and preclinical studies that have shown aortic plaque regression and metabolic improvements (Eilertsen et al., 2012; Hoper et al., 2014; Tande et al., 2016).

Before the three and six month investigations, participants were asked to count the number of supplements in their possession. During the clinical assessments, participants were asked four standardized supplement compliance questions:

1. Do you take the supplementation as prescribed? (YES/NO);
2. Have you experienced any side effects from the supplementation? (YES/NO)
3. How many unused capsules do you have at the present time? (Number recorded)
4. Have you started with another type of dietary supplementation (YES/NO)

The answers were recorded in the web-CRF by the investigators.

The content of the Calanus oil is described in detail in Table 1 (Wasserfurth, Nebl, Bosslau, et al., 2020) and the detailed analysis of the sun flower oil can be found in (Štěpán et al., 2022). Calanus-oil is extracted from the North Atlantic zooplankton *Calanus finmarchicus and* contains a high quantity of the long-chain omega-3 acids, eicosatetraenoic acid (EPA), and docosahexaenoic acid (DHA). Most (80–90%) of the *Calanus finmarchicus* oil consists of fatty acids that are esterified to long-chain fatty alcohols, with a small additional number of phytosterols, antioxidants, glycerol, and free fatty acids. The carotenoid astaxanthin gives the Calanus oil a deep red color, and the amount of lipids and wax esters in the harvested *Calanus*

173 *finmarchicus* depends on the geographic latitude with the highest amounts found in arctic
174 species (Gasmi et al., 2020; Pedersen et al., 2014; Schots et al., 2020; Wasserfurth, Nebl,
175 Schuchardt, et al., 2020).

176 The study staff and participants were blinded for study group affiliation during data collection
177 and data analyses. Boxes were labeled with a numbered code for identification and
178 distribution to the participants by the person responsible for randomization. The safety of
179 Calanus oil supplementation has previously been documented in a clinical study (Tande et al.,
180 2016). Compliance to the supplementation was pre-specified to 70%, based on knowledge of
181 ~ 50% long term compliance to prescription medication (Jimmy et al., 2011) and ~70-80%
182 compliance to fish oil oral nutritional supplementation (Hubbard et al., 2012).

183

184 ***Test procedures and clinical investigation***

185 ***Maximal oxygen uptake***

186 After an initial moderate-intensity warm-up of 10-15 minutes of walking or jogging at
187 approximately 70% of maximal heart rate, VO_{2max} was measured through an incremental
188 treadmill test to exhaustion using an indirect breath-by-breath ergospirometry system
189 (Metalyzer 2 A, Cortex Biophysik GmbH., Germany) at the NeXt Move core facility for
190 exercise, movement, neurophysiology and elite sport science at NTNU – The Norwegian
191 University of Science and Technology. Calibration procedures included high precision gas
192 calibration (15.00 ± 0.04% O_2 and 5.00 ± 0.1% CO_2, Aga AS, Trondheim, Norway) and
193 inspiratory flowmeter calibration using a three (3) L volume syringe (Metalyzer 2 A, Cortex
194 Biophysik GmbH., Germany). The test was performed on a treadmill (Woodway USA Inc.,
195 Waukesha, WI, USA) as a running or walking test depending on the participants' fitness
196 levels. The workload was increased every minute until exhaustion, and the mean of the three

highest consecutive 10 seconds VO_2 measurements was used to determine VO_{2max}. During a running test, subjects would start walking or jogging at 6 km · hr^{-1} and 4% treadmill inclination, and speed or grade would be increased approximately every 1-1:30 minutes until the subjects reached exhaustion. A plateau in oxygen uptake, despite increased workload, and a respiratory exchange ratio ≥1.05 were used as criteria for the determination of VO_{2max}. Maximal heart rate (HR_{max}) was measured by a heart rate monitor (Polar RS400, Polar Electro Oy, Kempele, Finland), and the BORG 6-20 scale was used to assess self-perceived effort (Borg et al., 2006). HR_{max} was defined as the highest recorded value during the termination of the test. Maximal oxygen pulse ($mL \cdot beat^{-1}$) was calculated as VO_{2max} ($mL \cdot min^{-1}$) divided by HR_{max} ($beats \cdot min^{-1}$) (Aspenes et al., 2011). Heart rate recovery (HRR) was calculated by subtracting the heart rate one (1) min after completion of the test from HR_{max}. Participants were standing still at the treadmill during the first minute after completing the test.

Blood sampling and fatty acid composition of red blood cell membranes

Venous serum and ethylenediaminetetraacetic acid (EDTA) samples were collected after an overnight fast (≥12 h). Subjects were asked to avoid food, alcohol, tobacco and only drink water during the fasting period. Subjects self-reported fasting hours when reporting for blood sampling. No other control of fasting or hydration status were made. EDTA samples were kept on ice, centrifuged for 10 min at 4°C and 2200 G, and aliquoted in cryotubes and frozen at -80°C for later analyses.

