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Randomized Control Trials

The effect of krill oil supplementation on skeletal muscle function and size in older adults: A randomised controlled trial

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SUMMARY

Background & aims: The aim of this study was to determine the effect of krill oil supplementation, on muscle function and size in healthy older adults.
Methods: Men and women, aged above 65 years, with a BMI less than 35kg/m2, who participated in less than 1h per week of structured self-reported exercise, were enrolled in the study (NCT04048096) between March 2018 and March 2020. Participants were randomised to either control or krill oil supplements (4g/day) for 6 months in this double blind randomised controlled trial. At baseline, 6 weeks and 6 months, knee extensor maximal torque was measured as the primary outcome of the study. Secondary outcomes measured were grip strength, vastus lateralis muscle thickness, short performance physical battery test, body fat, muscle mass, blood lipids, glucose, insulin, and C-Reactive Protein, neuromuscular (M-Wave, RMS and voluntary activation), and erythrocyte fatty acid composition.
Results: A total of 102 men and women were enrolled in the study. Ninety-four participants (krill group (26 women and 23 men) and placebo group (27 women and 18 men)) completed the study (mean (SD): age 71.2 (5.1) years and weight 71.8 (12.3) kg). Six months supplementation with krill oil resulted in, an increase in knee extensor maximal torque, grip strength and vastus lateralis muscle thickness, relative to control ($p<0.05$). The 6-month treatment effects were 9.3% (95%CI: 2.8, 15.8%), 10.9% (95%CI: 8.3, 13.6%) and 3.5% (95%CI: 2.1, 4.9%) respectively. Increases in erythrocyte fatty acid profile were seen with krill oil for EPA 214% (95%CI: 166, 262%), DHA 36% (95%CI: 24, 48%) and the omega-3 index 61% (95%CI: 49, 73%), relative to control ($p < 0.05$). Krill oil resulted in an increased, relative to control ($p < 0.05$), M-Wave of 17% (95%CI: 12.7, 38.1%) but there was no effect of krill oil on RMS, voluntary activation, or on any other secondary outcomes such as performance of the short performance physical battery test or quality of life.
Conclusion: Krill oil supplementation for 6 months results in statistically and clinically significant increases in muscle function and size in healthy older adults.
ClinicalTrials.gov Identifier: NCT04048096.

1. Introduction

The age-related loss of muscle mass and function, sarcopenia, has several deleterious effects, such as a reduction in the quality of life and increased incidence of falls, often leading to hospitalisation [1]. Moreover, recent estimates indicate that the excess cost of muscle weakness in the UK is £2.5 billion/year [2], whereas in the USA in 2000 it was estimated to be around $18.5 billion/year [3]. The prevalence of sarcopenia is unclear, but has been calculated to be between 4.6 and 7.9% [4]. Much of the variation in reported prevalence of sarcopenia is due to the definition employed. Whilst there is no universally agreed definition the criteria set out by the

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European Working Group on Sarcopenia in Older People is the most widely used [5]. This group defined probable sarcopenia as low muscle strength, confirmed sarcopenia as low muscle strength + low muscle quantity/quality and severe sarcopenia as low muscle strength + low muscle quantity/quality and low physical performance. With the percentage of older people (>65 y) predicted to rise from 17% in 2010 to 23% in 2035 in the UK, it is crucial to develop therapies to increase muscle mass and function in older adults. Even though resistance exercise improves muscle mass and function, even in nonagenarian women [6], it is less effective than in young people, the so-called "anabolic resistance" [7], and participation rates are low [8]. Alterations in nutrition, including protein and fatty acid intake, have been suggested to potentially be of therapeutic use for muscle function and health in older people [9].

Epidemiological data has shown that the consumption of fatty fish is positively associated with muscle function in older populations [10,11], indicating a potential role for long-chain n-3 polyunsaturated fatty acids (LCn-3 PUFA) in increasing muscle mass and function in older people. These findings were supported by cell culture and animal work [12,13]. Furthermore, human research has demonstrated that 8 weeks of LCn-3 PUFA supplementation (4 g/day) increased muscle protein synthesis (MPS) during a hyperaminoacidaemic-hyperinsulinaemic clamp [14] and recently that 6 months of LCn-3 PUFA supplementation (4 g/day) resulted in an 3.6% increase in muscle volume and a 2.3 kg increase in grip strength [15], both in older adults (>60 years). This requires confirmation in a larger and independent cohort. Most previous studies in this area have used fish oils as the source of LCn-3 PUFA, but Antarctic krill is also a rich source of these fatty acids. Fish oil supplements contain the majority of their LCn-3 PUFA in either triacylglycerol or ethyl ester form, while, on the other hand, krill oil has more than 40% of its LCn-3 PUFAs in phospholipid form [16]. On top of this, krill oil contains choline and asaxanthin which may also be important for muscle health [17,18]. In previous supplementation studies, similar increases in plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were seen with krill- and fish oil, despite lower doses of EPA and DHA in the krill oil supplements [19]. Furthermore, greater increases in the omega-3 index were seen with krill oil supplementation, compared to fish oil supplementation, when EPA and DHA in the supplements were matched [20].

