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Systematic Review

Impact of Omega-3 Fatty Acids Supplementation Combined with Resistance Training on Muscle Mass, Neuromuscular and Physical Function in Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

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Abstract: Age-related declines in muscle mass, neuromuscular, and physical function can be mitigated by resistance training (RT). Omega-3 polyunsaturated fatty acids (Ω-3 PUFAs) supplementation has shown benefits in older adults. However, it remains unclear if combining Ω-3 PUFAs with RT is more effective than RT alone or with placebo. This systematic review and meta-analysis examined the effects in randomized controlled trials (RCTs) of Ω-3 PUFAs combined with RT compared to RT alone or placebo on muscle mass and function in healthy older adults (≥65 y). Databases such as PubMed, Embase, SPORTDiscus, and Web of Science were searched on 11 April 2024. No restriction on language or publication date was implemented. Mean differences (MDs) or standardized mean differences (SMDs) with 95% confidence intervals and pooled effects were calculated. Nine studies (n = 286, 54% men) met the inclusion criteria. The meta-analysis found no significant effect of Ω-3 PUFAs on muscle mass or neuromuscular function but a large effect on chair-rise performance. Potential impact of Ω-3 PUFAs dose, duration, or sex were not observed. Most studies had varying levels of bias, and none met recommended quality standards for investigating Ω-3 PUFAs, but findings suggest no clear advantage of combining Ω-3 PUFAs with RT.

Keywords: omega-3 polyunsaturated fatty acids; resistance training; skeletal muscle mass; neuromuscular function; physical function; older adults

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1. Introduction

The global demographic is shifting toward an aging population [1]. By 2050, approximately 16% of the global population is projected to be over the age of 65 years old, up from 10% in 2024 [1]. Ensuring individuals remain physically functional and independent is crucial for personal well-being and a societal necessity to reduce the growing burden on

healthcare systems and caregivers [2,3]. Aging results in a progressive loss of skeletal muscle mass, as well as neuromuscular (e.g., muscle strength and power) and physical function (e.g., chair-rise) [4–6]. Physical inactivity and inadequate protein intake, two important modifiable lifestyle determinants, have been systematically reported to worsen such degeneration [7–9]. Also, chronic low-grade, systemic inflammation, a prevalent condition among older adults which has been defined as inflammaging [10], has been associated with accelerated loss of muscle mass and physical function [11–13]. Finally, the interaction between inflammation and physical inactivity has been highlighted in the recent years, and a new term, *inflamm–inactivity*, has been recently proposed [14,15].

Resistance training (RT) is a feasible strategy to minimize the rate of decline in neuromuscular and physical function, and to combat the onset of conditions such as sarcopenia and frailty [16–18]. Indeed, recent reviews have shown that RT is an effective method to increase muscle mass [19], neuromuscular, and physical function [20,21], in older adults. Additionally, RT has been considered a long-lasting anti-inflammatory therapy [22,23].

The use of anti-inflammatory nutrients, such as the omega-3 polyunsaturated fatty acids (Ω-3 PUFAs), has gained more attention in the past years to counteract accelerated aging [24,25]. The most-studied Ω-3 PUFAs are eicosapentaenoic acid (EPA; 20:5, Ω-3), docosahexaenoic acid (DHA; 22:6, Ω-3), and alpha-linoleic acid (ALA; 18:3, Ω-3). ALA is an essential fatty acid present in vegetables, nuts, and fruits [26] and the parent compound for Ω-3 PUFAs, which is metabolized to form EPA and DHA, found in fish (oils) and other sea foods [27]. Suggested mechanisms for the beneficial actions of Ω-3 PUFAs include: (i) a reduction in chronic low-grade inflammation [24], (ii) a hypertrophic effect likely due to an amino acid sensitizing effect via an increased mTOR signaling that controls muscle protein synthesis (MPS) in skeletal muscle [25], and (iii) improvement in muscle function (e.g., power) [28], possibly due to an increased nerve conduction velocity through modulation of sarcolemma ion channels [29]. While DHA and EPA can be directly absorbed and directly used by the neuromuscular system, it is likely that ALA needs to be converted into EPA or DHA, but the conversion may vary, with 8–10% and 0.05–4% into EPA and DHA, respectively [30,31].

The 2020–2025 Dietary Guidelines for Americans (DGA) recommends adults aged $\geq$ 51 years should consume a daily ALA intake of 1.6 g for males and 1.1 g for females. Furthermore, the DGA recommends consuming 8–10 oz. of a variety of seafood per week (providing approximately 250 mg EPA and DHA per day) [32]. Despite such recommendations, a relatively recent global survey using the Omega-3 Index (O3I), a gold standard method for assessing the percentage of EPA + DHA in erythrocyte membranes [33], highlighted that: (i) overall O3I indexes are from low to very low, and (ii) there is a markedly uneven geographic distribution ranging from an optimal index of $\geq$8% to very low EPA + DHA blood levels of $\leq$4% [34]. A range of factors such as age, sex, genetics, the presence of chronic conditions, and daily physical activity levels may influence the conversion of ALA into EPA and DHA, which will ultimately impact O3Is [35–37]. This also indicates the need to assess O3I levels to evaluate the actual Ω-3 PUFAs levels in erythrocyte membranes and the potential effect of supplementation strategies.

EPA and DHA obtained from diet or supplements as a part of an optimal diet have been included in multiple medical guidelines [38–41]; however, inconsistency in clinical outcomes has limited wider support of the benefits of EPA and DHA in guidelines and within the medical community [42]. If Ω-3 PUFAs combined with RT can be used to support healthy aging [43], it is important to explore the inconsistent findings in future clinical trials to determine if the results are due to a lack of effect from the Ω-3 PUFAs or because of methodological issues (e.g., low dose, inconsistent types, inappropriate timing, or short duration (DTTD modifiers)).

To our knowledge, in comparison to this present review, only one relatively similar systematic review with a meta-analysis has investigated whether the combined effects of Ω-3 PUFAs supplementation and RT may be superior to Ω-3 PUFAs supplementation only on muscle mass and function in older adults [44]. However, this is different from the present review that explores if the combined effects of Ω-3 PUFAs supplementation and RT are superior to RT only. Interestingly, the review showed improvements in some neuromuscular and functional outcomes in some studies [45–55] (i.e., body strength, up-and-go (UG), 30 s sit-to-stand performance), along with no changes in similar outcomes (i.e., muscle mass, upper body strength, and walking ability) in several other studies [45,46,48–50,54,56,57]. Important details that may potentially explain such mismatch (e.g., sex distribution [30], dose of EPA + DHA [33,36,58], type [59–61], duration [62–64], and timing of the supplementation [63,64]) remain largely unexplored [44]. In addition, when investigating Ω-3 PUFAs, other oils such as corn or olive oil are used as a placebo that may influence the study outcomes [59,65]. As an inert placebo seems unfeasible, this present review also included a study [54] without a placebo provided to the RT group, while accounting for this in the sensitivity analysis.

Therefore, the aims of this review were to (i) investigate whether the effect of Ω-3 PUFAs supplementation combined with RT is superior to RT alone or with placebo on muscle mass, as well as neuromuscular and physical function, in older adults aged $\geq$ 65 years; and (ii) to explore potential factors, including sex distribution, or DTTD modifiers, such as dose and duration of the Ω-3 PUFAs supplementation regimes, which may modify the effect of the supplementation.