The fatty acid composition of red blood cell (RBC) membranes was determined after methylation of the fatty acids (Jansen et al., 2019). Firstly, aliquots of RBC were taken from the cell pellets and frozen at -80°C. Upon analysis, cells were thawed in the fridge overnight and washed with cold phosphate-buffered saline (PBS). Washed RBC membranes were vortexed and pipetted into new vials and methylated with 3N methanolic hydrochloric acid.

The fatty acid methyl esters (FAME) were extracted with hexane, and the extracts neutralized with 3N potassium hydroxide in water. After mixing and centrifuging the hexane phase was injected into the gas chromatograph – flame ionizing detector (GC-FID). Analysis was performed on a 8890 GC with a split/splitless injector, a 7693A automatic liquid sampler, and flame ionization detector from Agilent Technologies (Palo Alto, CA, USA). Separations were performed on a TR-FAME (30 m × 0.25 mm i.d. × 0.25 µm film thickness) column from Thermo Fisher Scientific (Waltham, MA, USA). The content of the individual fatty acids in the samples was expressed in percent of total fatty acid content. Thus, omega-3 index is defined as the percentage of omega-3 fatty acids (including EPA and DHA) of total fatty acids in red blood cells.

Clinical assessments

Body composition and body mass were assessed by bioelectrical impedance analysis (InBody 720, Biospace Co, Ltd, Seoul, Korea). Resting systolic and diastolic blood pressure was measured using plethysmography (Casmed 740, CAS Medical Systems Inc., USA) with the cuff on the right arm adjusted according to the arm circumference, and after the participant had been sitting relaxed for five minutes. SBP and DBP were measured three times with 1-minute intervals, and the mean of the latter two was used in the analyses. Self-reported medical history, medication and lifestyle were recorded by the study investigators.

Physical activity

Self-reported weekly physical activity was recorded at baseline, three months, and six months using the short form of the international questionnaire for physical activity (IPAQ 7-days) (Kurtze et al., 2007). Duration and intensity of physical activity within the last seven days were recorded, and total physical activity in metabolic equivalents (MET) minutes per week

was calculated according to the IPAQ scoring protocol. A cut-off at 150 minutes per week of structured physical activity was used to define participants as physically active (≥150 minutes/week) or inactive (≤150 minutes/week). Subjects were instructed to continue their regular physical activity lifestyle during the study.

Adverse effects

Participants were asked if they had experienced any discomfort or adverse effects of supplementation at the 3- and 6-month clinical investigations. They were also instructed to contact the study coordinator by phone if any serious events occurred in-between visits.

Statistical analysis

Variables are presented as means or median with 95% confidence interval (CI). Normality was tested with normality curves, error bars, and Q-Q plots, and homogeneity of variances was checked with Levene's test. The between-group effect was tested using ANCOVA with baseline as covariate, and the two-sided level of significance was set to $p < 0.05$. Non-normally distributed variables were analyzed with non-parametric tests (Mann-Whitney-U test). A sample size of 32 participants in each group was estimated based on improvement in VO_{2max} from 32 ± 5 to 35.5 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$ (80% power and $p = 0.05$). All statistical analyses were performed using IBM SPSS Statistics software program version 27 (SPSS Inc. Chicago, IL., USA). Data analyses includes all participants completing the six months investigation.

RESULTS

Patient demographics

The baseline patient demographics are described in Table 2. There was no change in demographic variables during the intervention period. Self-reported prescription medication in nine of the participants included: common allergies (Calanus, n = 1; placebo, n = 2), psoriasis (placebo, n = 1), insomnia (placebo, n = 1), asthma (Calanus, n = 3), and hormone replacement therapy (placebo, n = 1).

Intervention

The inclusion and intervention ran from October 2016 to June 2017. Fifty-eight participants completed all three test time points, with fifty-one participants above the pre-specified 70% supplementation compliance. Thirteen participants (placebo, n = 7; Calanus, n = 6) were lost to follow-up: no reason provided (placebo, n = 3), lack of time (Calanus, n = 2; placebo, n = 1), pregnancy (Calanus, n = 1), high blood pressure (Calanus, n = 1), moved away (placebo, n = 1), unable to contact for re-testing (Calanus, n = 2; placebo, n = 2). A mean (min-max) of 600 (360-711) (~83% compliance) and 581 (270-692) (~81% compliance) (capsules were consumed in the Calanus and placebo groups, respectively. We lack information of whether the participants consumed less than the prescribed capsules on several days, or if they missed one or several days with supplementation, or when this occurred during the study timeline. Data analyses of participants with above 70% compliance, or of all participants with six months tests gave equal results. Thus, data from all participants with six months investigations is presented. Individual self-reported supplement compliance is displayed in Figure 2.

Maximal oxygen uptake

The VO_{2max} test results are presented in Figure 3 and Table 3. VO_{2max} was unchanged from baseline to six months. None of the other cardiopulmonary exercise test parameters; VE_{max}, RER, VE/VCO_2, O_2-puls, HR_{max}, HRR, treadmill speed and inclination or the Borg scale changed over the course of the study (Table 3). According to the prespecified criteria, all participants reached VO_{2max} at the cardiopulmonary exercise tests.