The aim of the current study, therefore, was to determine the effect of krill oil supplementation, on muscle function and size in healthy older adults. The primary outcome was the change in knee extensor muscle strength from baseline to 6 months. Secondary outcomes were the change in grip strength, muscle thickness, short performance physical battery test, blood lipids, fasting glucose, c-reactive protein, and erythrocyte fatty acid profiles. All other outcomes were exploratory. Our primary hypothesis was that krill oil supplementation would increase muscle function, based on the change in our primary outcome of knee extensor muscle strength over the 6-month trial period.

2. Materials and methods

2.1. Trial design

The current study was a double blind randomised controlled trial with participants randomly assigned, following baseline assessment, to either control or krill oil groups for the 6-month intervention period. Blinding efficacy was not assessed in the current study. Allocation was carried out in a 1:1 ratio with randomisation carried out, by an independent colleague using online software (sealedenvelope.com). The trial was registered at clintrials.gov (NCT04048096). Follow-up study visits took place at 6 weeks and 6 months. Participants were also asked to avoid exercise for 48 h before each study visit, which occurred after an overnight fast at the same time of day.

2.2. Participants

Participants were recruited from the Glasgow area between March 2018 and March 2020 via posters and newspaper/magazine adverts in the community. Inclusion criteria were BMI less than 35kg/m^2, an age above 65 years, and participation in less than 1 h per week of structured self-reported exercise (defined as planned, structured, repetitive and intentional movement). Exclusion criteria were diabetes mellitus, severe cardiovascular disease, seizure disorders, uncontrolled hypertension (>150/90 mmHg at baseline measurement), active cancer or cancer that has been in remission <5 years, ambulatory impairments which would limit ability to perform assessments of muscle function, dementia, taking medication known to affect muscle (e.g. steroids), having an implanted electronic device (e.g. pacemaker/defibrillator/insulin pump), being on anticoagulant therapy, taking any nutritional supplements, having allergies to seafood or regular consumption of more than 2 portions of oily fish per week. The study was approved by the University of Glasgow Medical, Veterinary, Life Sciences College Research Ethics Committee [Reference 200170067] and was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained after explaining the aims, risks, and potential discomfort associated with the study. The study was conducted in accordance with the Declaration of Helsinki.

2.3. Interventions

The supplementation period was 6 months, with participants instructed to maintain their normal dietary (other than the supplements) and physical activity habits. The control oil group consumed 4 g/day of mixed vegetable oil (a mixture of olive oil (extra virgin, cold pressed), maize oil (refined), palm kernel oil (refined) and medium chain triglycerides, in the ratio 4:4:3:2). The total LCn-3PUFA content of the control supplement was 4 mg/g, with <1 mg/g EPA and DHA. The fatty acid composition of this mixture was made in order to give a fatty acids ratio similar to the fatty acids in a normal, European diet. The krill oil group consumed 4 g/day krill oil (Superba*Boost*™). The total LCn-3PUFA content of the krill oil supplement was 322 mg/g, with 193 mg/g EPA and 96 mg/g DHA with each 1 g capsule also containing 79 mg choline. Participants were asked to take 2 capsules with lunch and 2 with dinner. The control and krill capsules were identical in look and taste, and both were provided by Aker Biomarine Antarctic AS (Lysaker, Norway). The manufacturer had no role in the design, conduct or analysis of the study.

2.4. Outcomes

The primary outcome was the change in knee extensor muscle strength from baseline to 6 months. Secondary outcomes were the change in grip strength, muscle thickness, short performance physical battery test, blood lipids, fasting glucose, c-reactive protein, and erythrocyte fatty acid profiles. All other outcomes were exploratory. Measurements were made by the lead researcher (SA), who was blinded to group allocation, at baseline, 6 weeks and 6 months (study completion), unless otherwise stated.

2.5. Knee extensor muscle strength

We measured muscle strength of the knee-extensor muscles of the right leg during an isometric maximal voluntary contraction

(MVC) with participants secured in a dynamometer, with a knee angle of 90° and force recorded via a load cell (Biometrics Ltd, Newport, UK). A minimum of three contractions (up to 10s, with 3 min rest between contractions) were carried out and the highest value was used in the subsequent analysis.

2.6. Grip strength

Grip strength was measured three times in each hand, using a Jamar dynamometer, with the participant seated and the arm supported, and the elbow flexed at 90°. The highest value was used in subsequent analysis.

2.7. Muscle thickness

Muscle thickness of the right vastus lateralis muscle was measured using an ultrasound imaging device, as detailed previously [21].

2.8. Short performance physical battery test [22]

Participants were asked to maintain a side-by-side, semi-tandem and tandem stances for 10s. Chair-stands were performed with participants rising from a chair with their arms across their chest 5 times; this was repeated 3 times and the quickest time is used for analysis. Following this, three separate 4-m walks were performed, with the fastest time recorded for analysis.

2.9. Body fat and muscle mass

Bioelectrical impedance was used to measure body fat and for the calculation of muscle mass, using previously published equations [23].

