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Review

The Influence of n-3PUFA Supplementation on Muscle Strength, Mass, and Function: A Systematic Review and Meta-Analysis

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A B S T R A C T

The effects of omega 3 polyunsaturated fatty acids (n-3PUFA) supplementation on skeletal muscle are currently unclear. The purpose of this systematic review was to synthesize all available evidence regarding the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults. Four databases were searched (Medline, Embase, Cochrane CENTRAL, and SportDiscus). Predefined eligibility criteria were determined according to Population, Intervention, Comparator, Outcomes, and Study Design. Only peer-reviewed studies were included. The Cochrane RoB2 Tool and the NutriGrade approach were used to access risk of bias and certainty in evidence. Effect sizes were calculated using pre–post scores and analyzed using a three-level, random-effects meta-analysis. When sufficient studies were available, subanalyses were performed in the muscle mass, strength, and function outcomes according to participant's age (<60 or ≥60 years), supplementation dosage (<2 or ≥2 g/day), and training intervention ("resistance training" vs. "none or other"). Overall, 14 individual studies were included, total 1443 participants (913 females; 520 males) and 52 outcomes measures. Studies had high overall risk of bias and consideration of all NutriGrade elements resulted in a certainty assessment of moderate meta-evidence for all outcomes. n-3PUFA supplementation had no significant effect on muscle mass (standard mean difference [SMD] = 0.07 [95% CI: −0.02, 0.17], $P = 0.11$) and muscle function (SMD = 0.03 [95% CI: −0.09, 0.15], $P = 0.58$), but it showed a very small albeit significant positive effect on muscle strength (SMD = 0.12 [95% CI: 0.006, 0.24], $P = 0.04$) in participants when compared with placebo. Subgroup analyses showed that age, supplementation dose, or cosupplementation alongside resistance training did not influence these responses. In conclusion, our analyses indicated that n-3PUFA supplementation may lead to very small increases in muscle strength but did not impact muscle mass and function in healthy young and older adults. To our knowledge, this is the first review and meta-analysis investigating whether n-3PUFA supplementation can lead to increases in muscle strength, mass, and function in healthy adults. Registered protocol: doi.org/10.17605/OSF.IO/2FWQT.

Keywords: n-3PUFA, Omega 3, strength, hypertrophy, muscle mass, muscle function

Statement of Significance

To our knowledge, this is the first review and meta-analysis investigating whether n-3PUFA supplementation can lead to increases in muscle strength, mass, and function in healthy adults.

Abbreviations used: ACSA, muscle cross-sectional area; ALA, alfa-linoleic fatty acid; DHA, docosahexaenoic fatty acid; DXA, dual-energy X-ray absorptiometry; EPA, eicosapentaenoic fatty acid; $F_{df1,df2}$, omnibus moderator test statistic; FFM, fat-free mass; FM, fat mass; FO, fish oil; n-3PUFA, omega 3 polyunsaturated fatty acids; OSF, open science framework; PLA, placebo; RoB2, Risk of Bias 2; 1-RM, one-repetition maximum test; SMD, standard mean difference.

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Introduction

Omega 3 polyunsaturated fatty acids (n-3PUFA) are an essential class of long-chain polyunsaturated fatty acids [1], whose main forms are the α-linolenic acid (18: 3n-3), present in oleaginous fruits and their resulting vegetable oils, such as flaxseed or chia seeds and canola oil; the eicosapentaenoic acid (EPA, 20: 5n-3); and the docosahexaenoic acid (DHA, 22: 6n-3), which are mainly present in oily, cold-water fish, fish oil, and crustaceans [2,3].

n-3PUFAs mediate several biological processes [4] and have been shown to benefit human health via improvements in immune function, inflammation, cognition, lipid profile, and neuromuscular function [5,6]. Since dietary sources of n-3PUFA are somewhat scant, its supplementation is widely prescribed by health professionals for patients with dyslipidemia, atherosclerosis, obesity, and metabolic syndrome [7].

In addition to its well-known effects on metabolic health, cell, animal, and even human studies have recently indicated that n-3PUFA supplementation could increase expression of genes and proteins involved in skeletal muscle hypertrophy [8–10], via increased incorporation into myocyte membrane phospholipids. To date, studies suggest that n-3PUFA may increase the uptake of amino acids by increasing skeletal muscle membrane fluidity and intracellular signaling of the mammalian Target Of Rapamycin—p70s6k pathway, the main regulatory pathway for protein synthesis [10–12]. Moreover, it has been suggested that n-3PUFA may reduce protein degradation by inhibiting Nuclear factor kappa-light-chain-enhancer of activated B cells and increase myocyte sensitivity to acetylcholine signaling, improving skeletal muscle contractility [13–19]. Theoretically, this could contribute to gains in muscle mass and strength; however, the practical application and efficacy of this approach have yet to be ascertained.

Several studies have investigated the influence of n-3PUFA supplementation on muscle mass, strength, and function, but results are conflicting. Although some studies report increased rates of protein synthesis, fat-free mass and physical capacity, and decreased pain and fatigue after n-3PUFA supplementation [10, 20–23] others show no effect on these outcomes [24–27]. Two recent meta-analyses on the topic also show contradictory results. Delpino and Figueiredo [28] found no effect of n-3PUFAs supplementation on lean body mass in adults and elderly participants who were either healthy or had type 2 diabetes and/or cardiovascular diseases [28]. In contrast, Huang et al. [29] showed minor increases in muscle mass and function after n-3 PUFA supplementation when compared with placebo in elderly participants, who were either healthy or had chronic diseases, with greater increases observed in studies with doses equal or higher than 2 g/day and a follow-up period longer than 6 months [29].

This heterogeneity in research findings may be because of different populations (e.g., younger vs. older adults and healthy vs. patients with chronic diseases) and protocols (e.g., n-3PUFA supplementation associated or not with resistance training) used in these studies. As such, it remains to be confirmed as to whether n-3PUFA supplementation can lead to increases in muscle strength, mass, and function in healthy populations.

Thus, the purpose of the current investigation is to synthesize all available evidence related to the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy adults and older adults using a systematic review and meta-analytic approach.

Methods

The protocol for this review was prospectively registered on the Open Science Framework (OSF, doi.org/10.17605/OSF.IO/2FWQT). It adheres to previously published guidelines [30] and includes all items described in the checklist of Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols [31]. The Population, Intervention, Comparator, Outcomes, and Study Design approach (Population, Intervention, Comparator, Outcomes, and Study Design) was used to guide the determination of the eligibility criteria for this review (see Table 1).

