Nutrition clinique et métabolisme 38 (2024) 158–167

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Original article

Effect of omega-3 fatty acids supplementation on inflammatory markers following exercise-induced muscle damage: Systematic review and meta-analysis of randomized controlled trials

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ARTICLE INFO

Article history:
Received 16 November 2023
Received in revised form 12 March 2024
Accepted 11 April 2024
Available online 9 August 2024

Keywords:
Omega-3 fat
Inflammation
Interleukin-6
Tumor necrosis factor-α
C-reactive protein
Exercise

ABSTRACT

Background. – Omega-3 fatty acids supplementation may protect against exercise-induced muscle damage (EIMD) through its anti-inflammatory properties. The purpose of the present meta-analysis was to evaluate the effects of omega-3 fatty acid supplementation on inflammatory markers following EIMD in trained and untrained individuals.
Methods. – Medline, Scopus, and Google Scholar databases were systematically searched up to April 2023. The Cochrane Collaboration tool was used to assess the risk of bias and evaluate the quality of the studies.
Results. – Omega-3 supplementation significantly reduced interleukin (IL) 6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP) concentrations.
Conclusion. – The current meta-analysis indicated the efficacy of omega-3 in reducing the serum levels of inflammatory markers in healthy individuals, overall, and in subgroup analysis. Thus, omega-3 may be a priority EIMD recovery agent for interventions.

1. Introduction

Regular physical activity is important for overall health, but it can sometimes cause muscle damage known as exercise-induced muscle damage (EIMD) [1]. EIMD happens when muscles experience intense or new physical stress, resulting in small tears in the muscle fibers [2,3]. This damage triggers an inflammatory response in the body to repair and rebuild the damaged muscle tissue [4].

The pathophysiology of EIMD starts with the mechanical stress placed on the muscle fibers during exercise. This stress results in micro-tears in the muscle tissue, triggering an inflammatory response as the body works to repair the damaged fibers [5].

Inflammation markers, such as tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) and interleukin-6 (IL-6), are key indicators of the body's immune response to stress, injury, or infection [6]. During physical activity and exercise, the body undergoes stress and strain, leading to an increase in inflammation markers as a natural response to EIMD [7]. Research has shown that elevated levels of inflammation markers can have a negative impact on athletic performance and recovery in several ways [8].

Firstly, increased inflammation can lead to muscle soreness and fatigue, which can impair an athlete's physical capabilities and overall performance. This can result in decreased strength, endurance, and agility during training or competition [9]. Moreover, heightened inflammation levels can delay the body's ability to repair and rebuild muscle tissue following intense exercise [10]. This delayed recovery process can prolong muscle soreness and fatigue, leading to longer recovery times between training sessions. As a result, athletes may struggle to maintain a consistent training schedule and make progress in their performance goals [11]. Chronic inflammation can also compromise the body's immune function, making athletes more susceptible to illness and injury. This can further disrupt training routines and hinder overall athletic performance [12]. Additionally, persistent inflammation has been associated with the development of overtraining syndrome, a condition characterized by decreased performance, persistent fatigue, and an increased risk of injury [13].

Overall, elevated levels of inflammation markers can disrupt the delicate balance between training stress and recovery, ultimately impairing athletic performance and hindering the athlete's ability to reach their full potential. It is essential for athletes to monitor their inflammation levels, implement strategies to reduce inflammation, and prioritize adequate recovery to optimize their performance and overall well-being.

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https://doi.org/10.1016/j.nupar.2024.04.006

Various studies have suggested that omega-3 LC-PUFAs have beneficial effects on human health and many inflammatory diseases [14], and may operate as important energetic molecules that can modulate oxidative stress and inflammatory responses to exercise [15]. The mechanism underlying the anti-inflammatory features of omega-3 LC-PUFA comprises membrane-derived omega-6 fats with omega-3 fats substrate competition for lipoxygenase (LOX) and cyclooxygenase (COX) enzymes, causing increased production of less inflammatory and decreased generation of inflammatory eicosanoids [16,17]. Moreover, omega-3 LC-PUFA have effects as nuclear ligands for nuclear factor kappa B (NF-KB) and peroxisome proliferator-activated receptors, thereby influencing the transcription of inflammatory factors such as adhesion molecules and cytokines [14,18].

Numerous studies have assessed whether omega-3 fatty acids supplementation can reduce the degree of muscle damage, inflammation, and oxidative stress following exercise [19–21]. However, more investigations have illustrated a positive effect of omega-3 LC-PUFA in relation to improving muscle damage, DOMS, inflammation, and oxidative stress following exercise [22–26], and some studies have shown no effect [27]. It is possible that differences in exercise protocols, supplementation duration and dosage, subject population, timing of supplementation, and measurement and biomarker selection are related to discrepancies in the outcomes between investigations.