2.10. Blood sample collection and analysis

At baseline and 6 months blood samples were collected from an antecubital vein and at 6 weeks a fingerprick blood sample was collected. For fatty acid analysis (baseline, 6 weeks and 6 months) a drop of blood was collected on filter paper that was pre-treated with a cocktail solution (Fatty Acid Preservative Solution, FAPS™) and allowed to dry at room temperature for 15 min before storage at −80 °C. Following completion of the study the dried blood spots (DBS) were shipped to OmegaQuant Analytics, LLC (Sioux Falls, USA) for fatty acid analysis. One punch of the DBS was transferred to a screw-cap glass vial followed by addition of BTM (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v) (Sigma–Aldrich, St. Louis, MO). The vial was briefly vortexed and heated in a hot bath at 100 °C for 45 min. After cooling, hexane (EMD Chemicals, USA) and HPLC grade water was added, the tubes were recapped, vortexed and centrifuged help to separate layers. An aliquot of the hexane layer was transferred to a gas chromatography (GC) vial and the extract analysed using a GC-2030 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA).

Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC OQ-A, NuCheck Prep, Elysian, MN) which was also used to construct individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); trans (16:1, 18:1*, 18:2* - see below for more details); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); cis n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition was expressed as a percent of total identified fatty acids. The omega-3 index is defined as the sum of 20:5n-3 (EPA) and 22:6n-3 (DHA) adjusted by a regression equation ($r = 0.97$) to predict the omega-3 index in the RBC.

*The chromatographic conditions used in this study were sufficient to isolate the C16:1 trans isomers and the C18:2 Δ 9t-12c, 9t-12t, and 9c-12t isomers; the latter is reported as C18:2n6t. However, each individual C18:1 trans molecular species (i.e., C18:1 Δ 6 thru Δ 13) could not be separated but appeared as two blended peaks that eluted just before oleic acid. The areas of these two peaks were summed and referred to a C18:1 trans.

At baseline and 6 months, samples were analysed for glucose, insulin, lipids, and C-reactive protein. Total and HDL-cholesterol, triglycerides, C-reactive protein, and glucose were measured on a c311 analyser and insulin on an e411 analyser (Roche diagnostics, Burgess Hill, UK) and LDL was calculated using the Friedwald equation. All assays were calibrated and quality controlled using the manufacturers reagents.

2.11. Neuromuscular function

During the measurement of knee extensor muscle strength a surface EMG (sEMG) electrode was positioned on the vastus lateralis muscle, in accordance with SENIAM guidelines [24], and recorded during each contraction; position was recorded for replication in future visits. sEMG signals were root mean square (RMS) processed, with average RMS calculated over a 500 ms period, 250 ms each side of peak force. During the isometric MVC the rate of torque development was also calculated as described previously [21]. With participants remaining seated in the dynamometer, an electrode was placed on the vastus lateralis muscle attached to a constant current variable voltage stimulator. Stimulation of the muscle was performed using an electrical stimulator (DS7A, Digitimer Ltd., Hertforshire, United Kingdom). Single stimuli, square wave pulse of 1 ms duration, were delivered to the muscle while participants maintained a 20% MVC, and the intensity of stimulation was increased until a plateau in twitch amplitude and M-wave occurred. Supramaximal stimulation was delivered by increasing the final stimulator output intensity by a further 30%. The sEMG electrode remained in place as resting twitch peak force was recorded and M-wave peak-to-peak amplitude calculated. Supramaximal stimulation was then applied on top of the MVC procedure to allow calculation of voluntary activation.

2.12. Quality of life

The EuroQol – EQ-5D-5L questionnaire was completed by participants at baseline, 6 weeks and 6 months.

2.13. Nutritional intake

Habitual diet was characterised using an online software (myfood24.org) for multi-pass 2-day dietary recalls at baseline and the end of the intervention, with an average of the 2 days reported at each time point.

2.14. Sample size

The sample size was based upon the primary outcome of knee extensor maximal strength. There is no established minimally clinically important difference (MCID) for knee extensor strength, but this has been estimated to be between 4 and 6% [25]. With an SD of 9% (based on data from our own lab) we required a sample size of 50 participants per group (80% power at $P < 0.05$) and aimed to recruit 120 to account for dropouts.

2.15. Statistical analysis

Statistical analysis was performed blinded to treatment allocation using Graphpad Prism and R. All data were tested for normality and skewness before selecting the appropriate test. Baseline data was compared between groups via unpaired t-tests. A 2-way ANOVA with repeated measures was performed to determine the effects of time, supplement and time*supplement interactions on the outcome variables. Where a significant interaction effect was observed in the ANOVA, a post-hoc t-test with Bonferroni correction was applied to locate differences between groups. $p < 0.05$ was considered statistically significant. Analysis was performed as intention to treat.

3. Results

3.1. Participant characteristics

A total of 102 men and women were enrolled in the study (NCT04048096) between March 2018 and March 2020 (Fig. 1 – participant flow diagram). After randomisation, 4 participants withdrew from the study. In March 2020 the UK was placed under a national lockdown due to COVID-19 with 4 participants final study visits, therefore, cancelled. Due to uncertainty around the duration of the lockdown and the pandemic, and time constraints on the researchers, the decision was taken to stop the trial at this point, although full recruitment had not been achieved. With the 8 participants lost to follow-up 94 participants (krill group (26 women and 23 men) and placebo group (27 women and 18 men)) completed the study. Participant characteristics and dietary intake data are presented in Table 1. There were no time, group or interaction effects seen for any of the outcome variables measured.