Search strategy

Four electronic databases were used for this review, namely Medline (Pubmed), Embase, Cochrane CENTRAL, and Sport Discus. This primary database search strategy was complemented by citation screening of all studies included in the review along with relevant reviews and book chapters [28, 32–37]. Free-text terms used for the search were n-3PUFA OR Omega 3 OR EPA OR DHA OR fish oil*) AND (supplementation) AND (muscle strength OR muscle mass OR protein synthesis OR performance OR hypertrophy OR lean body mass OR lean mass). Searches were limited to human studies, and no restrictions were placed on either date or language. Only peer-reviewed studies published in scientific journals were considered for inclusion. Search results from each database were downloaded as a .ris file then uploaded to a systematic review management software (rayyan.org) and deduplicated using the automatic option

TABLE 1
PICOS approach for eligibility criteria of studies assessing the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults

Population	Healthy adults of any age, sex or training status, without chronic diseases.
Intervention	Supplementation of n-3PUFA[12], irrespective of supplementation dose or length, conducted, or not, alongside any physical activity or exercise training intervention. Studies that supplemented n-3PUFA alongside other supplements were not considered, unless a n-3PUFA[1] only condition was included.
Comparator	A control group who took an inactive placebo supplement not containing n-3PUFA[12] (e.g., palm, olive, corn, safflower, or soy oil).
Outcomes	The primary outcome of interest was muscle mass, and secondary outcomes of interest were strength and muscle function. Muscle mass was considered if assessed using dual-energy X-ray Absorptiometry (DXA), Bod Pod or hydrostatic underwater weighing. Other measures of muscle mass, i.e., those assessed by ultrasound (muscle volume), or muscle biopsies (muscle cross-sectional area) were also considered. Muscle strength were considered if assessed through one-repetition maximum test (1RM), maximal isomeric torque, isokinetic tests, muscle endurance and power tests. Muscle function was considered if accessed by testes such as timed up and go, sit to stand, stand up, and gait speed tests, etc.
Study Design	Randomized, placebo-controlled, single or double-blinded trials.

n-3PUFA: Omega 3 polyunsaturated fatty acids.

provided therein. Searches were initially done on July 12, 2021, and later updated on September 16, 2022.

Selection process and data extraction

A 3-stage selection strategy was independently undertaken by HCSA and FL (title/abstract screen; full-text screen/full-text appraisal), and the results were filtered using the eligibility criteria described above. The independent screeners were not blinded to any study information and convened at the end of each screening stage to resolve any discrepancies. These discrepancies were resolved by discussion mediated by GPE. During the full-text screen and review stages, reasons for exclusion were categorized as 1 or more of the following: (1) inappropriate population, (2) inappropriate intervention, (3) inappropriate comparator, (4) inappropriate outcome, (5) inappropriate study design, and (6) others. Data were extracted from included studies into a prepiloted excel sheet.

Risk of bias and certainty of cumulative outcome

The risk of bias for each individual study was assessed using Version 9 of the Cochrane risk-of-bias tool for randomized trials 2 (RoB2) (www.riskofbias.info) [38], which is the recommended tool by Cochrane Reviews and considers 5 bias domains: (1) risk of bias arising from the randomization process; (2) risk of bias because of deviations from the intended interventions; (3) risk of bias because of missing outcome data; (4) risk of bias in measurement of the outcome; and (5) risk of bias in selection of the reported result. Certainty in evidence was accessed using the NutriGrade scoring system [39]. This instrument considers 7 items for meta-analyses (risk of bias, study quality and study limitations; Precision; Heterogenicity; Directness; Publication Bias; Funding Bias; and Study Design); the overall score is summarized as follows: 0–3.99: very low meta-evidence; 4–5.99: low meta-evidence; 6–7.99: moderate meta-evidence; ≥8: high meta-evidence.

Statistical analysis

As described in Table 1, selected outcomes of interest comprise measurements on different scales, but that closely relate to the same construct. Although this is a strong assumption, this allows for the inclusion of a broader range of studies and outcomes and for a better representation of the literature. As such, these measurements were extracted and converted into standardized effect sizes (standardized mean differences, SMD) and their variances using pre- and post-intervention scores from the placebo and intervention groups. The specific approach used was the effect size d_{ppc2} and its accompanying variance $\sigma^2_{(dppc2)}$ according to Morris, 2007 [40] (see equations 8 and 25 for specific formulas used), since this approach was shown to be most accurate when estimating sampling variance. To calculate the variance, a pre–post correlation value was required. Studies rarely report pre–post correlations associated with their studies; therefore, 1 correlation value for each outcome was used. To estimate adequate correlation values, preliminary data from an undergoing Randomized Clinical Trial within our own group was used, which investigates the role of n-3PUFA supplementation on muscle mass and strength in young adults undergoing resistance training. Using data from 9 participants, a correlation value of 0.95 for muscle mass and 0.65 for strength outcomes was found. As such, our selected correlation values were 0.90 for muscle mass outcomes (which is slightly more conservative than what was found), and 0.65 for both strength and function outcomes. We also report a sensitivity analysis with a correlation value of 0.7, which is a typically used default value for strength-related outcomes [41].

Studies included herein commonly reported data from >1 test related to the same outcome, with the same participants. Approaches to deal with these dependencies in the data are many [see chapter 24 in [42,43]], but a typically used approach is a 3-level random-effects model, in which outcomes and studies are considered the second and third levels of the model, respectively [44]. This was our selected approach, as it allowed for inclusion of all available studies, and thus, a better representation of the literature. The 3-level model was done by identifying each study and each outcome with an identification number, and then setting up the 3-level design within the rma.mv function. As described by Noortgate et al. [44], this approach models the sampling variation for each outcome (comprising the first level of the model); variation across outcomes within a study (comprising the second level); and variation across studies (comprising the third level). To achieve this, 3 separate equations are used, 1 for each level of the model [see equations 3, 4, and 5, respectively, in [44]]. Herein we report overall random-effects estimates obtained from each model, alongside 95% confidence intervals (95% CI), and the estimated variance within levels 2 and 3 (*i.e.,* variance between outcomes and variance between studies). Importantly, 3-level meta-analytic models have limitations and assumptions. As typical 2-level meta-analyses, these models assume that effect sizes are a random sample from a population of effects, which can be untrue in the case of publication bias. Additionally, multilevel models also assume that outcomes have a common between-study variance, and the same between-outcome covariance. Although these are strong assumptions that are commonly violated, Noortgate et al. [44] show that multilevel models are robust and can provide accurate effect size and error estimations even in these and other realistic conditions.