Thus, we hypothesized that omega-3 fatty acids supplementation affects muscle damage-related inflammatory markers after an exercise program. Long-chain omega-3 polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA; 22:6 n3) and eicosapentaenoic acid (EPA; 20:5 n3) are found in fish oil. It has been suggested that PUFAs, especially EPA and DHA, are incorporated into the cellular membranes. This procedure can modify the release of muscle enzymes, pro-inflammatory 2 series prostaglandins (PGs), thromboxanes, and prostacyclins [28,29]. Thus, the primary aim of the present review was to evaluate whether omega-3 fatty acids supplementation at different doses and during the days before and after different exercise protocols accelerated the recovery from EIMD and attenuated the increase in circulating plasma markers of inflammation. The current meta-analysis assessed markers of inflammation, including IL-6, TNF-α, and CRP in trained and untrained healthy adult participants of both sexes.

2. Methods

2.1. Search strategy

The current systematic review and meta-analysis were based on the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [30]. A computerized search was carried out from inception to April 2023 using diverse databases including PubMed, Scopus, ISI Web of Science, and Google Scholar. The literature search was restricted to articles published in English. The following MeSH and non-MeSH combinations were used: “fatty acids, omega-3”, “omega-3”, “n-3 polyunsaturated fatty acid”, “n-3 PUFA”, “docosahexaenoic acid”, “eicosapentaenoic acid”, “EPA”, “DHA”, “exercise”, “physical exercise”, “eccentric exercise”, “aerobic exercise”, “athlete”, “muscle soreness”, “muscle damage”, “C-reactive protein”, “tumor necrosis factor-alpha”, “interleukin-6”, “inflammation”, “controlled trial”, “random”, “randomly”, “randomized clinical trial”, “randomized”, “randomised”, “RCT”, “blinded”, “double blind”, “double blinded”, “trial”, “controlled clinical trial”, “crossover procedure”, “crossover trial”, “double blind procedure”, and “equivalence trial”. The reference lists of all the articles were checked to identify eligible articles.

2.2. Eligibility criteria

Studies were selected according to the population-intervention-comparator-outcomes-study design (PICOS) [30], including The Population (healthy participants aged > 18 years without a history of muscle damage or injury), Intervention (omega-3 supplementation), comparison (matched control group), and outcome (inflammation markers including CRP, IL-6, and TNF-α concentration), which were performed as a randomized controlled trial design.

All randomized controlled trials were included in the current meta-analysis if our inclusion criteria were: (1) original randomized controlled trial studies; (2) participants received oral omega-3 supplementation, as a nutritional strategy; (3) presented at least one outcome measure of inflammation markers (CRP, IL-6, and TNF-α); and (4) reported interest data as mean and standard deviation (SD) of CRP, IL-6, and TNF-α in the intervention and placebo groups. The exclusion criteria were: (1) consumption of omega-3 mixture only in the intervention group (vitamins such as Vitamin E and Vitamin C or amino acids like HMB, BCAA, etc.), not including a placebo group; (2) animal studies; (3) trials without control groups, non-randomized or semi-experimental trials; (4) case reports, editorial articles, or letters to the editor; and (5) duplicate articles with the same population (some studies reported the same data).

2.3. Selection strategy

After the initial search, all papers found from electronic or manual searches were recorded and entered into the EndNote software for screening (EndNote X6, Thomson Reuters, New York). The titles and abstracts of the articles were screened using a search strategy. Papers were assessed independently by two authors and were independently selected based on the inclusion criteria. Papers that met the eligibility criteria in the title and abstract screening were selected for full-text evaluation. All of the randomized controlled trials were included in the meta-analysis if the inclusion criteria were met. We used a standardized form to select trials eligible for inclusion in the review according to the data within the full text. Contradictions between reviewers were resolved by a third researcher or by consensus.

2.4. Data extraction

Two independent authors extracted the following data by applying a standardized electronic pre-designed form (Excel, Microsoft Office): first author’s name, country and year of publication, design of research, sample size, age and sex of participants, duration of intervention, and dose of omega-3. In addition, the authors extracted the baseline and post-intervention means and SD of inflammation markers (CRP, IL-6, and TNF-α). Standard errors of the mean (SEM) were converted to SDs using the following formula: ($SD = SEM \times \sqrt{n}$ [n is the number of subjects in each group]). Finally, in articles that reported data in graphical figures, data extraction was performed using the GetData Graph Digitizer 2.24 [31].