3.2. Knee extensor muscle strength

Muscle strength, thickness and neuromuscular function data are presented in Fig. 2 and Table 2. A time*group interaction ($p = 0.0049$), but not group ($p = 0.0646$) or time ($p = 0.6504$), effect for knee extensor maximal torque was noted, with post-hoc tests identifying that maximal torque was higher in the krill oil, relative to the control, group at 6 months. The change in maximal torque over the 6-month intervention period was $-2.2 \pm 14.1\%$ in the control group and $7.1 \pm 17.2\%$ in the krill oil group, giving a 6-month intervention effect of 9.3% (95%CI: 2.8,15.8%).

3.3. Grip strength

The ANOVA revealed group ($p = 0.030$), time ($p < 0.0001$) and interaction effects ($p < 0.0001$) for grip strength data, with post-hoc tests identifying that grip strength was higher ($p < 0.05$) in the krill oil, relative to the control group at 6 months. The change in grip strength over the 6-month intervention period was $-2.1 \pm 6.2\%$ in the control group and $8.8 \pm 7.2\%$ in the krill oil group, giving a 6-month intervention effect of 10.9% (95%CI: 8.3,13.6%).

3.4. Muscle thickness

Muscle thickness data are presented in Fig. 2. The ANOVA revealed an interaction ($p = 0.0124$), but not group ($p = 0.5457$) or time ($p = 0.2863$), effect for muscle thickness data. The change in

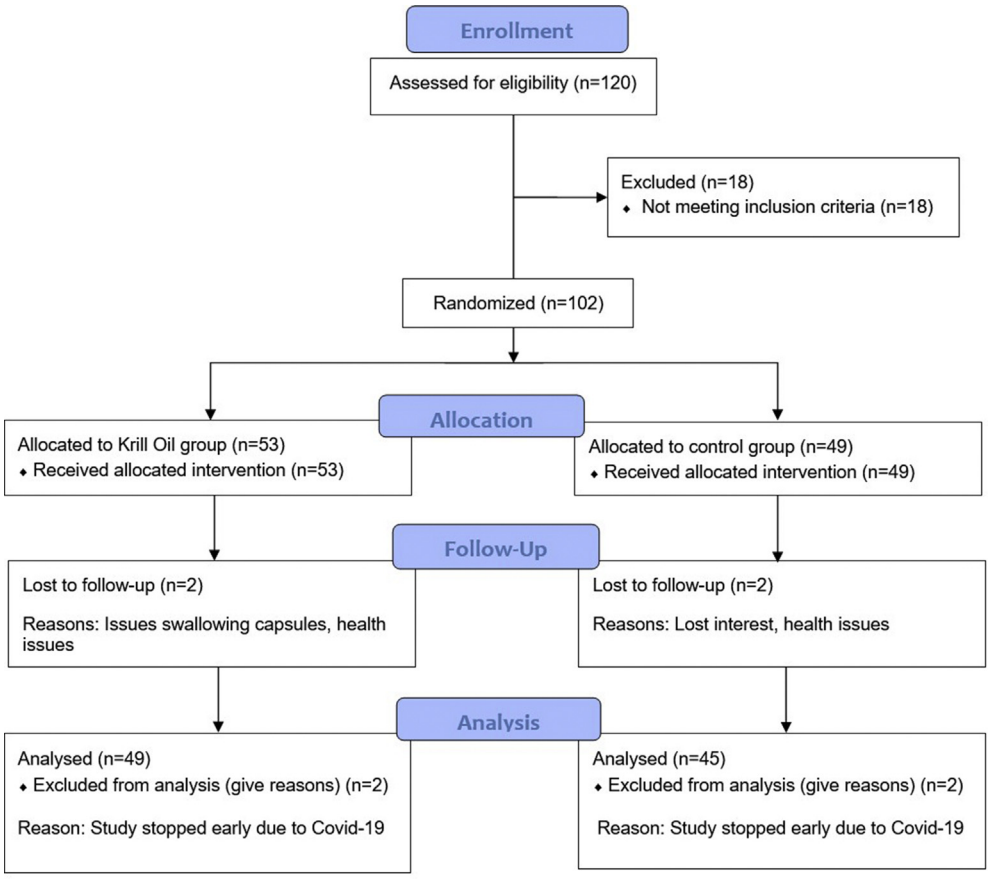

Fig. 1. Flow diagram illustrating the participant progress through the phases of the study.

Table 1
Participant characteristics, quality of life and dietary intake data in control and krill oil groups at baseline, 6 weeks and 6 months.

