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ORIGINAL ARTICLE

Chronic supplementation of omega-3 can improve body composition and maximal strength, but does not change the resistance to neuromuscular fatigue

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Abstract
Purpose The present study aimed to determine whether supplementation with omega-3 fatty acid (N-3) contributes to improving body composition, strength performance, and neuromuscular fatigue resistance in physically active men.
Methods The study was randomized, double–blind, and placebo controlled. 15 males were recruited and randomly assigned into two groups, N-3 supplementation (GN-3; $N = 7$) and placebo supplementation (GP; $N = 8$). Supplementation with N-3 or a placebo (safflower oil) was administered for 28-days at 1.4 g·day^{-1}. During this period, physical activity was monitored (internal load = volume × perceived exertion scale). Before and after the supplementation period, body composition, one maximum repetition of knee extension (1RM), and maximum repetitions of knee extension with 70 % of 1RM load (RMs) pre and post an incremental running protocol until exhaustion were measured.
Results ANOVA two way ($p < 0.05$) revealed a decrease in body fat mass (GP before: 8.3 ± 2.1 kg × after: 7.7 ± 2.4 kg; GN-3 before: 12.8 ± 9.4 kg × after: 11.8 ± 9.3 kg), increase in lean mass (GP before: 61.8 ± 4.1 kg × after: 62.7 ± 3.9 kg; GN-3 before: 64.2 ± 5.8 kg × after: 66.2 ± 6.0 kg), and 1RM (GP before: 111.3 ± 29.1 kg × after: 111.3 ± 25.9 kg; GN-3 before: 115.0 ± 36.2 kg × after: 129.1 ± 39.9 kg) in the GN-3, without significant alterations in the GP and no interactions between-groups. Moreover, the absolute variation of the RMs pre and post the incremental running protocol were not significantly altered for both group (GP before: −1.1 ± 2.9 repetitions × after: −1.1 ± 2.6 repetitions; GN-3 before: −3.9 ± 2.9 repetitions × after: −5.0 ± 4.6 repetitions), with no interactions between-groups.
Conclusion Four weeks of N-3 supplementation seems to improved body composition and maximal strength of knee extension, without influencing neuromuscular fatigue resistance.

Keywords Weight lifting · Strength training · Neuromuscular fatigue

Introduction

Omega-3 (N-3) is a polyunsaturated fatty acid present in several species of fish [1]. An intake of 1–2 g day^{-1} is indicated for the prevention of ischemic heart diseases such as atherosclerosis and thrombosis due to its action in reducing cholesterol and triglycerides plasma levels [1, 2]. Furthermore, N-3 contributes to attenuation of

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inflammatory and oxidative processes [3], including exercise-induced alterations [4, 5].

Due to the beneficial systemic effects of N-3 on cardiovascular and metabolic health, there is growing interest in the influence of N-3 supplementation in sports performance [2, 5, 6]. While some studies did not find any improvement after N-3 supplementation in exhaustive exercise performance [6], peak oxygen uptake (VO_2peak) or peak workload [7], other studies have linked N-3 supplementation with improvements in body composition and performance during aerobic [1] and anaerobic exercises [8], which could be important evidence of the beneficial effects of N-3 supplementation on neuromuscular performance.

The biological availability of N-3 has been associated with a reduction in free 5-hydroxytryptamine (serotonin), an important depressor of synaptic activity and consequently of the central nervous system (CNS), which contribute to negative modulation of sleepiness, mood, and fatigue [9]. The precursor of serotonin is free tryptophan, which is augmented in the blood stream during exercise by increasing lipolysis of fat free acids [9]. Free tryptophan can be translocated across the blood–brain membrane by a specific transporter and become abundant in the brain interstitial space, and presumably enhanced activity of serotonin in the CNS [6] and resulting in premature exercise fatigue [10, 11].

Decreasing the circulating serotonin by controlling the precursors (i.e., fat free acids and free tryptophan), may be an efficient strategy to reduce the harmful action in the CNS. N-3 supplementation has been shown to cause reductions in body fat mass [1], triglycerides, and fat free acids [12–14], characterizing it as an interesting strategy to maintain the quality of neuromuscular performance and consequently act as an ergogenic aid.

Thus, the purpose of the present study was to verify the effects of chronic N-3 supplementation on body composition, muscle strength performance, and neuromuscular fatigue resistance.