Anthropometric and clinical data

Anthropometric and clinical data are shown in Table 4, and the fatty acid composition of red blood cell membranes and the omega-3 index in table 5. There were no significant changes in systolic or diastolic blood pressure, resting heart rate, BMI, weight, fat mass, or muscle mass from baseline to six months (Table 4). The omega-3 index was relatively high (around 8%) at baseline and did not change significantly over the 6-month period ($p < 0.07$). Also, the fatty acid composition of RBC membranes was unchanged at six months (Table 5).

Physical activity

Self-reported vigorous physical activity, moderate physical activity, and total weekly MET-minutes were unchanged from baseline to 6-month. Self-reported weekly walking time was decreased by 97 min $\cdot$ $week^{-1}$ in the placebo group from baseline to 6 months, a significant decrease from baseline compared to in the Calanus group at six months ($p = 0.042$) (Table 6).

Safety and adverse effects

Three participants self-reported adverse events, including one subject with hives and a rash in the face (placebo) and two subjects with self-perceived atrial fibrillation (AF) and persisted elevated heart rate during exercise training (placebo and Calanus). All subjects were asked to contact their primary physician for follow-up and completed the study without further events. In the two subjects with self-reported AF, ECG evaluation during the 3-months CPET provided no indication of arrhythmias during exercise testing.

Nine participants reported discomforts. This included an upset stomach (placebo, n = 1; Calanus, n = 3), heartburn (Calanus, n = 2), and a fishy taste (Calanus, n = 2). One participant in the Calanus oil group suffering from severe insomnia reported normalization of the sleeping behavior after only one week of supplementation.

DISCUSSION

The main study finding was that VO_{2max} was unaffected by six months of Calanus oil supplementation in healthy middle-aged men and women. Our study supports the few previous studies demonstrating minor effects of other forms of omega-3 long-chain fatty acid rich supplementation on physical fitness and exercise performance in healthy humans (Bortolotti et al., 2007; Buckley et al., 2009; Da Boit et al., 2017; Da Boit et al., 2015; Peoples et al., 2008). Omega-3 polysaturated fatty acids supplementation is previously demonstrated to have no effect on neither resting metabolic rate (Noreen et al., 2010), submaximal energy expenditure (Bortolotti et al., 2007), or VO_{2max} (Bortolotti et al., 2007). Our findings are also supported by a recent study showing no effect of 16 weeks of combined Calanus oil supplementation and exercise training on VO_{2max} compared to placebo supplementation and exercise training in older women (Dadova et al., 2020; Štěpán et al., 2022).

We were unable to replicate preclinical findings of improved VO_{2max} after Calanus oil supplementation in diet-induced obese mice (Hoper et al., 2014). This could be due to physiological differences between species, differences in baseline levels of omega-3 fatty acids (Stark et al., 2016; Superko et al., 2013), or the fact that the preclinical study included high-fat feeding to induce obesity (Hoper et al., 2014), while no dietary or other lifestyle interventions beyond Calanus oil or placebo supplementation were introduced in our study. In addition, variability between humans and rodents in absorption of omega-3-fatty acids from dietary intake and oral supplementation, (Superko et al., 2013), or seasonal variation of omega-3-fatty acids (De Vriese et al., 2004) cannot be ruled out.

On average, our participants were healthy, physically active above current physical activity recommendations (Perk et al., 2012), and with higher average VO_{2max} than in Norwegian

reference data (Aspenes et al., 2011). The moderately high fitness- and physical activity levels could make changes in physical activity lifestyle less likely and may explain the discrepancy from the preclinical study (Hoper et al., 2014). We detected no change in self-reported weekly physical activity between baseline, three months, and six months that could have affected VO_{2max}. Physical activity behavior was not recorded in the preclinical study (Hoper et al., 2014); thus, it is unknown if Calanus oil supplementation could have changed the voluntary physical activity behavior in the caged mice and thereby could explain the increase in VO_{2max} in this study (Hoper et al., 2014). In addition to the timing of Calanus oil supplementation (Radak et al., 2017), its antioxidant effect may have abolished any favorable effect of the supplementation on VO_{2max}, by attenuating exercise-induced increase in reactive oxygen species and reactive nitrogen species, which are believed to be vital in the exercise adaptive responses (Merry et al., 2016).

Calanus oil supplementation in preclinical studies has shown beneficial health effects (Eilertsen et al., 2012; Schots et al., 2020), while omega-3-supplementation studied in diabetes and cardiovascular disease prevention show conflicting results (Chowdhury et al., 2012; Da Boit et al., 2017; Rizos et al., 2012; Wu et al., 2012). In our healthy participants, none of the measured cardiovascular risk factors, such as resting blood pressure, resting heart rate, heart rate recovery, or ventilatory to respiratory quotient gradient, changed during the 6-month intervention. This could be due to selection, as we included healthy middle-aged participants with few cardiovascular risk factors besides overweight. It also supports other studies documenting minor effects of omega-3 supplementation on risk factor reduction (Chowdhury et al., 2012; Wu et al., 2012) and cardiovascular disease outcome (Rizos et al., 2012).