	Control			Krill		
	Baseline	6 weeks	6 months	Baseline	6 weeks	6 months
Age (years)	70.9 (5.2)	–	–	71.3 (4.9)	–	–
Height (cm)	164.5 (10)	–	–	169.1 (8.5)	–	–
Weight (kg)	71.1 (14.2)	71.4 (14.2)	70.9 (14.0)	72.5 (10.4)	72.9 (10.5)	72.7 (10.8)
Systolic BP (mmHg)	131.7 (13.7)	127.3 (14.6)	129.4 (14.2)	135.4 (11.9)	131.8 (12.4)	132.7 (12.7)
Diastolic BP (mmHg)	78.4 (9.2)	75.3 (10.5)	76.5 (8.5)	78.6 (7.8)	75.2 (7.7)	77.1 (7.9)
Body fat (%)	30.85 (8.09)	30.92 (7.90)	31.41 (8.05)	30.05 (7.55)	29.75 (7.20)	29.98 (7.47)
Muscle mass (kg)	26.66 (6.54)	26.84 (5.59)	25.87 (5.24)	27.50 (5.33)	28.10 (5.13)	27.71 (5.31)
EQ5-5DL VAS	89.0 (9.6)	87.4 (9.0)	85.6 (11.9)	86.4 (10.6)	87.9 (8.8)	85.7 (9.9)
EQ5-5DL index score	0.89 (0.1)	0.90 (0.1)	0.89 (0.1)	0.88 (0.1)	0.88 (0.1)	0.87 (0.1)
Energy Intake (kJ)	7269.8 (3370.4)	–	6742.9 (2065.9)	6926.7 (2204.7)	–	6932.5 (2605.1)
Protein Intake (% of total energy)	17.3 (3.0)	–	16.7 (2.9)	16.9 (3.1)	–	16.3 (3.4)
Fat Intake (% of total energy)	36.1 (6.2)	–	37.1 (5.2)	36.2 (5.3)	–	37.4 (7.5)
Carbohydrate Intake (% of total energy)	46.9 (7.0)	–	46.3 (5.9)	46.5 (7.2)	–	45.6 (7.9)
Polyunsaturated fatty acid intake (% of total energy)	8.1 (6.4)	–	7.4 (5.8)	7.6 (6.3)	–	5.8 (5.1)

Data are mean (SD).

muscle thickness data over the 6-month intervention period was 0.05 ± 1.1% in the control group and 3.5 ± 4.5% in the krill oil group, giving a 6-month intervention effect of 3.5% (95%CI: 2.1,4.9%).

3.5. Short performance physical battery test

The short performance physical battery test data are presented in Table 2. All participants were able to complete the full balance tests, therefore these data are not presented here. There were no time, group or interaction effects (all $p > 0.05$) for the 4 m walk and chair rise tests.

3.6. Blood results

Data from blood samples are presented in Tables 3 and 4. There were no group, time or interaction effects found in the ANOVA for insulin, C-reactive protein, or triglycerides (all $p > 0.05$). However, the ANOVA did reveal time, but no group or interaction, effects for glucose, total cholesterol, LDL cholesterol, and HDL cholesterol ($p < 0.05$) with data lower at 6 months, compared to baseline. The analysis of the fatty acid data revealed group*time interaction effects (all $p < 0.0001$) for EPA, DHA and the omega-3 index, with post-hoc tests revealing that all were higher ($p < 0.05$) at 6 weeks and 6 months in the krill, compared to the control, group. Six-month intervention effects were 214% (95%CI: 166, 262%) for EPA, 36% (95%CI: 24, 48%) for DHA and 61% (95%CI: 49, 73%) for the omega-3 index. An interaction effect ($p < 0.0001$) was also observed for arachidonic acid with post-hoc comparisons finding no differences between groups at any specific time point. A six-month intervention effect of − 13% (95%CI: −16, −10%) for arachidonic acid was found. No other differences were found for other fatty acids measured.

3.7. Neuromuscular function

Effects of time ($p = 0.0084$)), but not group ($p = 0.1286$) or interaction ($p = 0.2123$), were found for voluntary activation and RMS during the MVC. No group, time or interaction effects were seen for the rate of torque development data (all $p > 0.05$). A time*group interaction ($p < 0.001$), but not group ($p = 0.6425$) or time ($p = 0.2464$), effect for M-wave was noted, with post-hoc tests identifying that the M-wave amplitude was higher in the krill oil, relative to the control, group at 6 months. The change in M-wave over the 6-month intervention period was −8.1 ± 23.6% in the control group and 17.4 ± 37.6% in the krill oil group, giving a 6-month intervention effect of 25.4% (95%CI: 12.7,38.1%).

3.8. Quality of life

No group, time or interaction effects (all $p > 0.05$) were noted for the EQ5-5DL VAS or index (Table 1).

4. Discussion

This double blind randomised controlled trial, has shown that 6 months of supplementation with 4 g/day of krill oil significantly increases knee extensor strength, grip strength and skeletal muscle thickness in healthy older men and women. This is in line with the previous work of Smith and colleagues [15] who, in a smaller cohort of older men and women, found increases in muscle volume and function after 6 months of fish oil supplementation (4 g/day). Together, these data show that LCn-3 PUFA supplementation may be efficacious as a therapy for the maintenance of skeletal muscle mass and function, thereby attenuate the age-related declines seen during the process of sarcopenia.