To analyze the role of potential moderators (age, supplementation dosage/day, and training intervention), these were included as fixed effects in the model. These moderator analyses were attempted only when a minimum of 4 outcomes per subgroup type were available [45]. All moderator analyses comprise categorical moderators containing 2 levels. Results are presented such that the first level represents the intercept (reference level), with the second level representing the average difference between the 2 levels of the moderator. Heterogeneity was mainly assessed using tau^2 values alongside its CIs. I^2 values, alongside corresponding CIs for each hierarchical level (outcome and study), are also reported as a supplementary material (**Supplementary File 1**—Supplementary Table 1). Small-study effects were assessed by visual inspection of funnel plots and by the Egger's regression–intercept test (44). SMDs of 0.01, 0.2, 0.5, 0.8, and 1.2 were considered as very small, medium, large, and very large, respectively [46]. Statistical significance was previously set at $P < 0.05$. All data were analyzed using Rstudio software (R version 4.2.0, Vienna, Austria; Rstudio version 1.4.1103, PBC, USA) and the rma.mv function from the metafor package [47]. R code and dataset utilized for the analyses and visualizations shown herein are fully available online (see Data Sharing section).

Results

A flowchart of the selection process is available in Figure 1. Seven studies had missing data in the published article and authors were contacted to request additional information. However, none of the authors replied. Three of these studies were still included, as the primary outcome data was available [48–50], and the other 4 were excluded from this review [51–54]. Thus, this systematic review included a total of 14 studies, comprising 1433 participants (913 females; 520 males); 3 studies with adults (mean age: 26.49 years) and 11 studies with older adults (mean age: 70.6 years) [21, 23, 48–50, 55–63]. Regarding concomitant exercise intervention, 5 studies provided the supplement alongside a resistance training intervention, 1 study had a daily walk intervention, and 8 studies did not have exercise. Thus, for the moderator analysis according to intervention type, we divided studies into 2 subgroups ("resistance training" vs. "none or other"). A summary of the study design, population, and dosing protocol of all included studies is available in Table 2. Sensitivity analysis assuming a correlation of 0.7 led to no major differences in results (**Supplementary File 1**—Supplementary Table 2), and the results presented herein were obtained using the correlation values obtained from our own sample of individuals.

Muscle mass

As described in Table 1, various outcomes representing muscle mass were included. In the interest of consistency, these will be collectively described as muscle mass throughout the

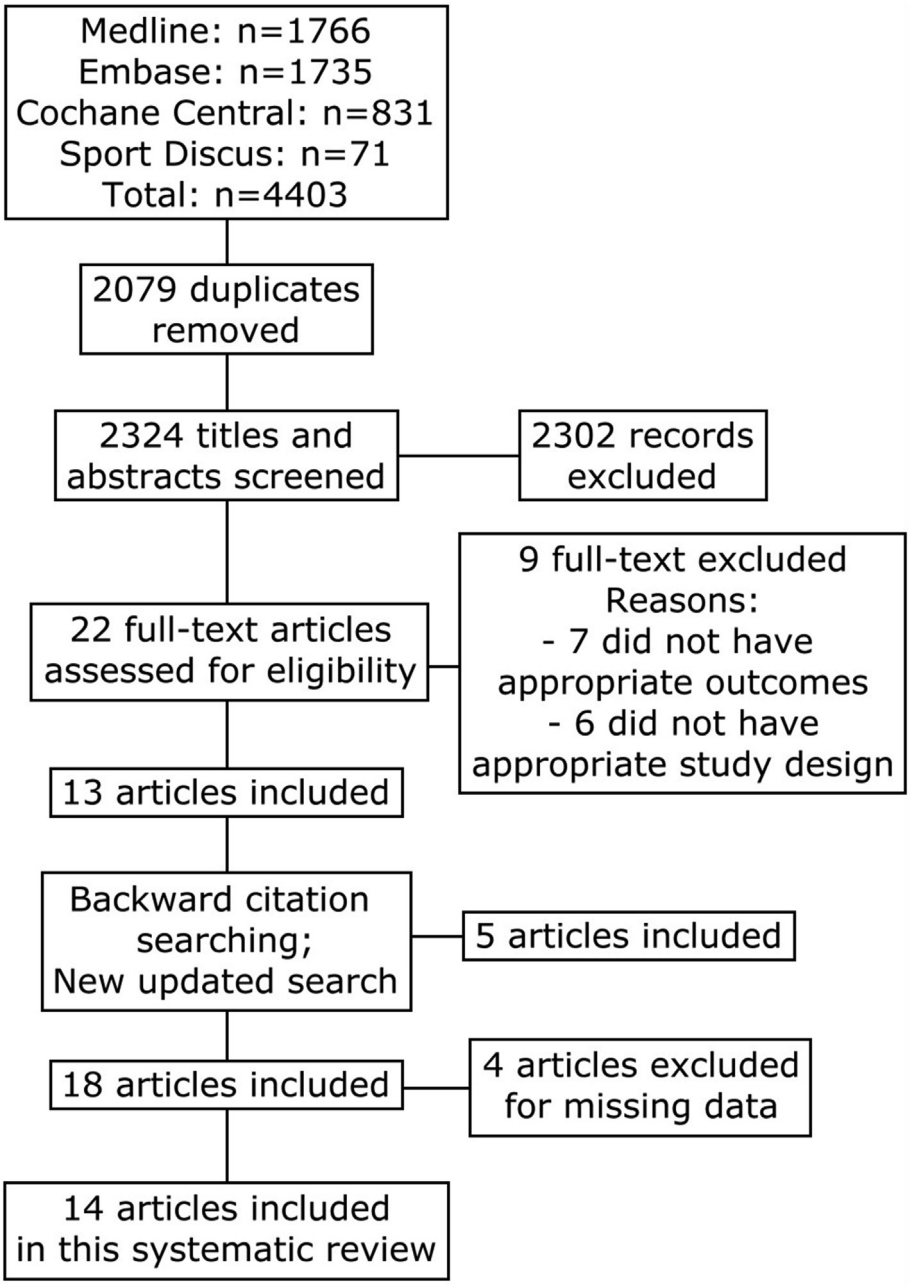

FIGURE 1. Flow diagram for screening and selection of studies assessing the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults.