2. Materials and Methods

2.1. Design

This literature review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [66,67]. The study protocol has been registered at PROSPERO (registration number: CRD42020221271). The electronic databases PubMed, Embase, Web of Science, and SPORTDiscus were searched, including all dates from inception up to 11 April 2024. Two authors (DLD and JAC) independently performed the search, screening, and data extraction process. Potential disagreements between the authors were resolved through a consensus reached with a senior researcher in the field (PC).

2.2. Search Strategy

Keywords to identify participant characteristics and study interventions included "older adults" or "aged" and "resistance training" or "strength training" and "fatty acids, omega-3". The search formulas for each database are provided in Supplementary Table S1.

2.3. Selection Criteria of Studies

Randomized controlled trials (RCTs) were included if they satisfied the defined inclusion criteria (Supplementary Table S2). Studies meeting the following participants, intervention, comparators, outcomes, timing, and study type (PICOTS) framework were included: Participants were aged $\geq$ 65 years (with individual participants aged $\geq$ 60 years) and without severe disease conditions (e.g., cancer, chronic obstructive pulmonary disease). To be considered for inclusion, RT needed to be the dominant component (exercise protocol prescribing RT $\geq$60% of the total training time) in the experimental group combined with Ω-3 PUFAs or an Ω-3-6-9 blend (e.g., plant- or fish-based oils containing ALA, EPA, and/or DHA, not including fish consumption as an intervention) expected to increase Ω-3 PUFAs

levels significantly and influence outcomes of interest by the original authors in a controlled set-up. The term Ω-3 PUFAs covers ALA, EPA, and DHA.

The control group had to receive a comparative intervention either (a) RT alone (i.e., no placebo supplementation used), or (b) RT combined with a placebo supplementation. The study had to report measures on at least one of the following primary outcomes: lean tissue (i.e., muscle) mass, measures of neuromuscular (i.e., lower-body or upper-body strength or power) or physical function (i.e., chair-rise, up-and-go, and walking ability testing). No restriction on language or publication date was implemented. Only RCTs and published material was included. Reference lists of included studies were also assessed for other relevant studies that fit our PICOTS framework.

2.4. Data Extraction and Quality Assessment

The data extracted included the following: first author and year of publication; study design (randomized controlled trial, RCT); participant characteristics, including sample size, sex distribution, and mean age; details of the experimental and control conditions, such as the RT and Ω-3 PUFAs supplementation (dose, type, timing, and duration) regime; compliance rates; outcome domains (muscle mass, neuromuscular function, and physical function); and baseline levels of Ω-3 PUFAs.

The methodological quality of the included studies was assessed according to the Cochrane Collaborations risk-of-bias tool version 2 (RoB 2) [68]. The intervention effect or 'intention-to-treat' was the effect of interested used. Due to compliance in all studies being high (>95%), we did not differentiate between studies that were 'intention-to-treat' or 'per-protocol' in the analysis. No study was excluded based on the risk of bias assessment.

Omega-3 scores for optimal study design in the studies in the meta-analysis were assessed on a 5-point quality assessment scale adapted from Anthony et al. (2021) [69] and based on recommendations from James et al. (2014) [70] to assess how differences in tissue levels of Ω-3 PUFAs between the control and intervention group(s) were maximized (Supplementary Table S3). Criteria included whether studies (i) excluded participants with a high baseline O3I, (ii) supplementation resulted in a change in O3I, (iii) excluded participants that consumed fish oil supplements prior to baseline, (iv) excluded participants consuming > 2 fish meals per week, and (v) had a minimum supplementation duration of four weeks. Each criterion was scored as either satisfied (1 point) or not satisfied (0 points), resulting in a score ranging from 0 to 5 [69].

2.5. Data Synthesis and Analysis

Meta-analyses were conducted using Review Manager Version 5.4 (RevMan), while RoB 2.0 graphs were conducted using the robvis tool [71]. The primary analyses assessed the effects of RT + Ω-3 PUFAs vs. RT + Placebo or alone on the primary outcomes (i.e., lean tissue (i.e., muscle) mass, measures of neuromuscular (i.e., lower-body or upper-body strength or power), or physical function (i.e., chair-rise, UG, and walking ability testing). Means and error bars (presented as standard deviation (SD) or standard error (SE)) were extracted using the WebPlotDigitzer software (Version 4.4., Ankit Rohatgi, CA, USA) when data were not included in text or tables. When only post-intervention data points and sample sizes were reported, the mean and SD values were calculated using the Microsoft Excel software (Version 16.60, Microsoft, 2022).

One trial [48] reported medians, ranges, and/or interquartile ranges, with the means and SDs being estimated using established methods [72]. To ensure all scales were measured in the same direction (e.g., an improvement was defined as a lower UG time), mean values were multiplied by −1 before standardization [73]. Studies presenting sample size, means,

and SDs separately for males and females were combined into a single sample size, mean, and SD using a Cochrane formulae for combining groups [74].

The effect size was estimated as mean difference (MD) or standardized mean difference (SMD) when different scales were used, along with their 95% confidence intervals (CIs). Statistical heterogeneity was assessed with the I^2 statistic [73]. A fixed-effect model was used unless statistical heterogeneity was high ($I^2 \geq 50\%$), in which case a random-effect model was used to yield more conservative results. The size of the pooled SMD was interpreted accordingly: $\leq$0.40 = small effect, 0.40–0.70 = moderate effect, $\geq$0.70 = large effect [75].

A subgroup analysis was conducted to explore the heterogeneity of the effect estimates according to participant characteristics (i.e., sex) and intervention components (i.e., duration of intervention). Regarding intervention durations, no recommendations for Ω-3 PUFAs currently exist. However, the approximately 16-week lifespan of erythrocytes provides a biologically relevant framework for determining the time required to achieve saturation of Ω-3 PUFAs in erythrocyte membranes [76,77]. Therefore, a cut point of $\geq$16 weeks was adopted, while Ω-3 PUFAs' saturation in erythrocyte membranes appears to reach a steady state at around 16 weeks [78]. Additionally, meta-regression analyses were conducted to investigate Ω-3 PUFAs doses (g/day) for the effects on RT + Ω-3 PUFAs compared to RT alone or with placebo on primary outcomes using standardized mean differences (SMDs). A mixed-effects meta-regression model was applied to account for both fixed effects (e.g., Ω-3 PUFA dose as a moderator) and random effects (to capture between-study heterogeneity). Results are presented using a bubble plot with a 95% confidence interval. The analysis was performed using the metafor package in R, version 4.2.2 (R Core Team, 2023, R Foundation for Statistical Computing, Vienna, Austria).

To avoid overtesting, only the three primary outcomes with the greatest effect sizes, muscle mass, neuromuscular function (lower-body), and physical function (chair-rise) were explored using dose–response relationships and subgroup effects based on sex and intervention duration. Sensitivity analysis was conducted by removing studies with a high risk of bias or publication bias (i.e., from funnel plots) to assess the robustness of the summary estimates [79]. Robustness analyses were performed, leaving one study out at a time. Publication bias was assessed by the visual inspection of funnel plots and Egger's bias test. The latter was performed using StataMP, version 14 (StataCorp; 2015; Stat Statistical Software: Release 14; College Station, TX, USA; StataCorp LP). For all analyses, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Search and Study Selection

The result of the search strategy is outlined in Figure 1. The electronic search strategy yielded a total of 912 studies, of which 11 studies [45–49,52,54,57,80–82] met the inclusion criteria and were included in the systematic review, and 9 (286 participants) were included in the meta-analysis [45–49,52,54,57,82]. Two studies were excluded due to a lack of access to data on functional outcomes (i.e., SPPB) from the author [80] and for being a preliminary study [81] that limited an adequate RoB assessment.