The current study is the first study to demonstrate a beneficial effect of krill oil supplementation on muscle size and strength in older adults. The magnitude of effects we observed can be compared to those previously reported in a smaller cohort [15] where 6 months of fish oil supplementation resulted in a treatment effect on grip strength of 2.3 kg, with the current study finding a treatment effect of 3.4 kg with krill oil. The increase in muscle thickness of 3.5% reported in the current study is similar to the 3.6% in muscle volume reported by Smith and colleagues [15]. Although the methods employed to measure muscle size were very different, which correlate at baseline but not following resistance exercise training [26], so making direct comparisons is not straightforward. Furthermore, whilst we found increases in muscle thickness, we did not find increases in bioelectrical impedance derived measures of muscle mass, likely due to relative lack of accuracy of the latter.

Whilst without a direct comparison between LCn-3 PUFA sources (fish vs krill) we cannot establish if krill oil is more effective than fish oil our data tentatively indicates krill may have superior effects, although both clearly have important physiological effects. There is some further evidence that may help explain this. For example, there is some indication that the exogenous choline and astaxanthin (found in krill but not fish oil) supplementation may have beneficial effects for skeletal muscle metabolism and health

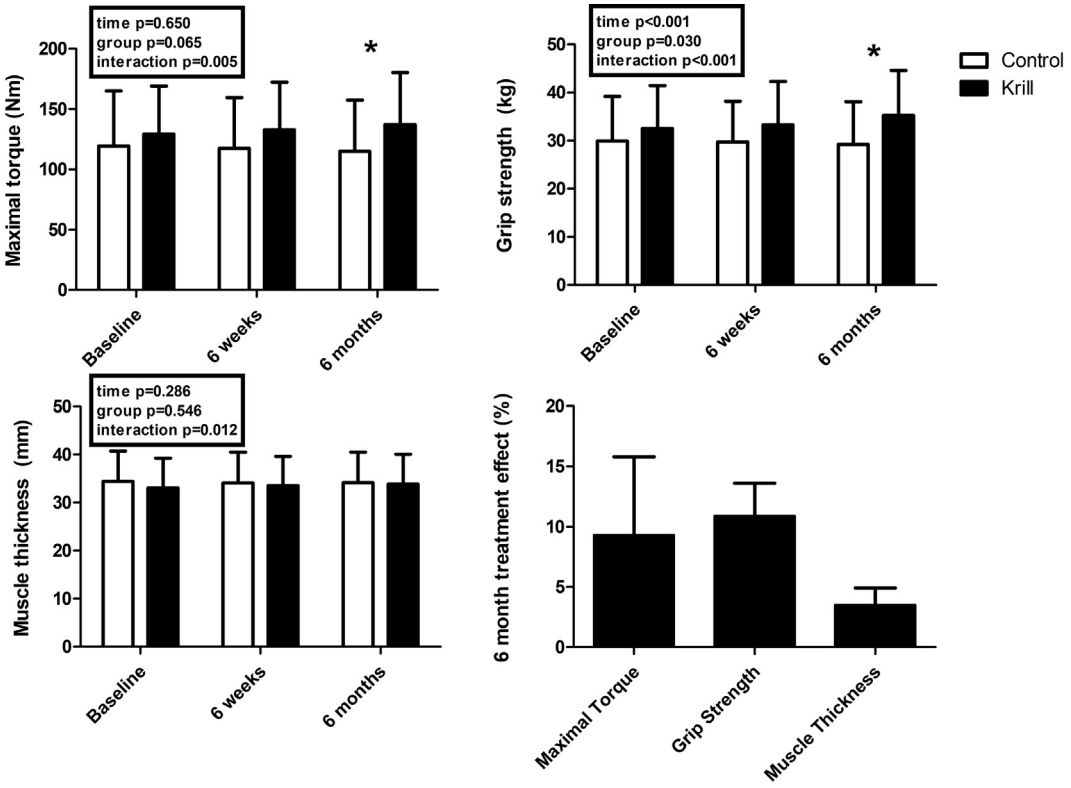

Fig. 2. Knee extensor maximal torque, grip strength and vastus lateralis muscle thickness at baseline, 6 weeks and 6 months and corresponding 6-month treatment effects in control and krill oil groups. Data are mean (SD) for baseline 6 week and 6-month data and mean (95%CI) for 6-month treatment effects. * denotes a significant difference from control at 6 months ($p < 0.05$).

Table 2
Short performance physical battery test, muscle strength, thickness and neuromuscular function data in control and krill oil groups at baseline, 6 weeks and 6 months.

	Control			Krill		
	Baseline	6 weeks	6 months	Baseline	6 weeks	6 months
4-m walk time (s)	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)	4.2 (0.6)	4.2 (0.5)	4.2 (0.6)
Chair rise time (s)	11.9 (1.2)	11.9 (1.1)	11.8 (1.1)	11.9 (1.2)	12.0 (1.2)	11.9 (1.2)
Voluntary activation (%)	77.8 (8.1)	78.6 (7.1)	79.8 (6.8)	75.9 (9.3)	78.5 (9.4)	81.0 (10.1)
RTD50 (N ms-1)	346.6 (444.0)	320.9 (309.0)	272.7 (280.0)	293.0 (258.3)	321.1 (268.9)	327.2 (268.8)
RTD100 (N ms-1)	205.3 (236.5)	212.7 (196.0)	186.6 (160.1)	184.0 (161.9)	201.4 (138.8)	216.3 (154.3)
RTD200 (N ms-1)	182.3 (172.5)	186.4 (145.0)	168.2 (133.1)	182.0 (118.0)	190.5 (116.3)	198.9 (120.0)
RTD300 (N ms-1)	162.7 (129.1)	167.1 (110.2)	153.2 (107.2)	168.1 (89.4)	173.0 (95.5)	181.5 (94.4)
RMS (uV)	93.8 (66.4)	95.9 (56.5)	86.7 (50.7)	106.2 (77.1)	122.5 (87.6)	109.7 (83.8)
M-wave (mV)	20.1 (5.1)	18.5 (4.6)	17.9 (4.3)	18.5 (4.8)	18.5 (5.0)	20.6 (5.4) *