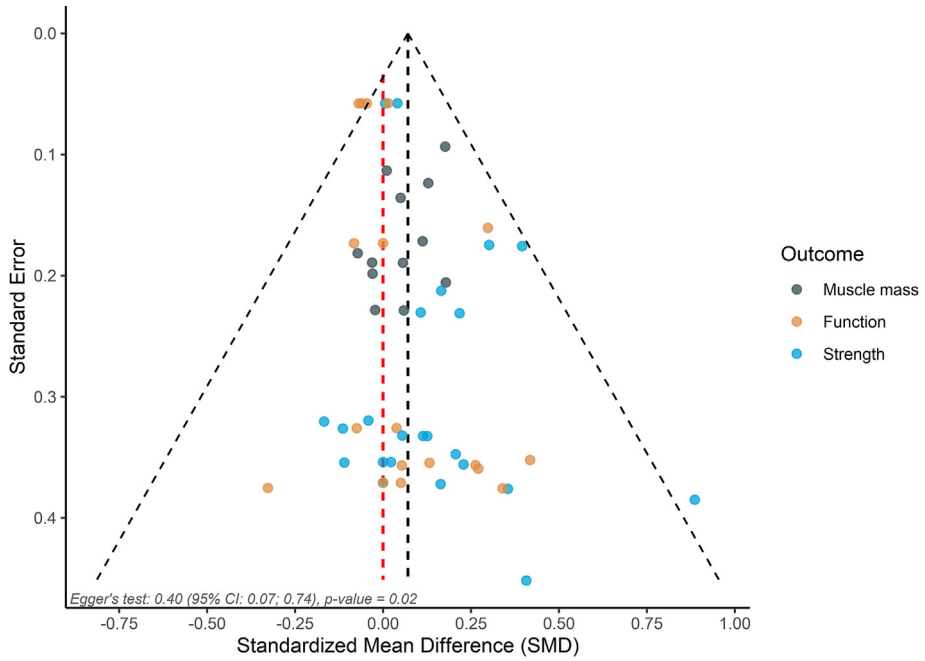

FIGURE 2. Funnel plot of studies assessing the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults. SMD: standardized mean differences.

manuscript. Nine studies were included in this analysis, totaling 12 outcomes [21,48,55–58,60,61,63]. Overall, n-3PUFA fatty acids had no effect on muscle mass when compared with placebo (SMD = 0.07 [95% CI: −0.02, 0.17], $P = 0.11$, Figure 3). Heterogeneity was low both between outcomes (level 2 variance, $\sigma^2 = 0.000$ [95% CI: 0.000, 0.02]) and between studies (level 3 variance, $\sigma^2 = 0.000$ [95% CI: 0.000, 0.02]). A subanalysis for the effect of intervention type showed no difference between interventions (none or other (reference): SMD = 0.10 [95% CI: −0.03, 0.22], $P = 0.11$; Resistance training: −0.07 [95% CI: −0.27, 0.13], p value = 0.45), with the test for moderators being nonsignificant (F = 0.59, $P = 0.45$).

Strength

In this analysis, 11 studies were included, with a total of 23 outcomes (23,48,49,55,57–63). N-3PUFA supplementation had a significant, albeit very effect on strength (SMD = 0.12 [95% CI: 0.004, 0.24], $P = 0.04$, Figure 3). Heterogeneity was low both between outcomes (level 2 variance, $\sigma^2 = 0.000$ [95% CI: 0.000, 0.023]) and between studies (level 3 variance, $\sigma^2 = 0.008$ [95% CI: 0.000, 0.058]). Moderator analyses were performed based on supplementation dosage category, the presence of a resistance training intervention, and age (see Table 3 for a summary of all models). The study done by Cornish and Chilibeck (57) was classified as "≥ 2 g/d", although it may be important to highlight that it supplemented individuals with alpha-Linoleic n-3 fatty acids (56). There was no significant effect of supplementation dosage (< 2 g/d SMD = 0.14 [95% CI: −0.05, 0.33], $P = 0.14$; >2 g/d (SMD = −0.01 [95% CI: −0.27, 0.24], $P = 0.87$), with the moderator test being nonsignificant (F = 0.02, $P = 0.87$). A sensitivity analysis done by removing Cornish and Chilibeck (57) from the ≥2g/d" group did not meaningfully influence these results (57). There was no effect of intervention type (none or other SMD = 0.14 [95% CI: −0.01, 0.28], $P = 0.06$); resistance training SMD = −0.05 [95% CI: −0.33, 0.24], $P = 0.74$), with the moderator test being nonsignificant ($F = 0.10$, $P = 0.74$). Finally, there was no significant effect of age group (<60 years SMD = 0.15 [95% CI: −0.25, 0.56], $P = 0.44$; ≥60 years (SMD = −0.03 [95% CI: −0.45, 0.40], $P = 0.88$), with the test for moderators being nonsignificant (F = 0.02, $P = 0.88$).

Function

Seven studies were included in this analysis, with a total of 17 outcomes (49,50,55,58,60–62). n-3PUFA supplementation did not significantly affect performance in functionality tests (SMD = 0.03 [95% CI: −0.09, 0.15], $P = 0.58$, Figure 3). Heterogeneity was low both between outcomes (level 2 variance, $\sigma^2 = 0.000$ [95% CI: 0.000, 0.011]) and between studies (level 3 variance, $\sigma^2 = 0.000$ [95% CI: 0.000, 0.087]). Supplementation dose had no significant effect (<2 g/d (SMD = −0.003 [95% CI: −0.12, 0.11], $P = 0.94$; ≥2 g/d (SMD = 0.11 [95% CI: −0.15, 0.38], $P = 0.38$)) on muscle function, with the moderator test being nonsignificant (F = 0.79, $P = 0.38$). Training intervention had no significant effect (none or other SMD = 0.02 [95% CI: −0.12, 0.17], $P = 0.72$; resistance training SMD = 0.05 [95% CI: −0.25, 0.35], $P = 0.72$), with the moderator test being nonsignificant (F = 0.12, $P = 0.72$). No moderator analyses for age were performed in this outcome because of all studies involving older subjects.

Reporting biases and certainty in outcomes

Results from the RoB2 assessment are available in Figure 4. Potential sources of bias within the selected studies were mainly because of a lack of detail in reporting, including a lack of information on randomization and concealment approaches (Domain 1), nonreporting of adherence or compliance information (Domain 2), missing information on outcome measurements (Domain 4), and lack of a preregistered protocol or analysis plan (Domain 5). The complete decision rationale of RoB2 is available in Supplementary File 2.