In a review of the literature, omega-3-oil supplementation has, to some degree, been shown to improve heart rate regulation, heart function, and vascular resistance in healthy young people

(Da Boit et al., 2017). In our study, we show no effect of Calanus supplementation on resting- or exercise heart rate, blood pressure, or oxygen-pulse, a surrogate measure for stroke volume (volume of blood ejected per cardiac cycle), giving physiological support to our finding of no change in VO_{2max}. It should be noted that both blood pressure and resting heart rate was in the normal to low range according to reference values and, therefore, are less likely to improve (Holmen et al., 2016; Nauman et al., 2012). A low resting heart rate has been found to be associated with high VO_{2peak} and lower mortality risk in prospective studies strengthening the notion that we studied healthy participants (Nauman et al., 2012; Nauman et al., 2011; Nauman et al., 2010).

In contradiction to the preclinical studies (Hoper et al., 2013; Hoper et al., 2014; Jansen et al., 2019), neither body weight nor body composition changed throughout the intervention. As neither muscle mass nor self-reported physical activity, with possible metabolic effects, increased throughout our study, the steady state in body composition was not unexpected. In a recent study of the combined effect of exercise training and Calanus oil supplementation fat mass decreased, and lean body mass increased (Wasserfurth, Nebl, Schuchardt, et al., 2020). As self-reported physical activity did not change, the discrepancies between studies might indicate a combined effect of supplementation with exercise (Wasserfurth, Nebl, Schuchardt, et al., 2020). Some previous omega-3 supplemental studies show increased muscular protein synthesis, volume, and strength in elderly participants, indicating that age might be a factor (Da Boit et al., 2017). Our findings are similar to a study of Calanus supplementation in older women reporting no change in body composition (Dadova et al., 2020). We detected no change in waist-to-hip ratio in our participants, and thereby our study does not support the finding of reduced body weight and abdominal visceral fat from preclinical studies (Hoper et al., 2013; Hoper et al., 2014; Jansen et al., 2019). Again, this could be due to more obesity in the preclinical studies and the use of a high-fat feeding model (Hoper et al., 2013; Hoper et

al., 2014; Jansen et al., 2019) as well as species differences. The high omega-3 index in our participants at baseline (Stark et al., 2016) is a complicating factor, but the somewhat lower index for the Calanus group at six months is indicative of a fair compliance to the Calanus oil supplementation. In comparison to a study of older women with lower baseline omega-3 index given 2.5 $g \cdot day^{-1}$ of Calanus oil supplementation (Štěpán et al., 2022), our data might indicate that more than two (2) $g \cdot day^{-1}$ of Calanus oil is needed to increase EPA and DHA red cell membrane content in healthy participants with already high baseline values (Patterson et al., 2015; Stark et al., 2016).

Strengths and weaknesses

Main strength was the randomized controlled double-blinded design and high self-reported adherence to the study supplementation in most participants, the six months duration of the study and directly measured VO_{2max}. Also, our study adds new evidence to previous studies as we studied the novel omega-3 supplementation Calanus oil, and we equally study men and women. The duration of the study was appropriate to access long-term changes in VO_{2max} and clinical parameters, and controls for any Hawthorn effect in the study. The randomized controlled blinded design was chosen to adjust for possible dietary and lifestyle group differences beyond the intervention. The study supplement compliance was ~81-83%, similar to the mean overall compliance found in a systematic review of compliance to oral nutritional supplements (Hubbard et al., 2012). A weakness of the study was the lack of objectively measured physical activity as well as the self-reported adherence to supplementation. Also, the relatively high fitness level and high omega-3 index of the participants at baseline might indicate that the study design was more attractive to fit and dietary conscious participants than unfit participants. A detailed dietary screening of omega-3 intake was also absent in the study.

Steady state submaximal exercise testing in our study would have allowed for investigation of possible changes in moderate exercise metabolic efficiency due to long-term Calanus oil supplementation. Also, we cannot confirm whole blood and muscle content of EPA and DHA due to lack of plasma and muscle biopsy samples for this analysis. The results from the study can be generalized to apply for physically fit, middle-aged men and women.

CONCLUSION

Six months of Calanus oil supplementation had no effect on maximal oxygen uptake in healthy, normal to overweight, 30 to 50 years old men and women.

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AUTHORSHIPS

All authors (LT, RENR, HD, TL, TK) were involved in the writing and final approval of the manuscript and were all involved in developing the research questions, study design, data acquisition, data analyses, and interpretation of the data.

DECLARATION OF FUNDING OR CONFLICT OF INTEREST

The study was funded by Calanus AS, Tromsø, Norway and NTNU - the Norwegian University of Science and Technology, Trondheim, Norway. None of the authors have any relevant conflicts of interest. The funder participated in the development of the project protocol and provided Calanus oil and placebo capsules to the study but had no role in data analyses and writing of the article. The authors declare that they have no competing interests.