3.2. Study Characteristics

General characteristics of the 11 included studies are summarized in Table 1. The studies included data from 841 participants, of whom 43% were men. Sample sizes ranged from 13 [81] to 542 participants [80]. We found that all participants were untrained older adults. The mean age of six studies was <70 years [45,52,54,57,81,82], while five studies had a mean age > 70 years [46–49,80]. Five studies included both men and women [45,47,49,52,80], and the remaining six studies included either men [46,48] or women [54,57,81,82]. All studies were RCTs [45–49,52,57,80–82], while one was without a placebo [54].

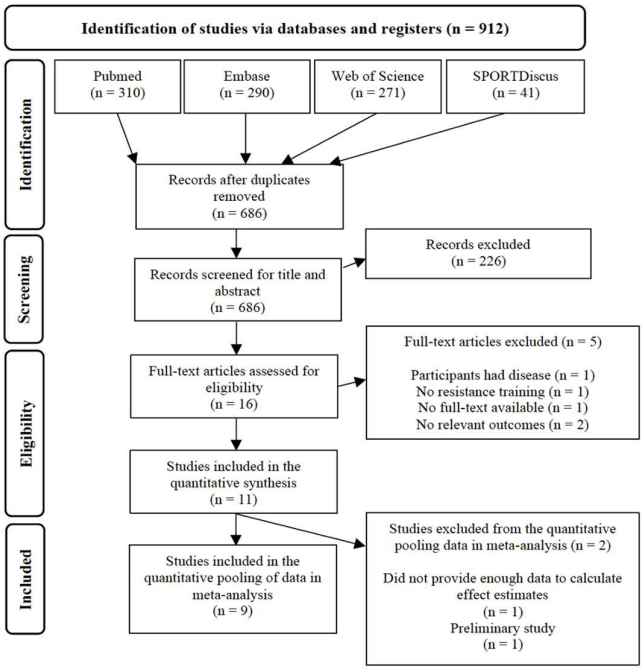

Figure 1. PRISMA flow chart of the study selection process.

Table 1. Characteristics of included RCTs in older adults.

Author	Design [a]	N (Men %)	Age	TTV (TS × WKs)	Ω-3 Dose [b] (g/Day)	PLA Dose (g/Day)	Outcome Domain [c]	Duration (WKs)
Bischoff-Ferrari et al. [80]	DB EG: RT + Ω-3 CG: RT	275 (38) 267 (38)	74.7	468 (3 × 156)	T-Ω-3: 1 EPA: 0.33 DHA: 0.66	NR	PF (CR + WA)	156
Brook et al. [57]	DB EG: RT + Ω-3 CG: RT + PLA	8 (0) 8 (0)	67	18 (3 × 6)	T-Ω-3: 3.68 EPA: 1.86 DHA: 1.54	Corn oil: NR	MM NMF (LB)	6
Cornish & Chilibeck [45]	DB EG: RT + Ω-3 CG: RT + PLA	25 (56) 26 (54)	65.5	36 (3 × 12)	T-Ω-3: 14 ALA: 14	Corn oil: 27.6	MM NMF	12
Cornish et al. [46]	DB EG: RT + Ω-3 CG: RT + PLA	11 (100) 12 (100)	71.2	36 (3 × 12)	T-Ω-3: 3 EPA: 1.98 DHA: 0.99	Omega-3-6-9 blend: 3	MM NMF PF (WA + UG)	12
Da Boit et al. [47]	DB EG: RT + Ω-3 CG: RT + PLA	23 (52) 27 (57)	70.7	36 (2 × 18)	T-Ω-3: 3 EPA: 2.1 DHA: 0.6	Safflower oil: 3	MM NMF (LB) PF (CR + WA)	18
Dadóva et al. [48]	DB EG: RT + Ω-3 CG: RT + PLA	27 (100) 26 (100)	71	32 (2 × 16)	T-Ω-3: NR EPA: 0.125 DHA: 0.105	Sunflower oil: NR	MM NMF PF (CR)	16
Dalle et al. [49]	DB EG: RT + Ω-3 CG: RT + PLA	12 (67) 11 (64)	70.9	36 (3 × 12)	T-Ω-3: 3.3 EPA: 1.6 DHA: 1.23	Corn oil: 1.1	MM NMF PF	12
Kamolrat et al. [81]	DB EG: RT + Ω-3 CG: RT + PLA	7 (0) 6 (0)	66.9	24 (2 × 12)	T-Ω-3: 4 EPA: 1.7 DHA: 0.4	Olive oil: 4	NMF (LB) PF (WA)	12
Lee et al. [52]	SB EG: RT + Ω-3 CG: RT + PLA	10 (40) 10 (30)	69.3	24 (2 × 12)	T-Ω-3: NR EPA: 2.1 DHA: 0.72	Safflower oil: NR	NMF PF	12
Lee et al. [82]	DB EG: RT + Ω-3 CG: RT + PLA	10 (0) 10 (0)	65.7	16 (2 × 8)	T-Ω-3: NR EPA: 2.1 DHA: 0.72	Safflower oil: NR	NMF (UB) PF	8

Table 1. *Cont.*

Author	Design [a]	N (Men %)	Age	TTV (TS × WKs)	Ω-3 Dose [b] (g/Day)	PLA Dose (g/Day)	Outcome Domain [c]	Duration (WKs)
Rodacki et al. [54]	NA EG: RT + Ω-3 CG: RT alone	15 (0) 15 (0)	64.4	36 (3 × 12)	T-Ω-3: 2 EPA: 1.2 DHA: 0.9	NR	NMF (LB) PF	12

[a] Included experimental and control groups are presented for each trial. [b] Omega-3 score from 1–5 (see Table S3). [c] If no additional information is provided, the outcome had measurements in all areas (e.g., 'PF', equals 'PF: CR + WA + UG'). ALA, alpha-linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CG, control group; CR, chair-rise; DB, double-blinded; EG, experimental group; LB, lower-body; MM, muscle mass; NA, not applicable (no placebo supplementation used); NMF, neuromuscular function; NR, not reported; Ω-3, omega-3; PF, physical function; PLA, placebo; RCT, randomized clinical trial; RT, resistance training; SB, single-blinded; TTV, total training volume; T-Ω-3, total omega-3; TS, training sessions; UB, upper-body; UG, up-and-go; WA, walk capability; WKs, weeks.