TABLE 2
Individual results of studies assessing the influence of n-3PUFA supplementation on lean mass, strength, and function in healthy young and older adults

Author	Reference number	Blind ing	*N*	Participant's characteristics	Supplementation	Exercise intervention	Study duration	Effects on body composition	Effects on muscle strength and function
Alkhedhairi et al.	55	Double-blinded randomized	94	Healthy, untrained older men and women	n-3 group: Krill Oil (4 g; 0.39 g EPA and 1.92 g DHA) PLA group: Mixed Vegetable Oil (4 g) Compliance not reported.	No exercise intervention	24 weeks	No group or time effect was detected on muscle thickness (via Ultrasound).	Knee extensor maximal torque and Hand grip strength were higher in the krill oil, relative to the control, group at 6 months. No differences between groups for measures of muscular function (Repeated chair rises, 4-m walking speed and short physical performance battery test).
Brook et al.	56	Double-blinded randomized	16	Healthy, untrained older women	n-3 group: n-3 supplement (3.68 g; 1.86 g EPA and 1.54 g DHA), 99% compliant PLA group: Corn Oil (3.68 g). 99% compliant.	Unilateral Resistance Training (3 times per week)	6 weeks	No changes in total mass, lean mass, bone mass or body fat percentage in either group (DXA).	No differences between groups for measures of muscular strength (1-RM).
Cornish & Chilibeck	57	Double-blinded randomized	51	Healthy, untrained older men and women	n-3 group: Flaxseed Oil (30 mL/d; 14 g/d ALA), 83.6 ± 14.4% compliant PLA group: Corn Oil (30 mL/d) 78.2 ± 21.0% compliant.	Resistance Training (3 times per week)	12 weeks	No differences between groups for measures of body composition (DXA). No differences between groups for measures of knee flexor muscle thickness (Ultrasound).	No differences between male or female ALA and placebo groups for measures of muscular strength (1-RM).
Cornish et al.	58	Double-blinded randomized	23	Healthy, untrained older men	n-3 group: n-3 supplement (3 g; 1.98 g EPA and 0.99 g DHA), 96.7% compliant PLA group: omega 3-6-9 blend (3 g), 87.3% compliant.	Resistance Training (3 times per week)	12 weeks	No differences between groups for measures of body composition (DXA).	No differences between groups for measures of muscular strength (1-RM) or function (Timed up and go test and 6-minute walk test).
Crestani et al.	59	Double-blinded randomized	15	Healthy adult men	n-3 group: n-3 supplement (1.4 g), 99.6% compliant PLA group: Safflower oil (4 g), 96.4% compliant.	No exercise intervention	4 weeks	No measurement included.	n-3 group had a significant improvement on 1-RM test, whereas PLA group did not have the same results. There were no significant between-group interaction.
Da Boit et al.	60	Double-blinded randomized	50	Healthy, untrained older men and women	n-3 group: n-3 capsules (3 g, 2.7 g EPA + DHA) PLA group: Safflower oil (3 g) Compliance not reported.	Resistance Training (2 times per week)	18 weeks	No differences between groups on muscle ACSA or fat. Women on n-3 group had a greater increase on muscle ACSA (27.0 ± 17.1%) than women on PLA group (8.8 ± 17.6%).	No differences between groups on maximal isokinetic torque, 4-m walk time, chair-rise time, or maximal isometric torque.
Dalle et al.	61	Double-blinded randomized	23	Healthy, untrained older men and women	n-3 group: n-3 soft gels (3.06 g, 1.23 g DHA and 1.62 g EPA) PLA group: Corn Oil (3.3 g) Compliance not reported.	Resistance Training (3 times per week) for 12 weeks	14 weeks	No differences between groups for measures of muscle volume.	No differences between groups for measures of muscular strength (1-RM[1]) or function. Isometric strength was increased in n-3 (+ 12.2%) and not in PLA group.
Gravina et al.	23	Double-blinded randomized	26	Men and women professional soccer players	n-3 group: 0.1 g/kg weight (mean intake 7 ± 2 capsules/day, each capsule 0.7 g EPA 0.2 g DHA) PLA group: 0.1 g/kg weight (mean intake 7 ± 2 capsules/day).	No exercise intervention	4 weeks	No measurement included.	No differences between groups for measures of muscular strength (1-RM) or on Vertical squat jump test. On Yo-yo test there was a significant increase only in the n-3 group ($P < 0.01$).

Hutchins-wiese et al.	50	Double-blinded randomized	118	Healthy, untrained older women	n-3 group: Fish Oil (2 g; 0.72 g EPA 0.48 g DHA), 82% compliance PLA group: Olive Oil (2 g; 1.8 g oleic acid), 78% compliance.	No exercise intervention	24 weeks	No measurement included.	No differences between groups for measures of muscular strength (Handgrip) or function (Repeated chair rises). Walking speed increased in the n-3 PUFA group compared with placebo.
Kunz et al.	63	Double-blinded randomized	63	Healthy, untrained older men and women	n-3 group: n-3 supplement (4 g; 2.7 g EPA and 1.20 g DHA), compliance demonstrated by increase on EPA and DHA serum levels PLA group: Corn Oil (4 g). Compliance demonstrated by no change on EPA and DHA serum levels.	No exercise intervention	24 weeks	No differences between groups for measures of body composition (DXA).	No differences between groups for measures of muscular strength (1-RM).
Logan & Spriet	62	Single-blinded randomized	24	Healthy, untrained older women	n-3 group: Fish Oil (5 g; 2 g EPA and 1 g DHA), compliance demonstrated by increase on EPA and DHA serum levels. PLA group: Olive Oil (3 g). Compliance demonstrated by no change on EPA and DHA serum levels.	No exercise intervention	12 weeks	No measurement included.	No differences between groups for measures of muscular strength (Handgrip). No differences between groups on 30-Second Chair Stand. Decrease in Timed Get Up and Go Test speed (7%; $P = 0.006$) of 0.5 ± 0.2 s was found only in the FO group. There were no significant between-group interaction.
Noreen et al.	21	Double-blinded randomized	44	Healthy, untrained adult men and women	n-3 group: Fish oil (4 g; 1.6 g EPA and 0.8 g DHA) PLA group: Safflower oil (4 g) Compliance not reported.	No exercise intervention	6 weeks	Change in fat-free mass and fat mass (BodPod) over time was significantly different between the treatments (FFM: SO = -0.1 ± 1.2 kg; FO = $+0.5 \pm 0.5$ kg; $P = 0.03$/FM: SO = 0.2 ± 1.2 kg; FO = -0.5 ± 1.3 kg; $P = 0.04$).	No measurement included.
Rolland et al.	49	Double-blinded randomized	842	Untrained older men and women	n-3 group: Fish Oil (2 g; 0.8 g EPA and 0.23 g DHA) PLA group: Flavored Paraffin oil (2 g). General compliance $\geq 75\%$[1].	30 minute/day walk	156 weeks (3 years)	No measurement included.	No differences between groups for measures of muscular strength (Handgrip) or function (Repeated chair rises, 4-m walking speed and short physical performance battery test).
Smith et al.	48	Double-blinded randomized	44	Healthy, untrained older men and women	n-3 group: n-3 supplement (4 g; 1.86 g EPA and 1.50 g DHA), 93.6% compliance PLA group: Corn Oil (4 g). 91.8% compliance.	No exercise intervention	24 weeks	No differences between groups for measures of body composition (DXA). n–3 therapy increased thigh muscle volume (treatment effect: 3.6%; 95% CI: 0.2%, 7.0%; *P*, 0.05).	n–3 therapy increased handgrip strength (2.3 kg; 95% CI: 0.8, 3.7 kg; *P*, 0.01), and 1-RM muscle strength (4.0%; 95% CI: 0.8%, 7.3%; *P*, 0.05). No significant differences on Isokinetic muscle power.