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626 Table 1. *Calanus finmarchicus* oil fatty acid composition (Wasserfurth, Nebl, Bosslau, et al.,
627 2020)

Fatty Acid	**Name**	**Mg · 2g^{-1} Calanus oil**
14:0	Myristic acid	125
15:0	Pentadeclic acid	6.5
16:0	Palmitic acid	105
16:3	-	7
18:0	Stearic acid	8
18:1n9	Oleic acid	36
18:2n6	Linoleic acid	11
18:3n3	Alpha-Linolenic acid	23
18:3n6	Gamma-Linolenic acid	3
18:4n3	Stearidonic acid	124
20:1n9	Gondoic acid	43
20:4n6	Arachidonic acid	3
20:5n3	Eicosapentaenoic acid	109
22:1n11	Cetoleic acid	70
22:5n3	Docosapentaenoic acid	8
22:6n3	Docosahexaenoic acid	87
24:1n9	-	8

628 80% of the fatty acids are present as wax esters

629

630

631 Table 2. Participant baseline demographics.

	Calanus oil, n=30	**Placebo, n=28**
Gender (M/F)	14 / 16	14 / 14
Age (years)	39.7 (38.0-41.4)	38.8 (36.8-40.9)
Weight (kg)	75.4 (72.1-78.8)	76.7 (71.5-82.0)
BMI ($kg \cdot m^2$)	24.8 (24.0-25.6)	24.8 (23.7-25.8)
Muscle mass (%)	42.8 (41.4-44.3)	43.2 (41.4-45.0)
Fat mass (%)	23.8 (21.4-26.2)	22.9 (19.9-25.9)
SBP (mmHg)	120 (116-123)	117 (112-122)
DBP (mmHg)	79 (76-81)	78 (74-82)
HR_{rest} ($beats \cdot min^{-1}$)	61 (58-65)	59 (55-63)
Current smokers (n)	0	1
Participants using any prescription medication (n)	4	5
Physical activity status (active/inactive)	26 / 4	26 / 2

632

633 Data are reported as mean and 95% Confidence Interval (CI). Abbreviations: BMI = body
634 mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR_{rest} = resting
635 heart rate. Training status was categorized by achieving/not achieving at least 150 min of
636 combined weekly moderate and/or vigorous physical activity (incl. time spent walking) based
637 on the IPAQ-7.

638

639 Table 3. Results from cardiopulmonary exercise testing at baseline, 3 months, and 6 months.

	Calanus oil			Placebo		
	Baseline	**three months**	**six months**	**Baseline**	**three months**	**six months**
VO_{2max} ($L \cdot min^{-1}$)	3.79 (3.47-4.11)	3.76 (3.46-4.06)	3.74 (3.44-4.04)	3.85 (3.48-4.23)	3.77 (3.41-4.12)	3.79 (3.44-4.14)
VE_{max} ($L \cdot min^{-1}$)	129.8 (119.1-140.5)	127.7 (116.4-139.0)	129.4 (117.9-140.8)	124.6(111.9-137.4)	123.4 (111.2-135.6)	123.0 (111.7-134.4)
HR_{max} ($beats \cdot min^{-1}$)	187 (184-190)	186 (183-188)	186 (183-188)	184 (180-188)	185 (182-188)	183 (179-186)
HRR ($beats \cdot min^{-1}$)	33 (30-37)	33 (29-37)	35 (31-39)	33 (28-38)	31 (27-35)	32 (27-36)
RER	1.10 (1.08-1.11)	1.09 (1.07-1.10)	1.08 (1.06-1.10)	1.10 (1.08-1.12)	1.10 (1.08-1.12)	1.08 (1.06-1.11)
VE/VCO_2	29.6 (28.7-30.4)	29.5 (28.6-30.4)	29.8 (28.6-31.1)	27.8 (26.9-28.8)	28.2 (27.1-29.3)	28.4 (27.3-29.5)
O_2-Pulse ($ml \cdot beat^{-1}$)	20.3 (18.6-22.1)	20.2 (18.7-21.8)	20.1 (18.6-21.7)	20.9 (19.0-22.8)	20.3 (18.5-22.2)	20.5 (18.7-22.4)
Speed ($km \cdot h^{-1}$)	11.4 (10.9-12.0)	11.6 (11.0-12.2)	11.7 (11.1-12.3)	11.4 (10.8-12.1)	11.5 (10.8-12.1)	11.5 (10.9-12.2)
Incline (%)	9.9 (9.7-10.1)	10.0 (9.8-10.2)	10.0 (9.8-10.2)	9.6 (9.3-10.0)	9.9 (9.6-10.1)	9.8 (9.5-10.1)
Borg scale	18 (18-19)	19 (18-19)	19 (19-19)	18 (18-19)	19 (18-19)	19 (18-19)

640

641 Data is reported as mean and 95% Confidence Interval (CI). Abbreviations: VO_{2max} = maximal oxygen uptake; VE_{max} = maximal pulmonary
642 ventilation; HR_{max} = maximal heart rate; HRR = heart rate recovery; RER = maximal respiratory exchange ratio; VE/VCO_2 = maximal minute
643 ventilation - carbon dioxide production relationship; O2-Pulse = maximal oxygen pulse; Speed = maximal treadmill speed; Incline = maximal
644 treadmill inclination. Borg scale = 6-20 scale of self-perceived exercise effort
645