Data are mean (SD). * denotes a significant interaction effect in the ANOVA ($p < 0.05$).

Table 3
Plasma metabolic analyses in control and krill oil groups at baseline and 6 months.

	Control		Krill	
	Baseline	6 months	Baseline	6 months
Insulin (μU/mL)	10.1 (7.6)	10.3 (7.2)	10.0 (6.0)	10.0 (6.0)
Glucose (mmol/L)	5.5 (1.1)	5.0 (0.9)	5.4 (0.5)	5.0 (0.8)
Total Cholesterol (mmol/L)	4.6 (1.0)	4.0 (1.0)	4.7 (1.1)	4.4 (1.0)
HDL (mmol/L)	1.3 (0.3)	1.2 (0.3)	1.4 (0.3)	1.2 (0.3)
LDL (mmol/L)	2.8 (0.9)	2.3 (0.9)	2.8 (0.9)	2.7 (0.9)
Triglycerides (mmol/L)	1.2 (0.7)	1.2 (0.6)	1.2 (0.7)	1.0 (0.6)
C-Reactive Protein (mg/L)	1.4 (1.6)	2.1 (4.6)	2.1 (3.5)	2.1 (3.8)

Data are mean (SD).

[17,18]. The precise role of these different nutrients of krill oil in the beneficial effect we have observed remains to be established and a compartive trial needed.

Whilst there is no agreed MCID in grip strength, the magnitude of effect that we have observed is similar to the difference in grip strength seen over a ~10 year age difference (65 vs 55 years of age) [27] and we would argue that such a difference is relevant. Similarly, the difference in knee extensor maximal isometric torque we have observed is greater than the previously reported as the MCID [25]. However, it is worth pointing out that while we found differences in these lab based measurements of muscle function, we did not find an effect of krill oil on our measurements of functional abilities, as measured by the short performance physical battery test [22]. The chosen scale is less responsive to change and so we were not powered to detect differences. In the current study we recruited relatively healthy older people and all participants achieved the maximum or near maximum score at all timepoints. It is therefore likely that the room for improvement was small and a ceiling effect for these tests was present. This suggestion is

Table 4
Fatty acid composition in control and krill oil groups at baseline, 6 weeks and 6 months.

	Control			Krill		
	Baseline	6 weeks	6 months	Baseline	6 weeks	6 months
Myristic Acid (%)	0.8 (0.3)	1.0 (0.5)	0.9 (0.4)	0.8 (0.4)	0.9 (0.5)	0.8 (0.2)
Palmitic Acid (%)	21.6 (3.6)	21.9 (3.7)	21.9 (3.6)	22.4 (1.7)	22.0 (3.6)	22.4 (1.4)
Palmitelaidic Acid (%)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
Palmitoleic Acid (%)	1.5 (0.6)	1.5 (0.7)	1.5 (0.6)	1.6 (0.7)	1.5 (0.7)	1.6 (0.7)
Stearic Acid (%)	10.2 (1.8)	10.0 (1.8)	10.1 (1.8)	10.5 (1.0)	10.0 (1.7)	10.2 (0.8)
Elaidic Acid (%)	0.4 (0.1)	0.4 (0.2)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
Oleic Acid (%)	20.3 (3.7)	20.9 (3.8)	20.8 (3.9)	20.6 (2.2)	19.4 (3.6)	20.1 (2.3)
Linoelaidic Acid (%)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)
Linoleic Acid (%)	20.6 (4.4)	20.4 (4.2)	20.2 (4.5)	20.6 (3.5)	19.1 (4.2)	20.3 (2.8)
Arachidic Acid (%)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.01)	0.2 (0.1)	0.2 (0.1)
gamma-Linolenic Acid (%)	0.3 (0.2)	0.3 (0.2)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)
Eicosenoic Acid (%)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
alpha-Linolenic Acid (%)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.5 (0.1)	0.5 (0.2)	0.5 (0.1)
Eicosadienoic Acid (%)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
Behenic Acid (%)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.2)	0.5 (0.1)
Dihomo-g-linolenic Acid (%)	1.6 (0.4)	1.5 (0.4)	1.6 (0.4)	1.5 (0.3)	1.2 (0.3)	1.3 (0.3)
Arachidonic Acid (%)	9.0 (1.9)	8.6 (1.8)	9.2 (1.9)	9.6 (1.5)	8.6 (1.7)	8.5 (1.3)*
Lignoceric Acid (%)	0.9 (0.3)	0.9 (0.3)	0.8 (0.3)	1.0 (0.3)	0.9 (0.3)	0.8 (0.3)
Eicosapentaenoic Acid (%)	1.3 (0.7)	1.1 (0.5)	1.1 (0.4)	1.2 (0.6)	3.2 (1.2)	3.2 (1.1)*
Nervonic Acid (%)	1.2 (0.4)	1.2 (0.3)	1.2 (0.4)	1.3 (0.3)	1.3 (0.4)	1.2 (0.4)
Docosatetraenoic Acid (%)	0.9 (0.3)	0.9 (0.3)	0.9 (0.3)	1.0 (0.3)	0.8 (0.3)	0.7 (0.2)
Docosapentaenoic Acid - n6 (%)	0.3 (0.1)	0.3 (0.1)	0.3 (0.9)	0.4 (0.9)	0.3 (0.1)	0.2 (0.1)
Docosapentaenoic Acid - n3 (%)	1.3 (0.3)	1.3 (0.3)	1.3 (0.3)	1.4 (0.2)	1.7 (0.4)	1.8 (0.3)
Docosahexaenoic Acid (%)	3.5 (1.0)	3.4 (1.1)	3.5 (1.0)	3.4 (1.0)	4.2 (1.2)	4.4 (0.9)*
Omega-3 Index (%)	6.7 (1.9)	6.4 (1.8)	6.5 (1.6)	6.5 (1.7)	9.7 (2.6)	10.0 (2.1)*