1-RM, one-repetition maximum test; ACSA, muscle cross-sectional area; ALA, alfa-linoleic fatty acid; DHA, docosahexaenoic fatty acid; DXA, dual-energy X-ray absorptiometry; EPA, eicosapentaenoic fatty acid; FFM: fat-free mass; FM, fat mass; FO, fish oil; PLA, placebo.

[1] Participants were deemed adherent if they attended at least 75% of the prescribed capsules.

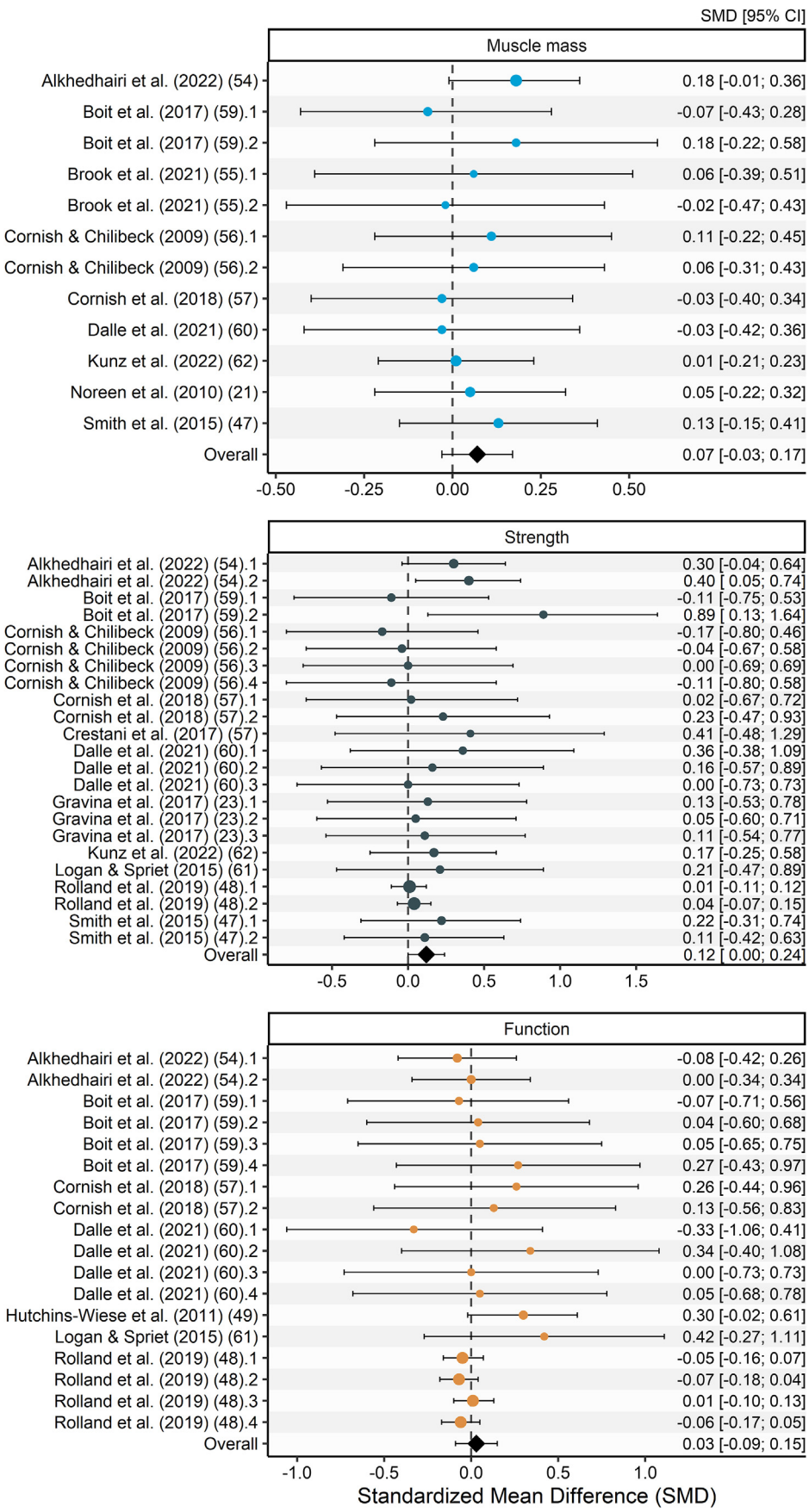

FIGURE 3. Forest plot of the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults, showing individual outcomes and results from the random-effects meta-analyses as standardized mean differences and 95% CIs. Data are separated according to outcome type. Size of points correspond to weight attributed to each outcome in the meta-analysis. Numbers after reference number denote multiple outcomes from the same study.

TABLE 3
Results of meta-analysis models and subgroup analyses of studies assessing the influence of n-3PUFA supplementation on muscle mass, strength, and function in healthy young and older adults