646 Table 4. Anthropometric and clinical data at baseline, three months, and six months.

	Calanus oil			Placebo		
	Baseline	**Three months**	**Six months**	**Baseline**	**Three months**	**Six months**
SBP (mmHg)	120 (116-123)	118 (115-122)	117 (113-121)	116 (112-122)	115 (111-120)	116(111-121)
DBP (mmHg)	79 (76-81)	77 (74-80)	78 (75-81)	78 (74-82)	77 (74-80)	78 (74-82)
HR_{rest} (beats·min^{-1})	61 (58-65)	60 (57-63)	58 (55-61)	60 (56-63)	58 (55-62)	58 (55-61)
Body weight (kg)	75.4 (72.2-78.6)	75.6 (72.4-79.1)	75.6 (72.3-78.6)	76.7 (72.0-81.8)	76.5 (71.5-81.6)	76.8 (71.9-81.6)
Body fat (%)	23.8 (21.4-26.2)	24.1 (21.4-26.7)	23.8 (21.0-26.7)	22.9 (19.9-25.9)	23.4 (20.2-26.6)	23.1 (19.8-26.4)
Visceral fat (Cm^2)	76.5 (68.4-84.6)	79.7 (70.8-88.6)	76.7 (67.1-86.2)	77.4 (65.3-89.8)	79.0 (65.9-92.1)	78.3 (64.6-92.0)
Muscle mass (%)	42.8 (41.4-44.3)	42.5 (40.9-44.1)	42.9 (41.1-44.6)	43.2 (41.4-45.0)	43.0 (41.1-45.0)	43.2 (41.2-45.2)
BMI (kg·m^2)	24.8 (24.0-25.6)	24.8 (24.0-25.7)	24.8 (24.0-25.6)	24.8 (23.7-25.8)	24.8 (23.6-25.9)	24.9 (23.6-26.1)
Waist to hip ratio	0.89 (0.86-0.91)	0.89 (0.87-0.92)	0.89 (0.87-0.91)	0.90 (0.87-0.92)	0.90 (0.88-0.93)	0.90 (0.87-0.93)

647

648 Data is reported as mean and 95% Confidence Interval (CI). Abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL
649 = high density lipoproteins; LDL = low density lipoproteins; HR_{rest} = resting heart rate; BMI = body mass index. SBP, DBP, and HR_{rest} display
650 resting measures. * = within group difference from baseline to 6 moths ($p < 0.001$).

651 Table 5. Fatty acid composition of red blood cell membranes at baseline and 6 months.

	Calanus		Placebo	
Fatty Acids (%)	**Baseline**	**Six months**	**Baseline**	**Six months**
16:0 (Palmitic acid, %)	25.5 (25.0-25.9)	27.1 (26.1-28.1)	25.5 (25.2-25.8)	27.4 (26.3-28.5)
18:0 (Stearic acid, %)	20.3 (19.9-20.7)	21.7 (20.8-22.6)	20.1 (19.7-20.4)	21.5 (20.8-22.2)
18:1n9 (Oleic acid, %)	14.9 (14.5-15.3)	15.8 (15.3-16.3)	15.2 (14.8-15.5)	16.0 (15.5-16.6)
18:2n6 (Linoleic acid, %)	10.7 (10.2-11.1)	11.2 (10.7-11.8)	10.9 (10.4-11.4)	11.1 (10.6-11.5)
18:3n3 (Alpha-Linolenic acid, %)	0.18 (0.16-0.20)	0.19 (0.17-0.20)	0.17 (0.16-0.19)	0.17 (0.16-0.19)
20:3,n6 (Dihomo-γ-linolenic acid, %)	1.66 (1.51-1.81)	1.50 (1.37-1.62)	1.63 (1.50-1.76)	1.55 (1.42-1.69)
20:4n6 (Arachidonic acid, %)	15.8 (15.2-16.5)	13.6 (12.5-14.6)	15.8 (15.1-16.4)	14.5 (13.4-15.5)
20:5n3 (Eicosapentaenoic acid, %)	1.28 (1.08-1.49)	1.28 (1.09-1.47)	1.34 (1.13-1.54)	0.90 (0.75-1.05)
22:5n3 (Docosapentaenoic acid, %)	2.91 (2.78-3.05)	2.41 (2.18-2.64)	2.95 (2.80-3.09)	2.28 (2.03-2.54)
22:6n3 (Docosahexaenoic acid, %)	6.74 (6.23-7.24)	5.11 (4.49-5.74)	6.47 (6.03-6.91)	4.50 (3.90-5.10)
Omega-3 index (%)	8.0 (7.3-8.7)	6.5 (5.7-7.3)	7.8 (7.1-8.4)	5.4 (4.6-6.1)