3.3. Ω-3PUFAs Supplementation Protocol and Omega-3 Score

Protocols for Ω-3 PUFAs are summarized in Table 1 and detailed in Supplementary Table S4. Omega-3 scores with comments for optimal study design can be assessed in Supplementary Table S3. Scores for optimal Ω-3 PUFAs study design ranged from 1 to 3. Ten studies combined RT and Ω-3 PUFAs, EPA, and DHA, and one study [45] provided a dose of ALA (~14 g). The conversion from ALA to EPA and DHA was not explored; therefore, the converted amount of EPA and DHA from this study [45] was not reported. EPA doses ranged from ≈0.1 g/d [48] to 2.1 g/d [47] with a mean of 1.5 g/d (SD 0.7). DHA doses from ≈0.1 g/d [48] to 1.5 g/d [57], with a mean of 0.8 g/d (SD 0.4), were used. Combined doses of EPA and DHA ranged from 0.2 g/d [48] to 3.4 g/d [57] with a mean of 2.2 g/d (SD 1). The majority (n = 9) of the interventions had EPA-dominated supplement formulations [45–49,52,54,57,81,82], while DHA [80] or ALA [45] were predominant in one study each. Ω-3 PUFAs supplements were derived from fish in all but one study [48], which supplemented with Calanus oil extracted from wax esters rich in Ω-3 PUFAs. Supplementation duration ranged from 6 to 156 weeks (Table 1). The following studies had short (<16 weeks) [45,46,49,52,57,81,82] and long duration (≥16 weeks) [47,48,80]. When the longest study was removed [80], the mean duration was 12 weeks (SD 3.4).

Overall, three studies reported Ω-3 blood levels with either erythrocyte (i.e., O3I) [47] or plasma measures [54,81] to investigate changes pre- and post-supplementation. All three studies reported significant changes in Ω-3 blood levels, two measured EPA + DHA levels [47,54], and one study [81] measured EPA exclusively. The two studies [47,54] had intervention groups with moderately high baseline O3I (i.e., EPA + DHA blood levels), at 7.4% and 7.6%, respectively.

3.4. Resistance Training Protocol

The RT protocols are summarized in Table 1 and detailed in Supplementary Table S5. Regarding the type of RT protocol, eight studies targeted lower- and upper-body exercises in their RT program [45,46,48,49,52,54,81,82], while one focused on lower-body exercises only [47], one combined RT with joint flexibility [80], and another followed a unilateral RT program [57]. The mean RT frequency was 2.5 sessions/week (ranging from 2 to 3 sessions/week), with the intervention duration ranging from 6 to 156 weeks. The RT intensity varied from 50% [52] to 85% [45,46] of the participants' one repetition maximum (1RM). Eight studies used a progressive RT protocol [45–47,49,52,54,57,82], while three studies [48,80,81] did not include a progression regime.

3.5. Placebo Characteristics

Placebo protocols are summarized in Table 1 and detailed in Supplementary Table S4. Six studies used corn oil [45,49,57] or safflower oil [47,52,82], and three used ei-

ther sunflower oil [48], olive oil [81], or an omega-3-6-9 blend [46]. Among the five studies [45–47,49,81] that reported dose, they ranged from 1.1 to 30.0 g/d. One study [80] did not provide information on the type of placebo used, while another study [54] did not use a placebo but provided RT alone to the control group.

3.6. Outcome Measures

The skeletal muscle mass measures were conducted using dual-energy X-ray absorptiometry (DXA) [45,46], whole-body magnetic resonance imaging (MRI) [47], immunofluorescence [57], computed tomography (CT) [49], or bioelectrical impedance (BIA) [48]. The measurements related to neuromuscular function (i.e., lower- and upper-body strength or power) included 1RM chest press [45,46], 1RM leg press using weight equipment [45,46,52,57], and knee extensor function by dynamometry (peak power output [48], maximal isometric muscle strength [49] and torque [47,54] by dynamometry). Furthermore, handgrip strength (HGS) was assessed using either an analogue [49,52] or digital dynamometer [48]. Physical function was assessed as short- and long-distance walking (gait speed (4-m, 6-m time), and timed 6 min walk distance) [45–47,49,52,54,82], chair-rise performance (five-times-sit-to-stand test (FTSST) and 30 s chair stand), [47–49,52,54,82], and UG performance (i.e., UG is used as an umbrella term to include, timed up-and-go (TUG) and 8-foot-up-and-go (8-FUGT)) [46,49,52,54,82]. Lower physical functional values following intervention period indicates better performance (e.g., FTSST and UG performance). The outcomes and measurements are detailed in Supplementary Tables S6 and S7.

3.7. Risk of Bias of Included Studies

The overall rating summarizing the quality within the studies is presented in Figure 2. One study achieved an overall low risk-of-bias score.

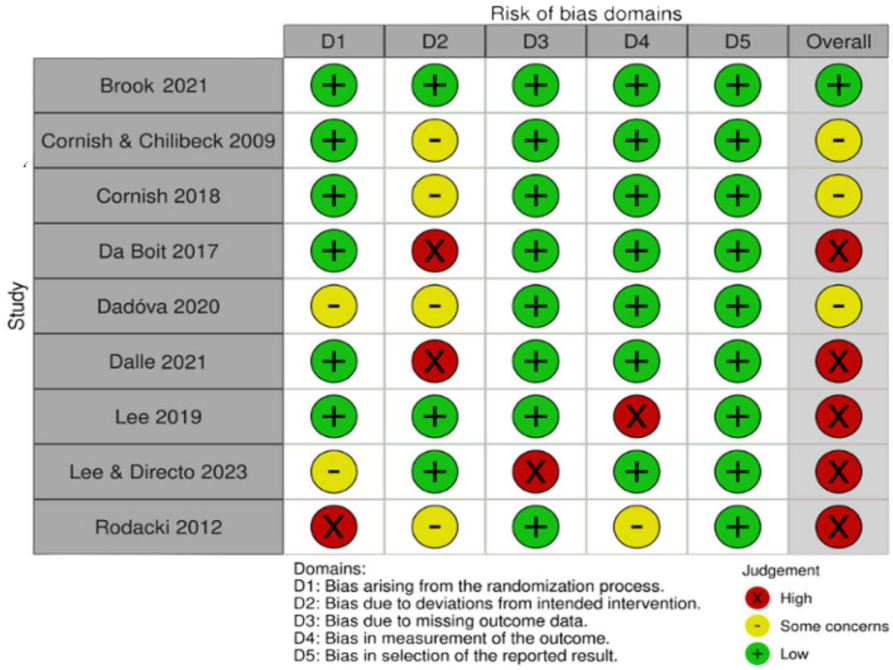

Figure 2. Risk of bias of the included studies [45–49,52,54,57,82].

There was generally a low risk of bias for most domains, including bias arising from the randomization process (n = 3 studies), bias in measurements of the outcome (n = 2 studies), and bias due to missing outcome data (n = 1 studies). The assessment revealed a low risk of bias due to the selection of the reported results in all studies. The bias due to deviations from intended interventions was at low risk in three studies, unclear in four, and at high

risk in two. The overall risk of the included studies had some concerns in three studies and a high risk in five.

3.8. Treatment Outcomes

3.8.1. Primary Results

A summary of the effects of RT + Ω-3 PUFAs vs. RT + placebo or alone on muscle mass, neuromuscular, and physical function is shown (Table 2). The table also focuses on investigating whether the significant effect on chair-rise (CR) performance is influenced by changes in dose, intervention duration, or sex through a dose–response and subgroup analysis.

Table 2. Summary of the effects of RT with Ω-3 PUFAs versus RT alone or with placebo on the outcomes of interest.