2.5. Study quality

It has been accepted that randomized controlled trial inclusion with a high risk of bias may distort the results of a meta-analysis study [32,33] and the Cochrane Collaboration tool was used to measure the risk of bias. The quality of the included trials was assessed using the following items: randomization sequence generation, allocation concealment, participants, personnel, assessor, and investigator blinding, attrition rates, and financial interest by companies. These items were rated as having a high, unclear, or low risk of bias, respectively, according to the key areas of alloca-

tion concealment, reporting of attrition rates, and participants and assessors (low = low risk of bias for all key areas, high = high risk of bias for one or more key areas, and medium = low or unclear risk of bias for all key domains) [32].

2.6. Analyses and measures of treatment effect

Mean differences and SD were computed for continuous variables in every trial. Standardized mean changes were used for variables pooled on different scales. For papers with no reported SD of the mean change, the following formula was applied: SD change = square root ([SD baseline^2 + SD final^2] – [2 × 0.8 × SD baseline × SD final]) [34]. The heterogeneity of studies was evaluated using the chi-squared (χ^2) test and quantified using the I^2 statistic, which reports the percentage of total variation across trials that is attributable to heterogeneity rather than to chance. Significant heterogeneity was defined as a P value < 0.05.

A random effects model was used to calculate the weighted mean differences (WMDs) with 95% confidence intervals (CIs) to estimate the overall effect. To evaluate whether the outcomes could have been distinctly influenced by a single study, sensitivity analysis was performed [35]. Subgroup analysis was also performed, based on follow-up measurements after exercise (immediately, < 24 h, 24 h, 48 h, 72 h, and 96 h post exercise), dose of omega-3 (lower than 2 g/day and 2 g/day or more), duration of trials (acute (single dose), less than one month, and more than one month), time of supplementation (before exercise, after exercise, and before and after exercise), exercise type (anaerobic and both anaerobic and aerobic combination), and training status (trained and untrained). Egger's regression asymmetry and Begg's rank correlation tests were used to evaluate publication bias. The effect sizes (differences in means) against their corresponding standard errors were depicted using funnel plots. Statistical analyses were conducted using the STATA 11.2 software (StataCorp, College Station, Texas, USA).

3. Results

3.1. Findings from search and overview of included studies

Our search yielded a total of 254 studies. After removing duplicates, extensive screening of titles and abstracts was conducted for 251 studies. Twenty relevant studies remained after considering the inclusion and exclusion criteria for eligibility. Twelve other studies were excluded following detailed screening of the full text:

- performed on elderly or unhealthy participants (n = 5);
- performed on animals (n = 4);
- publications that assessed the effect of omega-3 supplementation combined with other treatments (n = 2);
- studies that did not report random allocation (n = 1).

Finally, eight articles were identified for qualitative and quantitative analyses in the current meta-analysis, including 23 effect sizes for IL-6 concentration, 16 effect sizes for TNF-α concentration, and four effect sizes for CRP concentration, which studied 156, 96, and 76 participants, respectively. This number includes subjects who dropped out in some studies. All subjects were young, aged 18.2–23.5 years. Nevertheless, in one study, the age of participants was 65–83 and, therefore, excluded from the meta-analysis. In addition, the random allocation of subjects was not illustrated in one study. Furthermore, all subjects were male, except for one study in which both men and women participated (n = 10) [27].

Fig. 1 presents the selection process and reasons for excluding studies. Table 1 shows the basic characteristics of the studies in our systematic review and meta-analysis. Briefly, studies were published between 2002 and 2020. The total number of participants who completed the trials according to the inclusion criteria was 76 in the intervention group and 80 in the placebo group for IL-6 concentration, 48 in the intervention group and 48 in the placebo group for TNF-α concentration, and 36 in the intervention group and 40 in the placebo group for CRP concentration. The dose of omega-3 supplementation was 0.8–3 g/day in these studies, and the duration ranged between 1 and 62 days. All studies used a randomized placebo-controlled design, except for one [36] that used a randomized crossover design. The effect of omega-3 on IL-6, TNF-α, and CRP levels were examined in two studies [37,38] and three studies reported only IL-6 (39), TNF-α [39] and CRP [40] concentrations.

Most of the articles undertook multiple follow-up measurements for each outcome (e.g., muscle damage enzymes for intervention and placebo immediately, and 1, 2, 3, 24, 48, 72, and 96 h after exercise). We focused on the results reported immediately after completion of the recovery intervention and subsequent hours (24, 48, 72, and 96 h post exercise). Five effect sizes in five studies had a follow-up time immediately post exercise [22,27,36,38,41]; four effect sizes in three studies had a follow-up time immediately post exercise [27,36,39]; eight effect sizes in seven studies reported 24 h follow-up times [22,27,36,39–42]; five effect sizes in four studies had 48 hours follow-up times [22,36,39,41]; five effect sizes in four studies reported 72 h follow-up times [36,39,41,43] and four effect sizes in three studies reported 96 h follow-up times [36,39,42].