652 Data in mean (95% CI).

653

654 Table 6. Weekly physical activity at baseline, 3 months, and 6 months.

	Calanus oil			Placebo		
	Baseline	**Three months**	**Six months**	**Baseline**	**Three months**	**Six months**
Vigorous PA (min·wk^{-1})	90 (60-135)	90 (60-120)	105 (45-180)	120 (70-158)	80 (60-120)	155 (120-180)
Moderate PA (min·wk^{-1})	105 (60-240)	128 (105-160)	120 (60-180)	150 (90-240)	120 (60-240)	128 (105 – 240)
Walking (min·wk^{-1})	85 (40-180)	75 (60-120)	95 (40-150)	210 (120-325)	125 (60-195)	113 (75-210)*
Sitting (min·day^{-1})	480 (421-570)	480 (420-600)	480 (420-600)	495 (375-600)	480 (360-600)	480 (375-600)
MET-minutes·week^{-1}	1496 (1160-2160)	1538 (1334-1884)	1600 (1193-2337)	2324 (1680-3277)	1920 (1533-2673)	2260 (1923-2847)

655 Data in median (95% CI). PA = physical activity; walking = weekly time spent walking; sitting = daily time spent sedentary. MET = Metabolic
656 equivalent minutes per week. Walking time was reduced in the placebo versus the Calanus group from baseline to 6 months (*$p < 0.05$).

Figure legends and figure captions

Figure 1. Study flowchart.