Outcome	Studies (N)	N	Method	Effect Estimates (95% CI)	*p*-Value	I^2
Muscle mass	6	211	SMD. Fixed.	0.14 (−0.13, 0.42)	0.30	0%
Neuromuscular function (LB)	8	264	SMD. Random.	0.30 (−0.26, 0.85)	0.30	78%
Neuromuscular function (UB)	6	190	SMD. Fixed.	0.09 (−0.19, 0.38)	0.52	0%
Physical function (CR)	6	196	SMD. Random.	0.77 (0.10, 1.44)	0.02 *	78%
CR (Dose)	6	196	SMD. Mixed-effects [a]	β = 0.15 (−0.09, 0.40)	0.22	0%
CR (Duration)	6	196	SMD. Random.	0.77 (0.10, 1.44)	0.38	78%
CR (Sex)	4	110	SMD. Random.	0.92 (−0.20, 2.04)	0.46	87%
Physical function (WA)	6	166	SMD. Random.	0.03 (−0.58, 0.63)	0.93	72%
Physical function (UG)	5	116	MD. Random.	0.12 (−0.18, 0.42)	0.42	75%

[a] Meta-regression analysis was conducted. Results are presented as mean differences (MD) or standardized mean differences (SMD) with 95% confidence intervals. Heterogeneity is reported as I^2. Abbreviations: CR, chair-rise; LB, lower-body; MD, mean difference; SMD, standardized mean difference; UG, up-and-go; UB, upper-body; WA, walk ability. * $p < 0.05$ indicates statistical significance.

3.8.2. Muscle Mass

For muscle mass, six studies [45–49,57,83] with 211 participants (n = 144 males, 67 females) reported measures of skeletal muscle mass with intervention periods ranging from 6 to 18 weeks (Table 1). The meta-analysis revealed that pooled muscle mass measures were not significantly different when RT + Ω-3 PUFAs was compared to RT + placebo ($p = 0.30$), although no heterogeneity was present (I^2 = 0%, p = 0.46) (Figure 3). The non-significant SMD (p = 0.30) and heterogeneity (I^2 = 8%; p = 0.46) remained after excluding studies with design-related outliers, Cornish and Chilibeck [45] using ALA and Dadóva et al. [48] using Calanus oil as the intervention supplement. No publication bias was found ($p > 0.05$) using Egger's test for small study effects. In addition, visual inspection of the funnel plot analysis did not reveal any asymmetry (Supplementary Figure S1). When the meta-regression analysis was conducted, a positive but non-significant association (p = 0.22) between Ω-3 PUFAs intake (g/day) and pooled muscle mass changes (SMD) (β = 0.148 (CI: −0.09, 0.40)) was found (Supplementary Figure S2a). There was no subgroup difference present when studies were stratified by sex (p = 0.42) or duration (p = 0.73) (cut point; ≥16 weeks) (Supplementary Figure S2b,c).

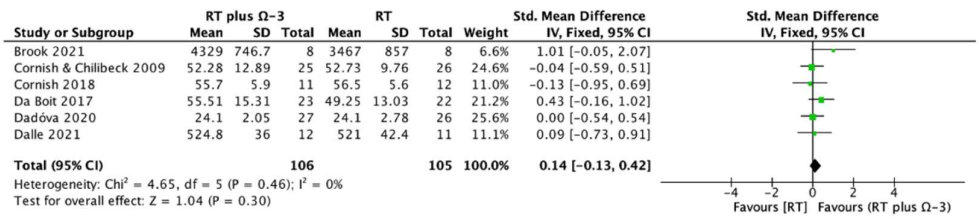

Figure 3. Forest plot of the effects of RT + Ω-3 PUFAs on muscle mass. IV: inverse-variance method; Fixed: fixed effect. Weight (in %), the influence of an individual study on the pooled result [45–49,57].

3.8.3. Neuromuscular Function

Pooled effects of RT + Ω-3 PUFAs compared to RT alone or with placebo on neuromuscular function in the lower extremities are shown in Figure 4. Eight studies [45–49,52,54,57], with 264 participants (n = 152 males, 112 females) using RT + Ω-3 PUFAs compared to RT alone or with a placebo, demonstrated a small, positive, but non-significant effect (p = 0.30), with significant heterogeneity (I^2 = 78%, p < 0.0001). Rodacki et al. [54] was the only study with publication bias and included a control group without a placebo supplement. Excluding this study reduced heterogeneity (I^2 = 0) and eliminated the positive effect (SMD = −0.05, p = 0.72). In contrast, removing other outliers, such as Cornish and Chilibeck [45] and Dadóva et al. [48], did not significantly impact our findings. No dose–response was found when investigating the association between Ω-3 PUFAs intake (g/day) and neuromuscular function in the lower extremities (β = −0.129 (CI: −1.03, 0.77), p = 0.78). A high heterogeneity was present between studies (I^2 = 92%) (Supplementary Figure S3a). Other potential sources of heterogeneity between studies were explored, but sex (p = 0.27) and duration (p = 0.20) showed no significant subgroup difference (Supplementary Figure S3b,c).

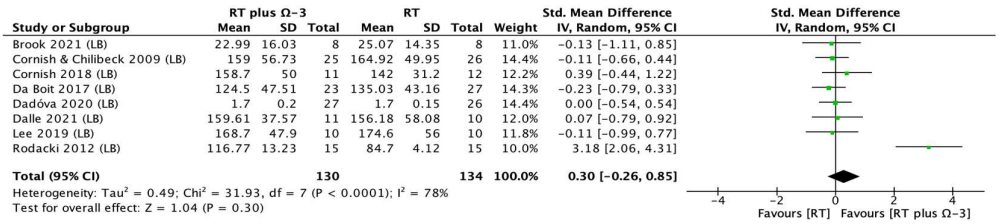

Figure 4. Forest plot of the effects of RT + Ω-3 PUFAs on neuromuscular function (lower-body). LB: lower-body; IV: inverse-variance method; Random: random effect. Weight (in %), the influence of an individual study on the pooled result [45–49,52,54,57].

Results for neuromuscular function in the upper body are shown in Figure 5 when using RT + Ω-3 PUFAs versus RT alone or with placebo. Six studies [45–49,52,54,57] were analyzed, which included 190 participants (n = 126 males, 64 females). A small but non-significant effect of RT + Ω-3 PUFAs was observed (SMD = 0.09, p = 0.52), without heterogeneity present (I^2 = 0). When Dadóva et al. [48], which used Calanus oil, was excluded, the small effect was also eliminated (SMD = −0.01). However, excluding other design-related outliers did not affect the results.

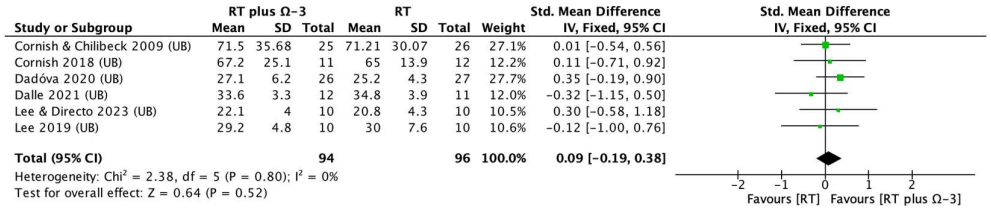

Figure 5. Forest plot of the effects of RT + Ω-3 PUFAs on neuromuscular function (upper-body). UB: upper-body; IV: inverse-variance method; Fixed: fixed effect. Weight (in %), the influence of an individual study on the pooled result [45,46,48,49,52,82].