In addition, the timing of when the omega-3 supplement must be consumed is debatable. Fourteen effect sizes in four studies were observed before and after exercise supplementation [22,36,41,43], and eight effect sizes in four studies were observed before exercise supplementation [27,38,40,42]. Jakeman et al. investigated the effects of the timing of supplement ingestion on inflammation after exercise using ten effect sizes [39].

3.2. Findings from quality assessments

Table 2 lists the quality details of the bias assessment. Briefly, random allocation of subjects was illustrated in all included studies. Nevertheless, two studies mentioned the method of random sequence generation and reported allocation concealment [39,41]. All studies had a low risk of bias due to incomplete outcomes. Most studies had a low risk of bias for selective outcome reporting, although three studies reported an unclear risk of bias [22,27,43] according to selective reporting. Moreover, all studies had an unclear or high risk of bias for blinding of participants and personnel and blinding of outcome assessors, except for two studies that indicated a low risk regarding blinding of participants, personnel, and outcome assessment [39,41]. Most studies reported a low risk of bias regarding other potential threats to validity, including a potential source of bias related to the particular study design applied, or a problem such as the study has been claimed to be fraudulent. Finally, most studies had a medium overall risk of bias, two studies had a low overall risk of bias [39,41] and two studies had a high overall risk of bias [27,42].

Findings from effects of omega-3 supplementation on inflammation markers meta-analysis.

3.3. Effects of omega-3 supplementation on IL-6 concentration

According to analysis of 23 effect sizes, overall, omega-3 supplementation had a significant reduction effect on IL-6 concentration (WMD = –0.97 pg/mL, 95% CI: –1.73, –0.22; P = 0.011). There was significant heterogeneity among the studies (Cochran's Q test = 689.08, P = 0.000, I^2 = 90.5%) (Fig. 2). Subgroup analysis was performed to assess whether the effect of omega-3 supple-