Methods

Participants

Twenty healthy men voluntarily began the study. Exclusion criteria were self-reported cardiovascular disease (i.e., hypertension), metabolic disease (i.e., diabetes), smoking, musculoskeletal problems or consumption habits of marine sources (i.e., fatty fish) or seeds/nuts more than twice per week. All participants were informed of the possible risks and benefits of the study prior to signing an informed consent that was in accordance with the Declaration of Helsinki and properly approved by the Research Ethics Committee of UNISALESIANO-Catholic Center University (Protocol 867.319/2014). During the study there was sample loss due to injuries (i.e., broken leg) or non compliance of, at least, 95 % of the supplementation program (Fig. 1). The final sample size consisted of fifteen men

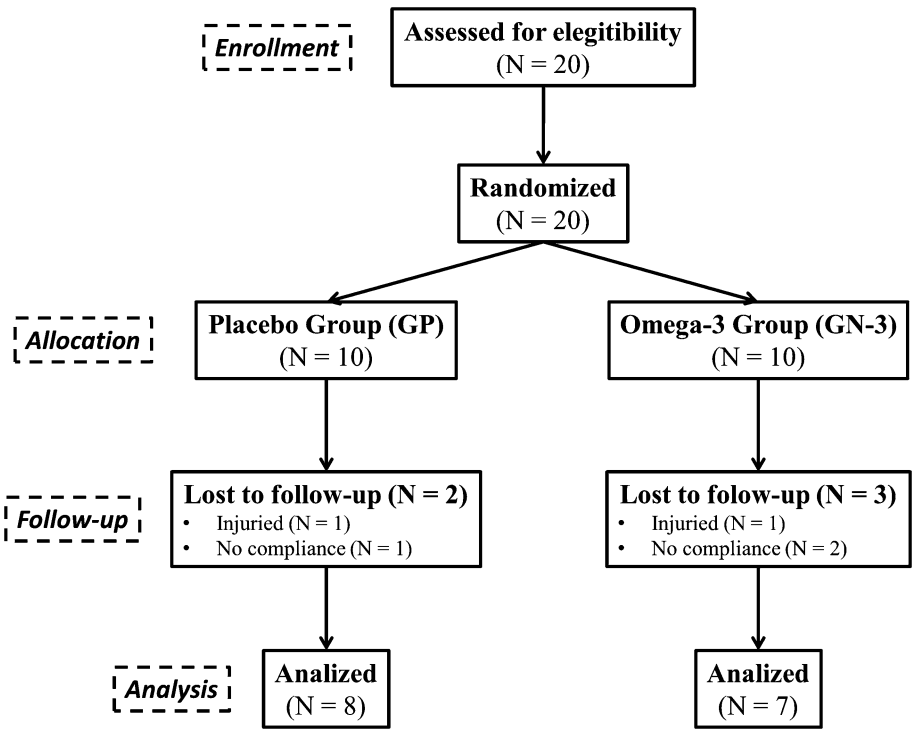

Fig. 1 Schematic recruitment of participants and progression of each stage of the study

(age: 23 ± 5 years; body mass: 73.3 ± 10.6 kg, height: 175 ± 6 cm).

Experimental design

Fifteen young men were randomly divided into two groups: placebo group (GP; safflower oil supplementation); and omega-3 group (GN-3; supplementation of omega-3). The study was conducted in a double blind, placebo controlled, and parallel manner and both groups received 1 g capsules of identical appearance, administered four times per day, for 28 days. Prior to the evaluations, participants were familiarized with the test protocols and during the supplementation period the physical activities were monitored for duration, characteristics, and perceived exertion scale (RPE) CR-10. The participants were instructed to consume a light meal ~2 h before the evaluations and not to modify their eating habits. The evaluations were performed at two different moments, before and after the supplementation period. On the first day of assessment, the body composition analysis and one maximum repetition (1RM) test of knee extension (i.e., 1RM half squat) were performed. After 48–72 h, the follow sequences were conducted: maximum repetitions (RMs) of knee extension with 70 % of 1RM load (i.e., RMs half squat), 5 min rest, an incremental treadmill-running test until exhaustion (IT), 5 min rest and RMs with the same load as previously performed.

Monitoring of daily physical activity level

All physical activity sessions performed by participants during the supplementation period were reported in a training diary for the duration, characteristics, and also the (RPE) CR-10 30 min after the end of each session. The training load was determined by the product between the duration (in minutes) of each session and the respective perceived exertion value [15].