3.8.4. Physical Function

Chair-rise performance was evaluated in six studies (n = 196; 102 males, 94 females) using two methods (Figure 6): four studies [47,49,52,82] assessed the time required to complete five sit-to-stand repetitions (5XSTS) as quickly as possible (improvement indicated by reduced time), while two studies [48,54] measured the maximum number of sit-to-stand repetitions completed in 30 s (30s STS). The pooled analysis to compare RT + Ω-3 PUFAs versus RT alone or with placebo indicated a large and significant effect

(SMD = 0.77, p = 0.02), although a significant heterogeneity was found (I^2 = 78%; p = 0.0003) favoring RT combined with Ω-3 PUFAs supplementation. When Rodacki et al. [54], which reported the largest effect (SMD = 3.16) and lacked a placebo supplement, was excluded due to potential publication bias, the effect of RT combined with Ω-3 PUFAs decreased but remained significant (SMD = 0.40, p = 0.01), and heterogeneity was eliminated (I^2 = 0). However, removing the findings of Dadóva et al. [48] meant this result was no longer significant (SMD = 0.84, p = 0.06), with heterogeneity being reduced but still significant (I^2 = 83%, p = 0.0001). Meta-regression analysis revealed a negative but non-significant association on Ω-3 PUFAs intake (g/day) and the effects of RT + Ω-3 PUFAs on chair-rise performance (β = −0.160 (CI: −1.23, 0.91), p = 0.77), with high heterogeneity present (I^2 = 91%) (Supplementary Figure S4a). Only a single study [47] assessed men exclusively in chair-rise performance; therefore, we did not perform subgroup analysis for sex differences. When considering, duration, no subgroup difference was evident (p = 0.31), although only long duration (≥16 weeks) found a significant effect (SMD = 0.40, p = 0.05) (Supplementary Figure S4b).

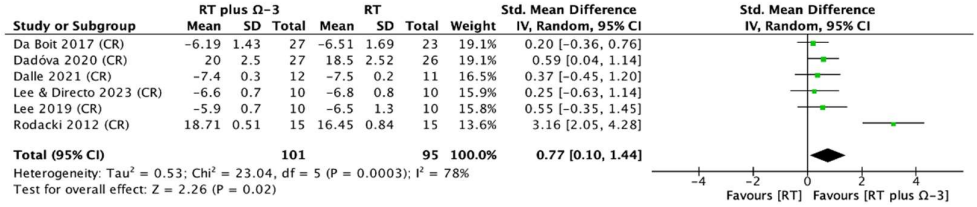

Figure 6. Forest plot of the effects of RT + Ω-3 PUFAs on physical function (chair-rise). CR: chair-rise; IV: inverse-variance method; Random: random effect. Weight (in %), the influence of an individual study on the pooled result [47–49,52,54,82].

Six studies assessed walking ability, with four studies evaluating gait speed (time used to walk 4 or 6 m, improvement indicated by reduced time) [47,49,52,82], and two studies evaluating distance (meters walked in 6 min, improvement indicated by increased distance) [48,54], with 166 participants (n = 72 males, 94 females) (Figure 7). A small but non-significant effect was observed (SMD = 0.03, p = 0.93) when Ω-3 PUFAs was combined with RT versus RT alone or with placebo. These results were unchanged, including heterogeneity, when Rodacki et al. [54] without a placebo supplement was removed.

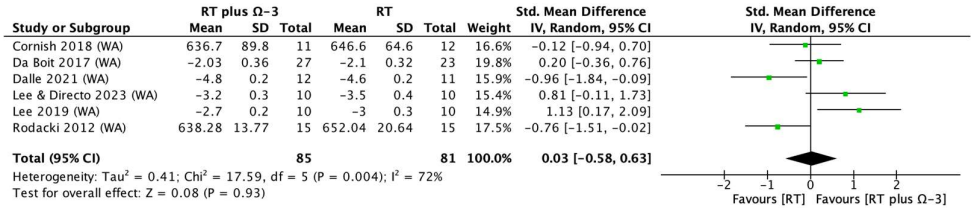

Figure 7. Forest plot of the effects of RT + Ω-3 PUFAs on physical function (walking ability). WA: chair-rise; IV: inverse-variance method; Random: random effect. Weight (in %), the influence of an individual study on the pooled result [46,47,49,52,54,82].

Five studies reported up-and-go results, such as TUG [46,49,52,82] and 8-FUGT [54] (improvement indicated by reduced time), involving 116 participants (n = 45 males, 71 females) (Figure 8). No significant effect of RT + Ω-3 was observed (p = 0.42), although significant heterogeneity was observed (I^2 = 75%; p = 0.003). When removing Rodacki et al. [54], the findings were not changed significantly (p = 0.68, I^2 = 72%).

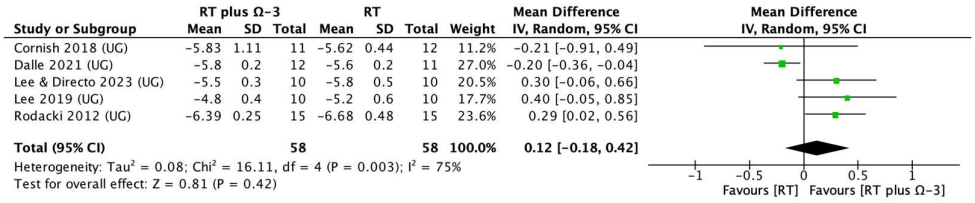

Figure 8. Forest plot of the effects of RT + Ω-3 PUFAs on physical function (up-and-go). UG: up-and-go; IV: inverse-variance method; Random: random effect. Weight (in %), the influence of an individual study on the pooled result [46,49,52,54,82].

4. Discussion

The primary findings of this systematic review and meta-analysis showed that combining RT with Ω-3 PUFAs (RT + Ω-3 PUFAs) did not result in additional effects on muscle mass, neuromuscular function, walking ability, and UG compared to RT alone or with placebo (RT + placebo) in older adults, but a large effect of RT + Ω-3 PUFAs was observed in chair-rise performance (SMD = 0.77, 0.10–1.44, Figure 6). However, Cochrane's risk of bias tool revealed that 56% and 33% of the included studies had a 'high risk' and some concerns of bias, respectively (Figure 2). In addition, the quality score for an optimal Ω-3 PUFAs study design only reached a mean of 2 (SD 0.9), with no study obtaining the maximum score of 5 (Supplementary Table S3).

Effect modifiers such as sex, dose, and duration of Ω-3 PUFAs supplementation were statistically investigated, but no significant differences were observed among groups.

4.1. Omega-3 Study Design Concerns

The assessment of study designs using the Omega-3 scoring system (Supplementary Table S3) revealed several imitations in the included studies. Most studies failed to establish a low baseline intake of Ω-3 PUFAs or measure baseline levels and subsequent changes in tissue Ω-3 PUFAs levels. This raises concerns about group separation validity [70], even though seven out of eleven studies used doses > 2 g per day, which have been shown to increase muscle mass [84].

Notably, only Da Boit et al. [47] measured the O3I, the measure of EPA + DHA found in erythrocytes and a reliable marker of long-term Ω-3 PUFAs intake. Other studies relied on plasma measures, which are more sensitive to short-term dietary intake and may not accurately reflect muscle tissue incorporation due to potential variability if not correctly sampled [85]. Measurement of the O3I both at baseline and post-supplementation is preferable as it provides a clearer picture of long-term Ω-3 PUFAs' status [62,85].