The figure describes the participants' flow in the study

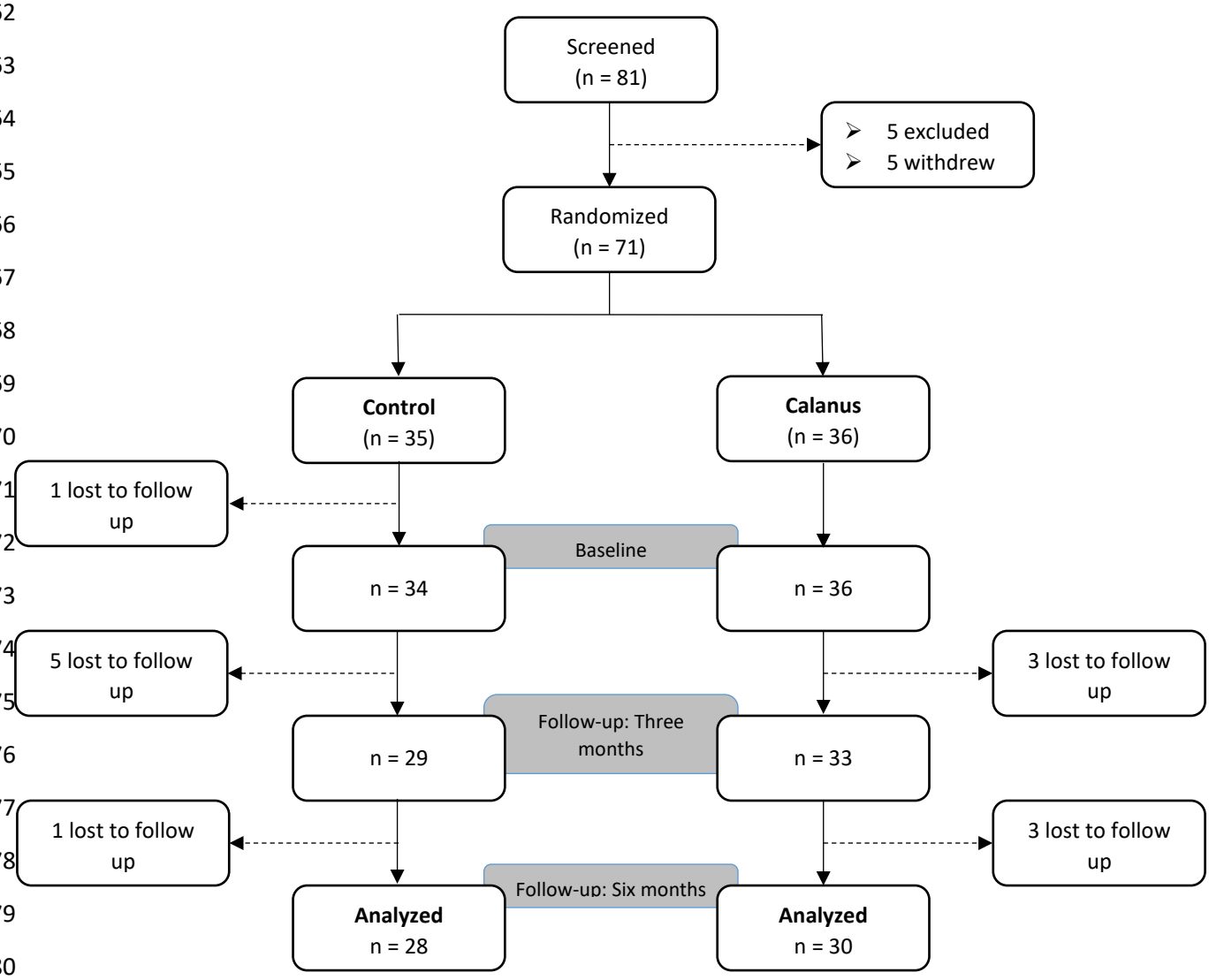
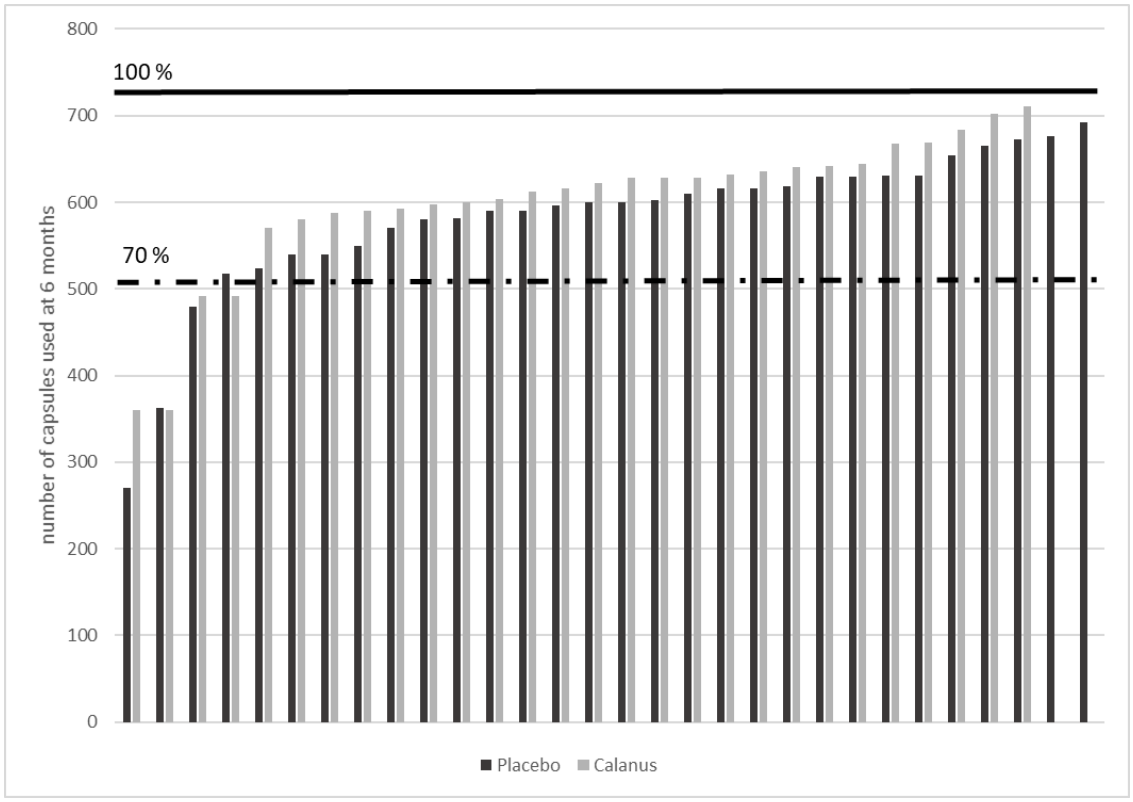
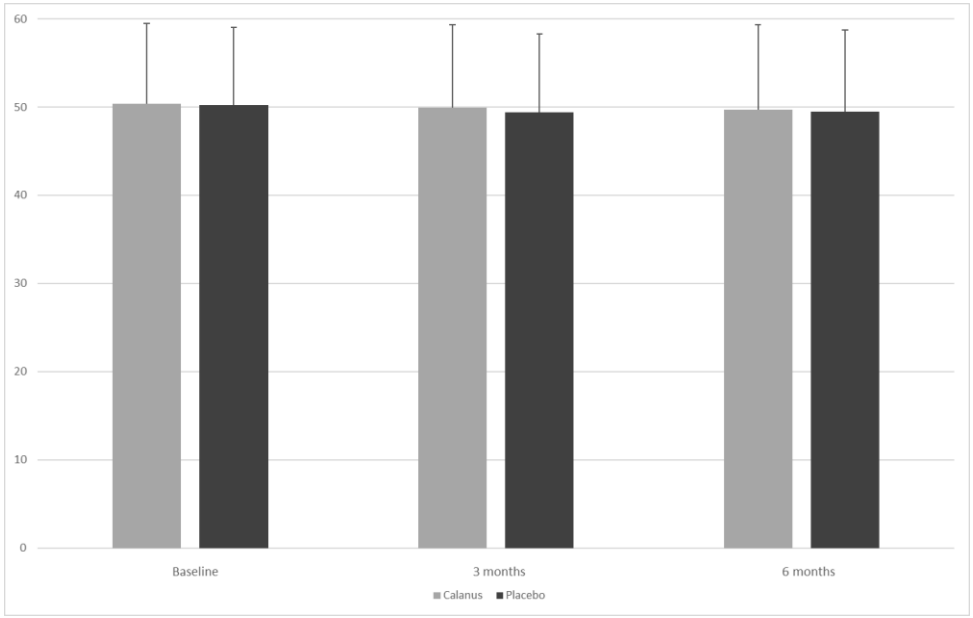


Figure 2. Individual compliance supplementation



Individual compliance to the supplementation in the placebo (dark grey) and Calanus group (light grey).

Figure 3. Maximal oxygen uptake at baseline, three months and 6 months



Maximal oxygen uptake (± standard deviation) at baseline, 3 months and 6 months in the Calanus (light grey) and the placebo group (dark grey).