The participants did not receive any instruction or training prescription from the evaluator at any time during the experimental design. The training frequency was 2–4 times per week and despite the participants having experience in strength training, at the time of the study, they were not performing any specific training program for muscle hypertrophy or strength improvements.

Supplementation protocol

The supplementation strategies were adapted from Hill et al. [1] (i.e., amount for health purposes) and Huffman et al. [6] (i.e., capsule administration protocol). The N-3 (Florien, Piracicaba, SP, Brazil) is preferably administered at mealtimes in 1 g soft-gel capsules that provide ~200 mg of eicosapentaenoic acid (EPA) and ~150 mg of docosahexaenoic acid (DHA), totaling ~1.4 g of N-3 per day. The placebo capsules were identical in appearance and contained 1 g of safflower oil (Florien, Piracicaba, SP, Brazil). The participants received the supplements in weekly-packages and were asked to return the capsules when not consumed. The level of compliance to the supplementation protocol was monitored by capsule counting and the rate of adherence was 98.0 % (GN-3: 99.6 %; GP: 96.4 %). There were no reports of side effects or any discomfort during the supplementation period for either group.

Body composition measurement

The body weight and height were measured and the body mass index calculated [BMI = body weight (kg)/height (m^2)]. The triceps brachii, subscapular, supra-iliac, and medial leg skinfolds were assessed using an adipometer (Cescorf, Porto Alegre, RS, Brazil) according to the guidelines of Harrison et al. [16], and the body fat mass and lean mass were estimated using the model proposed by Slaughter et al. [17].

Strength and performance evaluations

All evaluations of lower limb strength were held in half squat using a guided bar to ensure the safety of participants. The warm-up was composed of stretching and two series of ten repetitions with an unloaded bar (only the bar weight). After a 5 min rest, participants were required to start in full extension of the knee (180°), flex until 90° (among thigh and leg), and then extend again, returning to 180°.

One maximum repetition of knee extension (1RM)

The initial load was subjectively chosen according to the self-reported practice experience of each participant; the participant should be able to perform only one complete repetition of knee extension. Five attempts per day were allowed with a 5 min rest. If 1RM was not determined, the participant was asked to perform the test after a 48 h interval [18].

Maximum repetitions of knee extension (RMs)

Participants performed the greatest number possible of complete repetitions of half squat exercise with 70 % 1RM load. The participants were required to start in full extension of the knee (180°), flex until 90° (among thigh and leg), and return to full extension. Concentric and eccentric phases lasted one second each, according to a verbal command from the evaluator.

Incremental treadmill-running test (IT)

The participants underwent the incremental treadmill-running test (IT) on a motorized treadmill (Inbramed ATL, Porto Alegre, RS, Brazil), with a 1 % inclination, to determine the maximum velocity reached (V_{max}). After a 5 min warm-up at 7 km·h^{-1}, participants started the IT at 8 km·h^{-1} with increments of 1 km·h^{-1} each minute until voluntary exhaustion.

Statistical analysis

The results are presented as mean ± standard deviation (SD). Initially the homoscedasticity of the data was confirmed by the Shapiro–Wilk test. Then, an analysis of variance (ANOVA) two way (moment × groups) was performed to verify the possible differences between the supplementation moments (within-group − before × after) and the interaction between-groups (between-group − GP × GN-3). In addition to analysis of variance, Mauchly's sphericity test was applied and sphericity was assumed when no significant F value was observed. In case of violation of sphericity the Greenhouse–Geisser Epsilon correction was used. The analyses were completed with the Bonferroni post hoc.

To verify the possible differences within-group for RMs performed pre and post IT the paired t test was applied; and to test the internal load between-group the unpaired t test was applied. In all cases the level of significance was assumed as 5 % ($p < 0.05$). Statistical analyses were performed using SPSS 17.0 software for Windows.

Results

The mean of 28-days of the training load was not statistically different between-groups (GP: 777.2 ± 701.6 a.u. × GN-3: 515.4 ± 463.0 a.u.; $p = 0.42$).