Outcome (Moderator)	Parameter estimate (95% CI)	SE	$F_{df1,df2}$	Between-outcome variance (95% CI)	Between-study variance (95% CI)	QE_{df}	NutriGrade quality assessment
Muscle mass (overall)	0.073 (−0.026; 0.171)	0.045		0 (0; 0.017)	0 (0; 0.018)	3.42	Moderate Meta-Evidence
(Training intervention)							
None or other	0.101 (−0.028; 0.231)	0.058	$0.6_{1,10}$	0 (0; 0.019)	0 (0; 0.02)	2.82	
Resistance training	−0.07 (−0.272; 0.132)	0.091					
Strength (overall)	0.124 (0.004; 0.244)	0.058		0 (0; 0.023)	0.008 (0; 0.058)	14.60	Moderate Meta-Evidence
(Supplementation dosage)							
<2 g/d	0.142 (−0.051; 0.334)	0.092	$0.02_{1,21}$	0 (0; 0.024)	0.012 (0; 0.078)	14.14	
≥2 g/d	−0.02 (−0.282; 0.242)	0.126					
(Training intervention)							
None or other	0.141 (−0.008; 0.289)	0.071	$0.11_{1,21}$	0 (0; 0.023)	0.01 (0; 0.071)	14.57	
Resistance training	−0.045 (−0.333; 0.242)	0.138					
(Age)							
<60 years	0.153 (−0.251; 0.557)	0.194	$0.02_{1,21}$	0 (0; 0.023)	0.009 (0; 0.069)	14.41	
≥60 years	−0.029 (−0.454; 0.395)	0.204					
Function (overall)	0.032 (−0.089; 0.153)	0.057		0 (0; 0.011)	0.006 (0; 0.087)	10.59	Moderate Meta-Evidence
(Supplementation dosage)							
<2 g/d	−0.004 (−0.124; 0.116)	0.056	$0.79_{1,16}$	0 (0; 0.012)	0.004 (0; 0.116)	9.10	
≥2 g/d	0.112 (−0.154; 0.378)	0.126					
(Training intervention)							
None or other	0.025 (−0.121; 0.171)	0.069	$0.13_{1,16}$	0 (0; 0.011)	0.007 (0; 0.13)	9.84	
Resistance training	0.05 (−0.249; 0.349)	0.141					

$F_{df1,df2}$, omnibus moderator test statistic; QE_{df}, residual heterogeneity test statistic.
The second level of each moderator represents the difference between the first (reference level) and second level of the moderator.

Figure 2 shows a funnel plot of all outcomes. A visual inspection was made by checking how large the effect is for each separate outcome from the study with the highest precision (i.e., at the top of the funnel), and then checking how many dots are larger from that high-precision effect. Overall, it suggests a fairly symmetrical funnel plot, with some studies showing particularly large effects and standard errors. An Egger's intercept test for all outcomes was statistically significant (Intercept = 0.404 [95% CI: 0.072, 0.736], $P = 0.0180$), suggesting small-study effects. These results were considered during the NutriGrade assessment.

Regarding the NutriGrade assessment, certainty in results was downgraded because of risk of bias, heterogeneity, and publication bias. Two outcomes were downgraded on precision because of the number of participants included and CI overlapping the null value. On publication bias, all 3 outcomes were downgraded because of small-study effects; this decision was made using the Egger's regression–intercept test and the visual inspection of the funnel plots available on Figure 2. Thus, muscle mass, muscle strength, and muscle function outcomes were deemed to have "moderate meta-evidence." The NutriGrade quality assessment is described in Table 3 and the complete decision rationale is available in Supplementary File 3.

Discussion

The findings of this systematic review and meta-analysis showed that n-3PUFA supplementation may lead to very small increases in muscle strength, but it does not impact muscle mass and function in healthy young and older adults.

Our results are in contrast with the meta-analysis published by Huang et al [29], who found minor increases in muscle mass and function in the elderly after n-3 PUFA supplementation [29]. The fact that they included measurements of muscle mass based on dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis, and computed tomography, whereas we only considered gold-standard measurements (i.e., DXA, Bod Pod, hydrostatic underwater weighing, and computed tomography), may help to explain the discrepancy between our findings. More importantly, they evaluated elderly participants who were either healthy or had chronic diseases, which in turn may influence muscle protein synthesis. Indeed, previous literature suggests that n-3PUFA supplementation may be more likely to provide an anabolic stimulus in situations whereby muscle protein synthesis is compromised [64], such as when protein intake is suboptimal [65], within older adults who have higher degrees of anabolic resistance [66] and in conditions of increased systemic inflammation, such as chronic diseases [67]. In our review, we only included studies evaluating otherwise healthy participants, which may help to explain the discrepant findings.

We did find a positive, albeit very small, effect of n-3 PUFA supplementation on muscle strength when compared with placebo. We do urge caution in interpreting this result. First, of the 11 studies included in this analysis, 5 reported a positive effect of n-3 PUFA supplementation on muscle strength [48,55,59–61,63]. One of these studies [59] detected intra-group differences on n-3PUFA and placebo interventions only, but not a between-group interaction. It should be noted that, in any placebo-controlled study, an intra-group difference between the pre- and post-time points in the treatment group only should not be interpreted as evidence in favor of the intervention [68,69], and rather, the difference between treatments (i.e., the interaction term) should be considered. The other 4 studies [48,55,60,61] detected between-group differences across different strength tests. Alkhedhairi et al. [55], da Boit et al. [60], and Dalle et al. [61] detected an effect of n-3PUFA on maximal isometric torque, and Smith et al. [48] detected an effect of n-3PUFA supplementation on handgrip strength, 1-RM muscle strength and thigh muscle volume after 24 weeks of n-3PUFA supplementation. Importantly,