The lack of O3I measurements complicated the validation of participants' O3I baseline levels. For instance, excluding individuals with high baseline levels due to Ω-3 PUFA supplementation or frequent fish consumption (≥2 fish meals per week) could help ensure low baseline O3I levels.

Conversely, measuring O3I may reveal participants with unexpectedly high baseline levels despite no reported supplementation and/or frequent fish intake, potentially identifying them as high responders to Ω-3 PUFAs. Indeed, multiple studies have reported high inter-individual variability in Ω-3 PUFAs [36,70,86]. Stratifying participants into "high" and "low responders" to Ω-3 PUFAs has been suggested as a strategy to maximize differences in tissue levels of Ω-3 PUFAs between control and intervention group [70,86].

Furthermore, while all studies in this review met the minimum duration criterion of ≥4 weeks based on the Omega-3 score system, achieving an optimal Omega-3 score might have allowed for better stratification of participants into responder categories. This stratification could enable tailored interventions with adequate duration and doses to produce significant changes in O3I. Additionally, measuring O3I would allow future

studies to assess the effectiveness of actual Ω-3 PUFA concentrations rather than intake alone, which may not be valid due to group separation issues. This could potentially reveal and amplify effects of Ω-3 PUFAs and RT on muscle mass, neuromuscular and physical function.

4.2. Effects of Ω-3 PUFAs Alone or Combined with RT on Muscle Mass, Neuromuscular, and Physical Function

Our findings did not reveal any potentiating effect on muscle mass when Ω-3 PUFAs supplementation was added to RT compared to RT + placebo. Similar findings were reported in an earlier meta-analysis that investigated whether RT + Ω-3 PUFAs was superior to Ω-3 PUFAs only [44]. Regardless, another meta-analysis [84] found that muscle mass was increased after supplementation of Ω-3 PUFAs alone, but only if the dose exceeded 2 g/d of Ω-3 PUFAs. This may be due to a higher incorporation of Ω-3 PUFAs into skeletal muscle phospholipids; however, unlike in blood [87], no study has established a dose–response in skeletal muscle phospholipids [88]. Nevertheless, Ω-3 PUFAs have been reported to improve uptake of amino acids, upregulating cell signaling proteins known to control MPS, and ultimately contributing to increases in muscle mass [28,89,90].

Ω-3 PUFAs may also reduce muscle protein breakdown by blocking NF-κB-mediated inflammatory pathways [88], potentially decelerating skeletal muscle atrophy [91].

According to our meta-regression analysis on dose–response, higher dose did not influence muscle mass. Nevertheless, a critical point is that the doses were stratified by Ω-3 PUFAs intake (e.g., 2 g/d) rather than the actual Ω-3 PUFAs concentrations in erythrocyte membranes (i.e., assessed in the O3I, e.g., O3I of 5%). This may blur the results due to the differential Ω-3 PUFAs uptake, which have been reported to vary from 50 to 190% [86]. Indeed, inter-individual differences in Ω-3 PUFAs uptake may have resulted in overlaps between intervention and control groups [61,70]. Importantly, O3I baseline values may be a critical point as O3I above 8% is considered optimal [33]. Two studies included in this systematic review [47,54] had intervention groups with baseline O3Is at 7.4% and 7.6%, respectively, limiting the potential hypertrophic effects of Ω-3 PUFAs intake.

Neuromuscular function was assessed through upper and lower body strength measures. Ω-3 PUFAs may enhance muscle strength through neural modulation, even without hypertrophic effects [47], as they have been shown to increase nerve conduction velocity, improving muscle contractility [92–94]. Indeed, a 6-month supplementation of Ω-3 PUFAs in healthy older adults has been shown to significantly increase multiple neuromuscular outcomes, including handgrip strength, 1-RM muscle strength, and power [28]. However, in our meta-analysis, no effect on muscle strength was observed when RT was combined with Ω-3 PUFAs supplementation. In contrast to our findings, a similar meta-analysis [44] reported Ω-3 PUFAs with RT improved lower body strength when compared to Ω-3 PUFAs only. One possible explanation for this discrepancy may be that one of the additional studies [50] started with lower baseline O3Is (not measured), giving individuals a greater potential to increase their O3Is and, as a result, improve their lower body strength. Our findings did not reveal a sex difference when assessing neuromuscular function in the lower extremities. However, one of the included studies [47] demonstrated that Ω-3 PUFAs supplementation enhanced the effects of an RT increase in lower body strength in older women but not in men. Despite similar EPA and DHA concentrations in blood and muscle membranes in both groups being observed, it is unclear which potential mechanisms may determine a sex-specific response. Sex was identified as a factor influencing the O3I response to Ω-3 PUFA supplementation, with females showing a higher response compared to males. Additionally, variability in O3I response was partly explained by differences in physical activity levels [36]. It has been reported that estrogen may contribute to a higher conversion from ALA to EPA in women [30]. Sex difference responses to RT have

also been reported, although inconsistently [95,96], and based on these findings, controlling or stratifying by sex in future studies may provide insights into the role of sex as an effect modifier.

Three common measures of physical function were assessed in this meta-analysis, including chair-rise, UG, and walking ability, which are strong predictors of functional ability in older adults [6]. Our meta-analysis indicated that only the chair-rise performance improved, which the findings of a similar meta-analysis [44] also showed. Surprisingly, one study [48] using Calanus oil in a low-dose manner improved chair-rise performance compared to high-dose studies (Supplementary Figure S3a). Although the erythrocyte membrane Ω-3 PUFAs' composition was not measured, it is possible that the higher bioavailability of their Ω-3 PUFAs supplement contributed to the improved performance. Indeed, bioavailability has been shown to vary among different forms of Ω-3 PUFAs [97,98]. Conversely to our finding, the meta-analysis [44] reported improvements in UG performance; however, different studies were used, and not all included RT regimes. DHA-rich fish oil has been reported to improve reaction time efficiency [99] in active individuals, which may partly explain Ω-3 PUFAs' effect on UG. Another meta-analysis [84], investigating Ω-3 PUFAs only, reported improvements in walking ability only when the intervention period exceeded 24 weeks. Our meta-analysis did not find a duration to influence our outcomes; however, none of the included studies exceeded 18 weeks, which suggests that this period may have been too short for the optimal incorporation of Ω-3 PUFAs. Therefore, the intervention duration may still be an important modifier, depending on the research question, given the varying turnover rates of Ω-3 PUFAs in tissues [64]. For instance, durations longer than 16 weeks have been recommended to improve reaction time [100]. Additionally, DHA is an important component in the brain (50:1 ratio of DHA vs. EPA) [101] and highly involved in neuromuscular function [29,102]. Therefore, longer supplementation durations may lead to a higher DHA incorporation and improved muscle performance.

4.3. Impact of Daily Energy and Protein Intake on Muscle Mass, Neuromuscular, and Physical Function

Habitual diet may also contribute to inconsistent results. Older adults may be predisposed to undernutrition and protein energy malnutrition [8,103]. A daily energy deficit of ~500 kcal has been shown to hinder lean mass gains during RT, as highlighted in a recent meta-analysis [104]. While healthy older adults are advised to consume at least 1.0 g/kg/day of protein [9,105], only four studies in our review [45,46,54,57] controlled for this intake. As a result, some studies may not have provided sufficient energy or protein to support muscle mass increase, despite optimal Ω-3 PUFA supplementation and RT.