The GN-3 group showed significant differences within-group for body fat mass (GP = before: 8.3 ± 2.1 kg × after: 7.7 ± 2.4 kg, GN-3 = before: 12.8 ± 9.4 kg × after: 11.8 ± 9.4 kg; $F_{(1,13)} = 7.4$, $p < 0.02$, post hoc: GP $p = 0.17$, GN-3 $p = 0.04$) and lean mass (GP = before: 61.8 ± 4.1 kg × after: 62.7 ± 3.9 kg, GN-3 = before: 64.2 ± 5.8 kg × after: 66.2 ± 6.0 kg; $F_{(1,13)} = 7.9$, $p = 0.02$, post hoc: GP $p = 0.21$, GN-3 $p = 0.02$), with no significant alterations for body weight (GP = before: 70.0 ± 5.3 kg × after: 70.4 ± 5.0 kg, GN-3 = before: 77.0 ± 14.2 kg × after: 78.0 ± 14.3 kg; $F_{(1,13)} = 2.4$, $p = 0.15$) or BMI (GP = before: 21.1 ± 1.3 kg m^{-2} × - after: 21.2 ± 1.0 kg m^{-2}, GN-3 = before: 22.6 ± 3.9 kg m^{-2} × after: 22.8 ± 4.1 kg m^{-2}; $F_{(1,13)} = 2.0$, $p = 0.19$). There were no significant interactions between-group for body composition variables ($F_{(1,13)} < 1.0$, $p > 0.59$) (Fig. 2).

The 1RM test was significantly altered within-group for the GN-3 group after the supplementation period with no significant differences for the GP (GP = before: 111.3 ± 29.1 kg × after: 111.3 ± 25.9 kg, GN-3 = before: 115.0 ± 36.2 kg × after: 129.1 ± 39.9 kg; $F_{(1,13)} = 4.9$, $p = 0.04$, post hoc: GP $p = 0.99$, GN-3 $p = 0.01$). There was no interaction between-group ($F_{(1,13)} = 4.9$, $p = 0.05$) (Fig. 3).

The maximum velocity reached in the IT (GP = before: 12.8 ± 2.2 km h^{-1} × after: 13.1 ± 2.1 km h^{-1}, GN-3 =

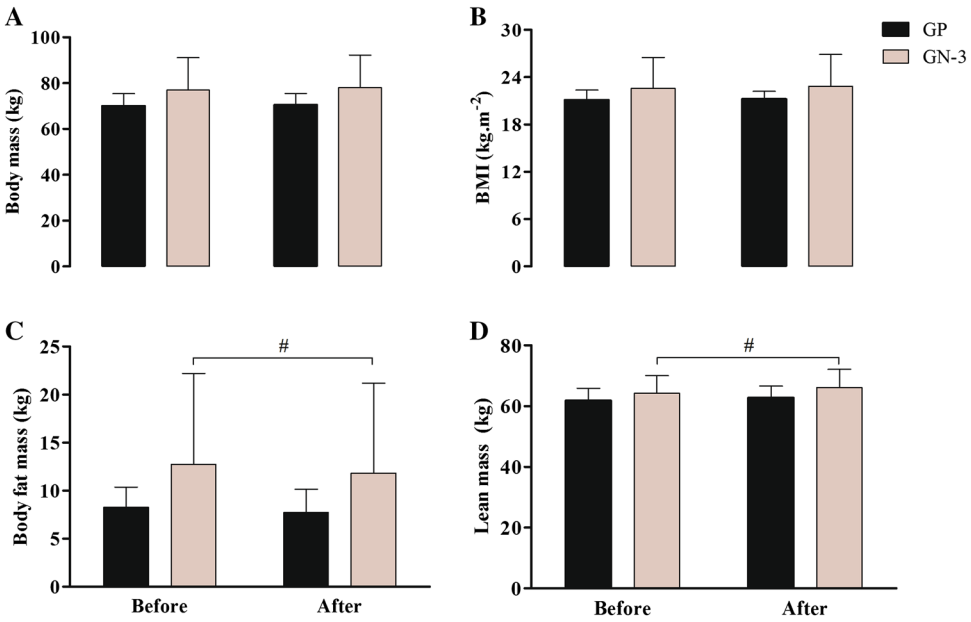

Fig. 2 Body composition variables. **a** Body mass; **b** body mass index (BMI); **c** body fat mass; **d** lean mass. #Within-group significant difference for GN-3 ($p < 0.05$)