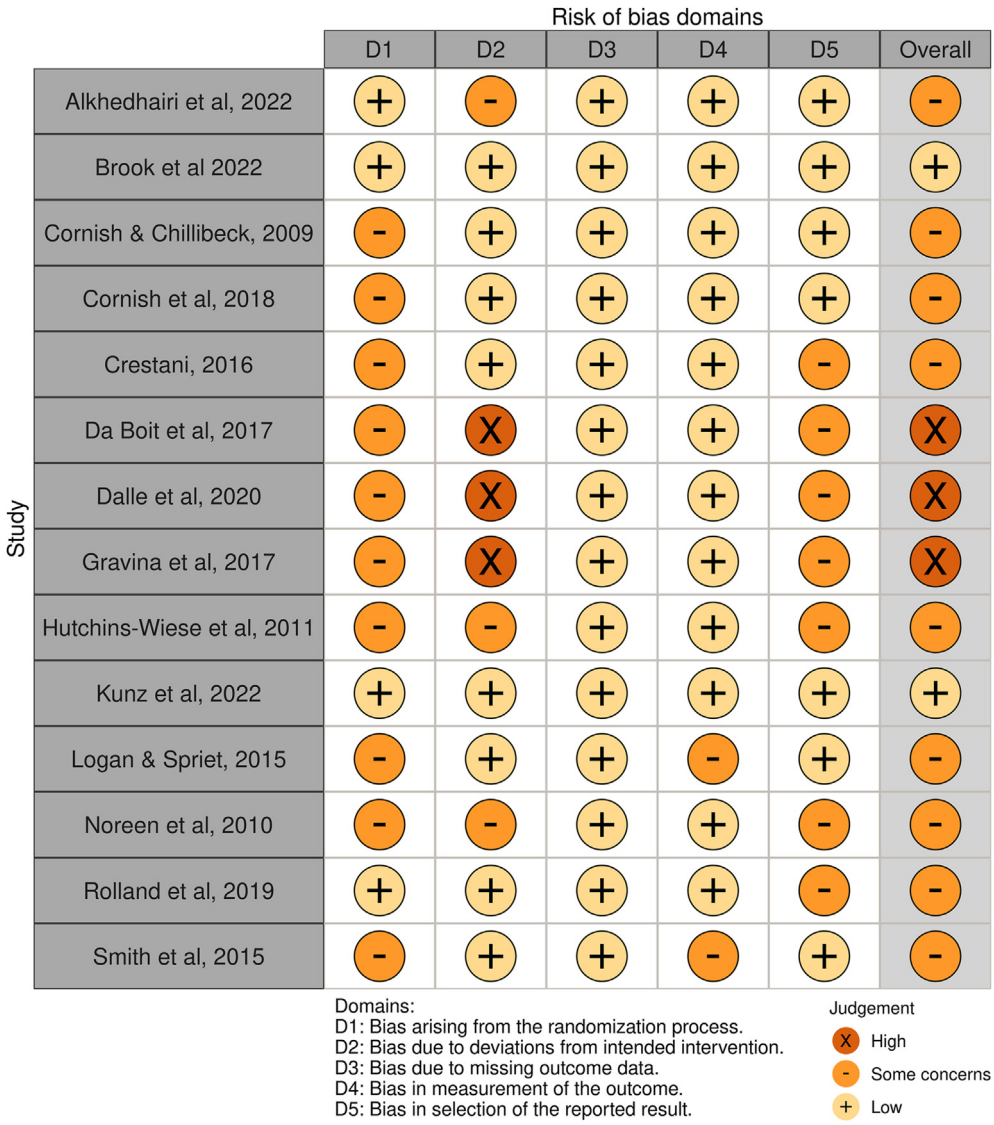

FIGURE 4. Risk of bias assessment for all studies identified in the systematic review. Phrases not in bold font are sources of bias. Assessed using the Cochrane risk-of-bias assessment tool version 2.0. Plot produced using robvis [71].

these studies were conducted on older adults, corroborating the hypothesis that in individuals with a higher degree of anabolic resistance, n-3PUFA supplementation may be more efficacious. Second and most importantly, the lower bound estimate of the CI was extremely small (0.004). As such, the effect of n-3PUFA supplementation on strength is compatible with trivially small benefits, and it is unlikely that such small changes would be clinically meaningful or relevant. Third, when considering the funnel plot (Figure 2), we notice that 2 studies (59,60) showed very large variances, suggesting that a set of small studies are most likely responsible for bringing the overall effect to the positive side. Finally, the NutriGrade quality assessment for muscle strength resulted in moderate certainty. Thus, we conclude that more well-conducted trials are necessary to confirm these findings, and most importantly, the clinical relevance of n-3PUFAs on strength in healthy individuals.

Despite the very small positive effect of n-3 PUFA supplementation on muscle strength, we did not find an effect on muscle mass and muscle function. This could be explained, at least in part, by the fact that increases in muscle strength are not necessarily correlated with changes in muscle size, since neural motor control, and/or cellular and molecular adaptations of muscle fibers may lead to increases in muscle strength in the absence of significant increases in muscle mass [70]. Moreover, muscle strength was the outcome with the largest number of available studies in this meta-analysis (i.e., 10 studies and 22 outcomes). One could argue that, because of insufficient available studies, and to studies with large variances/low sample sizes, we lacked appropriate statistical power to observe positive effects of n-3PUFA supplementation on the muscle mass and muscle function outcomes. However, none of the studies included in these analyses reported a treatment effect (between-group interactions) of n-3PUFA supplementation on muscle mass and function; thus, our overall estimate is what best represents the current stage of the literature. Therefore, our data indicate a lack of evidence of the benefits of n-3PUFA supplementation on lean/muscle mass and muscle function compared with placebo.

Our findings are in line with a narrative review by Rossato et al. [33], in which the authors concluded that studies with older adults do not show significant associations between

n-3PUFA intake and muscle mass or muscle function. Notably, the authors question whether n-3PUFA supplementation could have a greater effect on younger adults or sedentary individuals [33]. Our data reject this suggestion, as we did not show an impact of n-3PUFA supplementation on muscle mass, strength, or function neither in younger nor in older adults, irrespective of the presence of a resistance training intervention.

This study's strengths include the fact this was the first meta-analysis performed considering only healthy individuals, and the fact that we performed subgroup analyses according to age, supplementation dosage/day, and cosupplementation alongside resistance training to try and gather knowledge on their potential effect on our findings. Limitations of this review include the high overall risk of bias from the included studies, mostly because of the lack of information about the randomization process and blinding and the lack of plan trial registries, which resulted in only moderate certainty in study outcomes. The relatively small number of studies available is also an issue, and the analysis may have lacked the power to detect smaller effects, or to identify potential differences within subgroups (if they exist). Future studies should accomplish rigorous methodology, disclose their study protocol before recruitment via preregistration, and make a complete reporting of specific randomization, concealment, and compliance approaches, augmenting reproducibility and quality of evidence on research on this topic.

Conclusion

This review and meta-analysis concluded that n-3PUFA supplementation had no significant effect on muscle mass and muscle function, despite a very small positive effect on muscle strength in healthy adults and older adults when compared with placebo. Because of the low certainty in findings, we believe future studies with rigorous methodology and reporting of data are necessary to expand and confirm these results.

Author disclosures

The authors report no conflicts of interest.

Acknowledgments

HCSA, ED, and FBB designed the research; HCSA, FL, GPE, and GCB conducted the research; GPE and GCB analyzed the data; and HCSA, GPE, GCB, ED, and FBB wrote the mauscript. FBB had primary responsibility for final content. All authors read and approved the final manuscript. Our research group did not receive any financial support to the present investigation. The authors have no other potential conflicts of interest to declare. HCSA, GPE, GCB, ED, and FBB are supported by research grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant numbers: 2020/02741-1, 2020/07860-9, 2020/12036-3 & 2021/12116-0, 2019/05616-6 & 2019/26899-6, and 2019/17912-9). FL received a grant from Conselho Nacional de Desenvolvimento Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.advnut.2022.11.005.

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Data Availability

Data described in the manuscript and analytic code is publicly and freely available without restriction at GitHub [https://github.com/gp-esteves/meta-analysis-n3PUFA] and at the project's OSF page [https://osf.io/3dcwr/, DOI: 10.17605/OSF.IO/3DCWR].

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