4.4. Considerations for Future Research

The effectiveness of Ω-3 PUFAs combined with RT in older adults to improve muscle mass, neuromuscular, and physical function remains inconclusive. Methodological challenges, such as high bias risk and low Ω-3 PUFA study design scores, may have influenced our meta-analysis findings. While the potential effect modifiers showed no impact, the absence of O3I measurements is a critical limitation. Factors like intake vs. uptake, diet, protein intake, placebo type, Ω-3 PUFA forms, and timing may also affect outcomes. For example, using corn or olive oil as placebos might alter the net effect of Ω-3 PUFAs [59,65]. To enhance bioavailability, formulations with equal EPA and DHA ratios [106,107], low oxidation rates [108–113], and high levels of polyphenols [59], as well as ingestion with high-fat foods, may be beneficial [114]. A micro-dose of Ω-3 PUFAs could help blind participants without providing an overly potent placebo, as both EPA and DHA are present in the experimental and control groups [70]. Since no human has been measured with an O3I $\leq$ 2%, the absence of EPA and DHA cannot be tested against their presence [115].

Investigating timing, e.g., with a run-in period with the Ω-3 PUFAs supplementation prior to a RT intervention, may be a strategy to ensure a comparison between an intervention group with an optimal O3I (e.g., ≥8%) compared to a control group with a lower O3I (e.g., ≤4%).

Finally, Ω-3 PUFAs may amplify the effects of RT, and RT, in turn, may enhance Ω-3 PUFA incorporation into cell membranes [116]. Achieving a significant O3I difference between groups prior to RT is advisable to minimize confounding effects. These considerations are outlined (Supplementary Figure S5) in a hypothetical study design (Figure 9) to maximize treatment differences and assist future research.

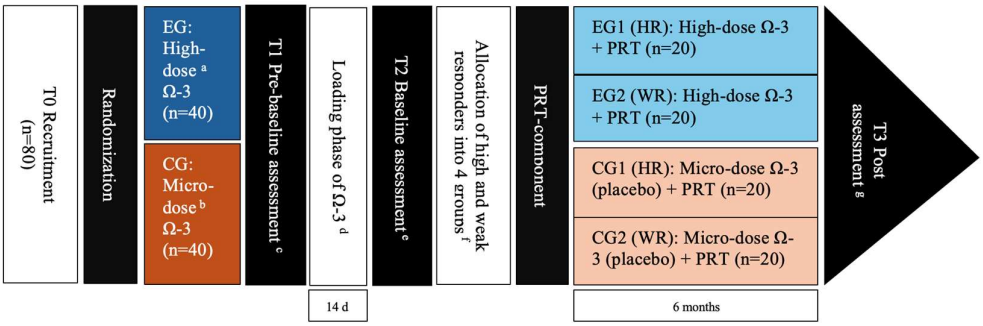

Figure 9. Hypothetical study design to investigate RT + Ω-3 PUFAs to maximize O3I differences. [a] High-dose refers to consuming a supplementation high enough to achieve an O3I of ≥8%. [b] Micro-dose refers to consuming same type of supplementation (e.g., EPA + DHA) as the intervention dose, targeting an O3I of ≤4%. Assessment ([c] pre-baseline, [e] baseline, and [g] post): Ω-3 measurements (O3I, half-maximum of Ω-3 levels and bioavailability of Ω-3 based on O3I) and relevant outcomes (e.g., muscle mass). [d] Loading phase of Ω-3 for minimum 14 days to reach half-maximum levels of EPA and DHA). [f] Allocation into high- (>50% increase in O3I) and weak responders (≤50% increase in O3I). Abbreviations: CG, control group; EG, experimental group; HR, high response to Ω-3 PUFAs; O3I, Omega-3 Index; omega-3 polyunsaturated fatty acids; Ω-3 PUFAs; PRT, progressive resistance training; T0–3, test sessions; WR, weak response to Ω-3 PUFAs.

4.5. Limitations and Strengths

A limitation of this systematic review is the limited number of studies (n = 9 in the meta-analysis) and the heterogeneity in Ω-3 PUFAs (ALA, EPA, and DHA in different dosages), RT, and placebo protocols (Table 1). The lack of control over RT duration and volume may have also affected the meta-analysis, as certain programs, like those including walking, might influence outcomes. Additionally, it was not possible to control for the impact of daily energy and protein intake, NSAIDs, or variability in placebo effects. However, a key strength of this study is that this is the first meta-analysis, to our knowledge, to examine the effects of sex, intervention dose, and duration on the impact of Ω-3 PUFAs combined with RT on muscle mass, neuromuscular-, and physical function, in older adults compared to RT alone or with placebo.

5. Conclusions

Overall, the present meta-analysis did not find conclusive evidence that RT combined with Ω-3 PUFAs is superior to RT alone or with placebo for improving muscle mass, as well as neuromuscular and physical function, in older adults. Additionally, no significant differences were observed based on sex, higher Ω-3 PUFAs doses, or longer intervention durations. Future clinical trials should consider designing interventions with low RoB, optimal Ω-3 PUFAs scores (e.g., by excluding participants with high O3Is), and standardizing for the potential DTTD modifiers (i.e., dose, type, timing, and duration).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jal5010004/s1, Figure S1. Funnel plots of the effect of Ω-3 PUFAs supplementation on muscle mass (a), NMF (LB) (b), NMF (UB) (c), PF (CR) (d), PF (UG) (e), and PF (WA) (f). Figure S2. Bubble and forest plots of the included studies assessing effect of Ω-3 PUFA supplementation on muscle mass stratified by dose (a), sex (b) and duration (c) of Ω-3 PUFAs. Figure S3. Bubble and forest plots of the included studies assessing effect of Ω-3 PUFA supplementation on neuromuscular function (lower-body) stratified by Ω-3 PUFAs dose (a), sex (b) and Ω-3 PUFAs duration (c). Figure S4. Bubble and forest plots of the included studies assessing effect of Ω-3 PUFA supplementation on chair-rise stratified by Ω-3 PUFAs dose (a) and duration (b). Figure S5. Ω-3 DTTD-factors based considerations applied in a hypothetical study design. Table S1. Search Strategy. Table S2. Eligibility criteria. Table S3. Omega-3 scores with comments for included studies. Table S4. Summary of Ω-3 PUFAs supplementation and placebo protocols in the included studies. Table S5. Summary of exercise training protocols in the included studies. Table S6. Men combined with women—Tables for muscle mass, neuromuscular- and physical function outcomes in included studies and reasons for outcome selection. Table S7. Men vs. women subgroup—Tables for muscle mass, neuromuscular- and physical function outcomes in included studies and reasons for outcome selection.

Author Contributions: Conceptualization, D.L.D.; design, D.L.D.; supervision: P.C., P.Ø.O., J.J.W. and M.A.T.; materials: D.L.D.; data collection and/or processing: D.L.D. and J.A.C.; analysis and/or interpretation: D.L.D.; literature search: D.L.D. and J.A.C.; writing manuscript: D.L.D.; critical review: D.L.D., P.C., P.Ø.O., J.J.W., S.F.B., J.A.C. and M.A.T. All authors have read and agreed to the published version of the manuscript.

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