Data are mean (SD). * denotes a significant interaction effect in the ANOVA ($p < 0.05$).

supported by the recent findings of the DO-HEALTH study [28], where it was found that 1 year of LCn-3 PUFA (1 g/day) supplementation had no effect on short performance physical battery test scores in older people ($n = 2175$), but similarly to the current study the baseline scores were ~11 out of 12 and the sample also had high levels of physical activity. Measures of grip or knee-extensor strength were not reported, as yet, in the DO-HEALTH study.

Further work should look at the effects of LCn-3 PUFA on muscle mass, function, and functional abilities in people with low(er) physical function at baseline [5,29]. Elaborating on the functional status of our participants none of them would meet the criteria for probable sarcopenia, confirmed sarcopenia or severe sarcopenia [5]. This does not, however, detract from the importance of the current findings in our participant group which demonstrates that krill oil may be useful in preventing people from developing sarcopenia and/or crossing the so-called disability threshold during ageing [30]. The development and evaluation of such a prevention programme is, therefore, in our opinion warranted. This could have profound benefits to the individual and society as sarcopenia/muscle weakness is associated with a reduction in the quality of life, an increase in the incidence of falls and hospitalisation 1, increases mortality risk and has large economic costs 2,3. Whilst we know that resistance exercise is the most efficacious method to increase muscle mass and function, we know that it is challenging to increase population level participation. Increasing LCn-3 PUFA intake, whether through an increase in krill oil, fish oil or fatty fish consumption, which is low in many populations, may represent an effective public health strategy for prevention of the development of sarcopenia. Large scale preventative trials in at-risk groups are needed to investigate this further.

Further studies are also required to understand the mechanisms through which krill oil are acting on muscle. The early hypothesis was that increased LCn-3 PUFA intake would have an anti-inflammatory effect [31], which would attenuate the negative effects of age-associated chronic low-grade inflammation, which contributes to the loss of both muscle mass and strength [32]. However, studies which have found benefits of LCn-3 PUFA on muscle, have done so in the absence of any change in circulating markers of inflammation [33,34], with the current study finding no effect of krill oil supplementation on C-Reactive Protein. However, effects of LCn-3 PUFA on inflammation at the level of muscle may still be present and could contribute to the beneficial effects of LCn-3 PUFA rich supplements on both muscle mass and strength. Other potential mechanisms which may increase muscle strength include effects of LCn-3 PUFA on neuromuscular function [35]; in this study we found increases in M-Wave (muscle excitability) in the krill oil vs. placebo group likely due to: i) presence of choline which has been shown to increase acetylcholine synthesis which enhances neuromuscular junction excitability [36] and ii) observed increased muscle thickness is likely to be from enlarged fibre diameter which enhances electrical conducting properties along the fibre nerve [37]. Further potential mechanisms include the extracellular matrix [38], mitochondria [39], and cross-bridge kinetics [40]. With krill oil in particular there is also the potential beneficial effect, as mentioned previously, of choline and astaxanthin [17,18]. The mechanisms through which LCn-3 PUFA increase muscle mass likely rely on the changes in muscle protein metabolism, increase in MPS [14] mentioned in the introduction, which may be due to increases in nutritive blood flow [41], although the more molecular mechanisms which drive these changes in muscle protein metabolism remain to be elucidated.

In summary, in this double-blind randomised-controlled trial of relatively large size and long duration, we have demonstrated that krill oil supplementation results in increases in muscle function, excitability, and size, although functional abilities were not affected, in healthy older adults. This indicates that LCn-3 PUFA could be an effective preventive strategy to counter the age-related decline in muscle mass and function. Further work is needed to determine, if LCn-3 PUFA can be an effective treatment in people with sarcopenia and/or frailty.

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Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.04.007.

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