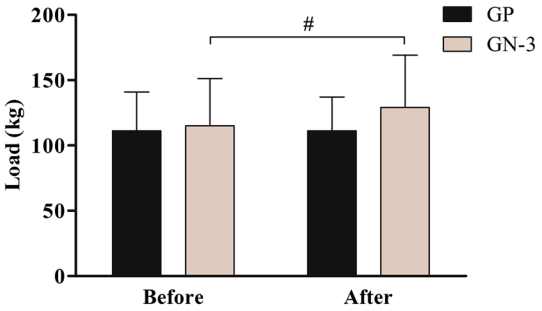

Fig. 3 One maximum repetition of knee extension (1RM). #Within-group significant difference for GN-3 ($p < 0.05$)

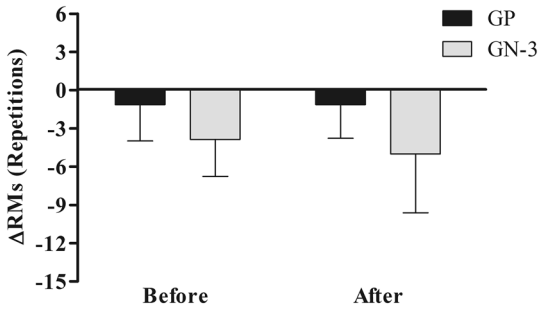

Fig. 4 Absolute variation in number of repetitions of knee extension pre and post incremental treadmill-running test before and after supplementation period

before: 12.9 ± 1.5 km h^{-1} × after: 13.1 ± 1.5 km h^{-1}) was not significantly different either within-group ($F_{(1,13)} = 1.2$, $p = 0.30$) or between-group ($F_{(1,13)} = 0.1$, $p = 0.77$). The paired t test revealed that the GP did not present significant alterations in the RMs either before (pre IT: 13.4 ± 5.5 repetitions × post IT: 12.3 ± 5.9 repetitions; $p = 0.30$) or after (pre IT: 12.6 ± 3.4 repetitions × post IT: 11.5 ± 4.3 repetitions; $p = 0.27$) the supplementation period. However GN-3 was significantly affected by IT and a decrease was observed in the number of RMs performed before (pre IT: 10.1 ± 3.9 repetitions × post IT: 6.3 ± 3.5 repetitions; $p = 0.01$) and after (pre IT: 12.1 ± 5.7 repetitions × post IT: 7.1 ± 4.7 repetitions; $p = 0.03$) the supplementation period (Table 1).

Furthermore, the absolute variation in RMs (ΔRMs = post IT − pre IT) did not demonstrate significant alterations (GP = before: -1.1 ± 2.9 repetitions × after: -1.1 ± 2.6 repetitions, GN-3 = before: -3.9 ± 2.9 repetitions × after: -5.0 ± 4.6 repetitions) either within-group ($F_{(1,13)} = 0.52$, $p = 0.49$) or between-group ($F_{(1,13)} = 0.52$, $p = 0.49$) (Fig. 4).

Discussion

The present study aimed to verify the possible influences of N-3 supplementation on body composition, maximum strength of leg extension, and protection against the neuromuscular fatigue process. The main findings were the improvement in maximum strength performance of the leg extension for the GN-3 but without any significant effect against neuromuscular fatigue. Additionally, the GN-3 presented a decrease in percentage of body fat with a concomitant increase in lean mass.

There is no consensus in the literature regarding N-3 supplementation and positive changes in body composition. DeFina et al. [19] found no effects of N-3 supplementation combined with aerobic exercise to improve body composition in sedentary individuals, however Hill et al. [1] conducted a study with similar interventions and observed significant decreases in body fat mass. Furthermore, Smith et al. [20] did not verify significant improvements in body weight, total body fat mass or intermuscular fat content in elderly people after 6-months of supplementation, however there were significant increases in thigh muscle volume, handgrip strength, and 1RM strength of leg press, chest press, knee extension, and knee flexion.

Therefore, the absence of studies verifying the influence of N-3 supplementation on body composition in healthy adults or athletes makes conclusions difficult. In the present study we found decreased body fat mass (pre: 12.8 ± 9.4 kg; post: 11.8 ± 9.3 %) and increased lean mass (pre: 64.2 ± 5.8 kg; post: 66.2 ± 6.0 kg) for the GN-3, with no significant changes in the GP. The main hypothesis regarding the mechanism of N-3 in decreasing body fat mass is regarding the up-regulation of several

Table 1 Mean ± SD of neuromuscular fatigue resistance pre and post incremental treadmill-running test before and after supplementation period

	Before supplementation			After supplementation		
	Pre IT (repetitions)	Post IT (repetitions)	V_{max} IT (km h^{-1})	Pre IT (repetitions)	Post IT (repetitions)	V_{max} IT (km h^{-1})
GP	13.4 ± 5.5	12.3 ± 5.9	12.8 ± 2.2	12.6 ± 3.4	11.5 ± 4.3	13.1 ± 2.1
GN-3	10.1 ± 3.9	$6.3 \pm 3.5^{\#}$	12.9 ± 1.5	12.1 ± 5.7	$7.1 \pm 4.7^{\#}$	13.1 ± 1.5

Pre IT number of repetition of knee extension performed before incremental treadmill-running test, *post IT* number of repetition of knee extension performed after incremental treadmill-running test; V_{max} maximum velocity reached on incremental treadmill-running test

Significant differences for pre IT within-group ($p < 0.05$)

nuclear receptors that contribute to an increase in fatty acid mobilization and the effectiveness of β-oxidation in muscle [21]. Flachs et al. [22] verified in mice that N-3 supplementation induced mitochondrial biosynthesis (i.e., by increasing peroxisome proliferator-activated receptor gamma co-activator 1 alpha—PGC-1α) as well as fatty acid mobilization (i.e., by increasing carnitine palmitoyltransferase 1), however exercise has similar effects in addition to prompting muscle hypertrophy. In this way the absence of a non-exercise group (i.e., group that did not practice any kind of physical activities during the supplementation period) could generate a confounding factor, especially with respect to the lean mass results. Based on our results and those in the literature [3] it is possible to suggest that N-3 supplementation may represent an interesting strategy to improve body composition by increasing lean mass and decreasing fat mass.

To our knowledge there are few investigations on the effects of N-3 supplementation and muscle strength performance. Rodacki et al. [23] verified a significant improvement in maximal strength of the knee extensor, knee flexor, plantar flexor and dorsiflexor muscles in elderly women after a long-term N-3 supplementation program (2 g·day^{-1} more than 90-day). Lewis et al. [8] verified an unclear effect of N-3 supplementation on maximal voluntary contraction of the knee extensors after 21-day of N-3 supplementation; however, the same study verified a beneficial increase in electromyography activity during maximal voluntary contraction in the N-3 group.

The present study verified a significant increase in 1RM for the GN-3 group (pre: 115.0 ± 36.2 kg; post: 129.1 ± 39.9 kg). The improvement in muscle strength concomitant with lean mass for the GN-3 could be a direct result of the N-3 supplementation since it was verified that N-3 may induce an improvement in the rate of synthesis, muscle content, phosphorylation, and protein-to-DNA ratio of some target proteins such as mTOR and p70s6k, which are directly involved in muscle anabolic signaling [24, 25].

Furthermore, no significant effect of N-3 supplementation was verified as a protective factor against neuromuscular fatigue, since there were no differences for the absolute variations in number of repetitions of knee extension and maximum velocity reached in the incremental treadmill-running test. Similarly, Huffman et al. [6] administered 4 g·day^{-1} of N-3 to 10 active individuals for 4 weeks and found no increase in time-to-exhaustion in running on a treadmill performed immediately after 75 min running at submaximal intensity, and Buckley et al. [2], found no improvement in performance of exercise until exhaustion and recovery time after 5 weeks of N-3 supplementation. Despite the fact that N-3 may improve muscular strength, it is also postulated that N-3 could increase insulin sensitivity, decreasing the triglycerides store and affecting the energetic sources during exercise [6]. This might be a possible explanation for the GN-3 presenting higher absolute variation in the number of repetitions of knee extension than the GP. However, these results should be viewed with caution, since the number of participants and period of supplementation were small.

The main limitations of the present study were the absence of direct measurement of N-3 plasma concentrations and fine control of training characteristics. Future research could include a non-exercise group, and investigation into the characteristics of neuromuscular fatigue induced by an incremental treadmill-running test (i.e.; use of the twitch interpolation technique for identification of the central or peripheral characteristics of neuromuscular fatigue).

Thus, it is concluded that 28 days of supplementation with 1.4 g·day^{-1} of N-3 seems to contribute to maximum strength of knee extension (1RM) and improvement in body composition, however it is unclear if N-3 supplementation can act as a protection factor for neuromuscular fatigue.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Committee of the UNISALESIANO-Catholic Center University (Protocol 867.319/2014) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in study.

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