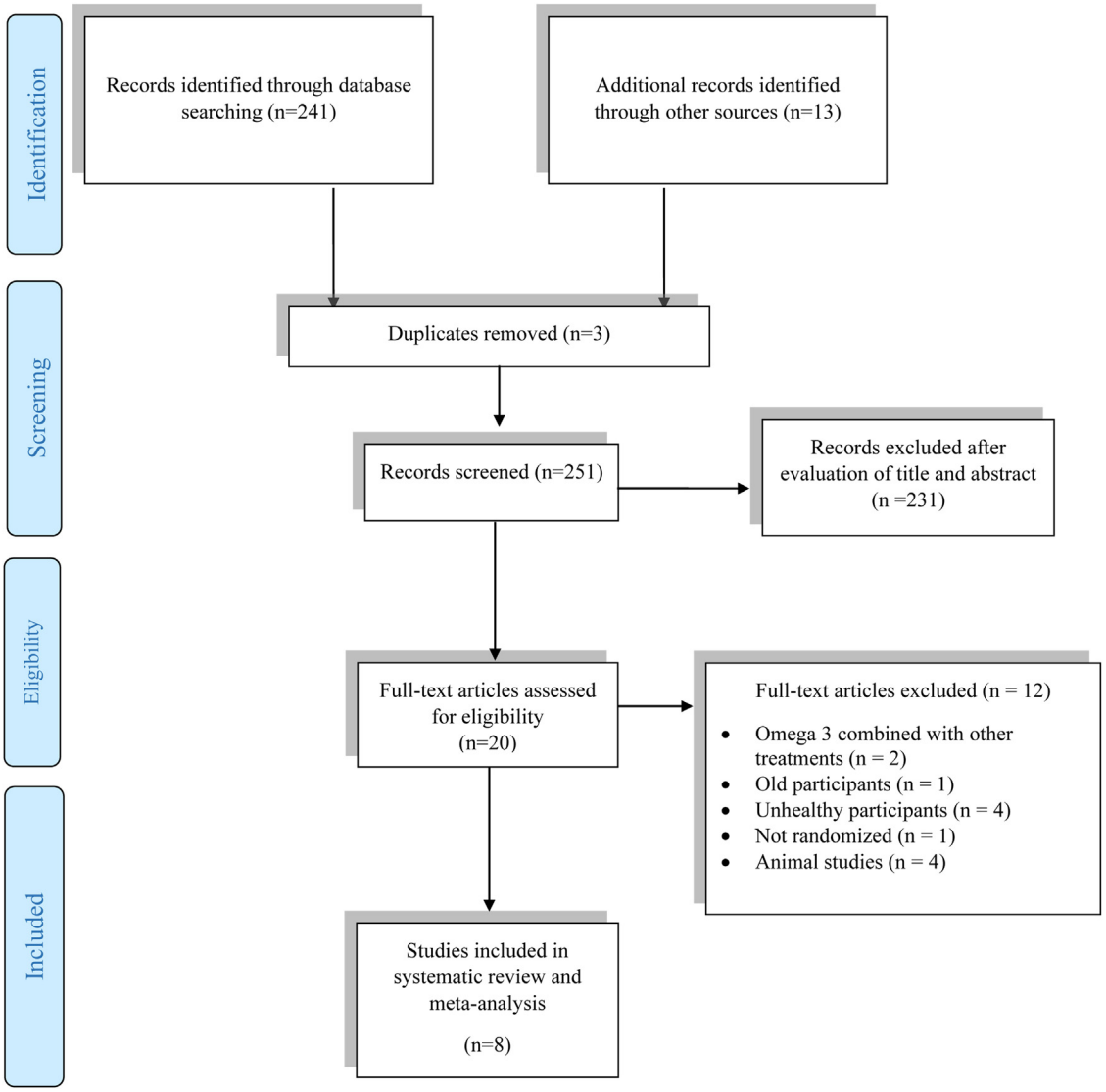

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of study selection process.

Table 1
Characteristics of the included studies.

Author (year)	Study design characteristics								Average age (y)	Sample size		Exercise type	Outcomes
	Design	Country	Training status	Omega-3 dose (g/d)	Omega-3 source	Duration (d)	Consumption time	Gender		Omega-3	Control		
Dalle et al. (2020)[a]	RP	Belgium	T	3	Fish oil	14	B.Ex	M and F	68	11	11	R	CRP, IL-6, TNF-α
Jakeman et al. (2017)	RP	UK	T	3	Fish oil	1	A.Ex	M	26	9	9	R	IL-6
Tsuchiya et al. (2016)	RP	Japan	U	2.4	Fish oil	62	B.Ex, A.Ex	M	19.5	12	12	R	IL-6, TNF-α
Mickleborough et al. (2015)	CP	USA	U	0.8	Mussel oil blend	30	B.Ex, A.Ex	M	22	16	16	A	TNF-α
Marques et al. (2015)[a,b]	CP	Brazil	T	3	Fish oil	30	B.Ex	M	33.8	8	8	A	CRP, IL-6, TNF-α
DiLorenzo et al. (2014)	RP	USA	U	2	Algal species	28	B.Ex	M	21.8	10	11	R	CRP, IL-6
Atashak et al. (2013)	RP	Iran	T	3	Fish oil	7	B.Ex	M	21.7	10	10	R	CRP
Tartibian et al. (2011)	RP	Iran	U	1.8	Fish oil	30	B.Ex	M	29.7	15	15	R	IL-6, TNF-α
Phillips et al. (2003)	RP	USA	U	0.8	Fish oil	14	B.Ex, A.Ex	M	22.1	16	19	R	CRP, IL-6
Lenn et al. (2002)	RP	USA	U	1.8	Fish oil	30	B.Ex	M and F	23.5	5	5	R	IL-6, TNF-α

B.Ex: before exercise; A.Ex: after exercise; A: aerobic training; R: resistance training; RP: randomized controlled clinical trial; CP: crossover study; M: male; F: female; D: days; Y: years; T: trained; U: untrained; ˆ unspecified or unknown; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: tumor necrosis factor-α.

[a] Excluded from meta-analysis.

[b] Not randomized.

Table 2
Cochrane risk of bias assessment.

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias	Overall risk of bias
Jakeman et al. (2017)	L	L	L	L	L	L	L	Low
Tsuchiya et al. (2016)	L	L	L	L	L	L	L	Low
Mickleborough et al. (2015)	U	U	U	U	L	L	L	Medium
DiLorenzo et al. (2014)	U	U	H	H	L	L	U	High
Atashak et al. (2013)	U	U	U	U	L	L	L	Medium
Tartibian et al. (2011)	U	U	U	U	L	U	L	Medium
Phillips et al. (2003)	U	U	U	U	L	U	L	Medium
Lenn et al. (2002)	U	U	H	H	L	U	L	High

L: low risk of bias; H: high risk of bias; M: medium risk of bias; U: unclear risk of bias.

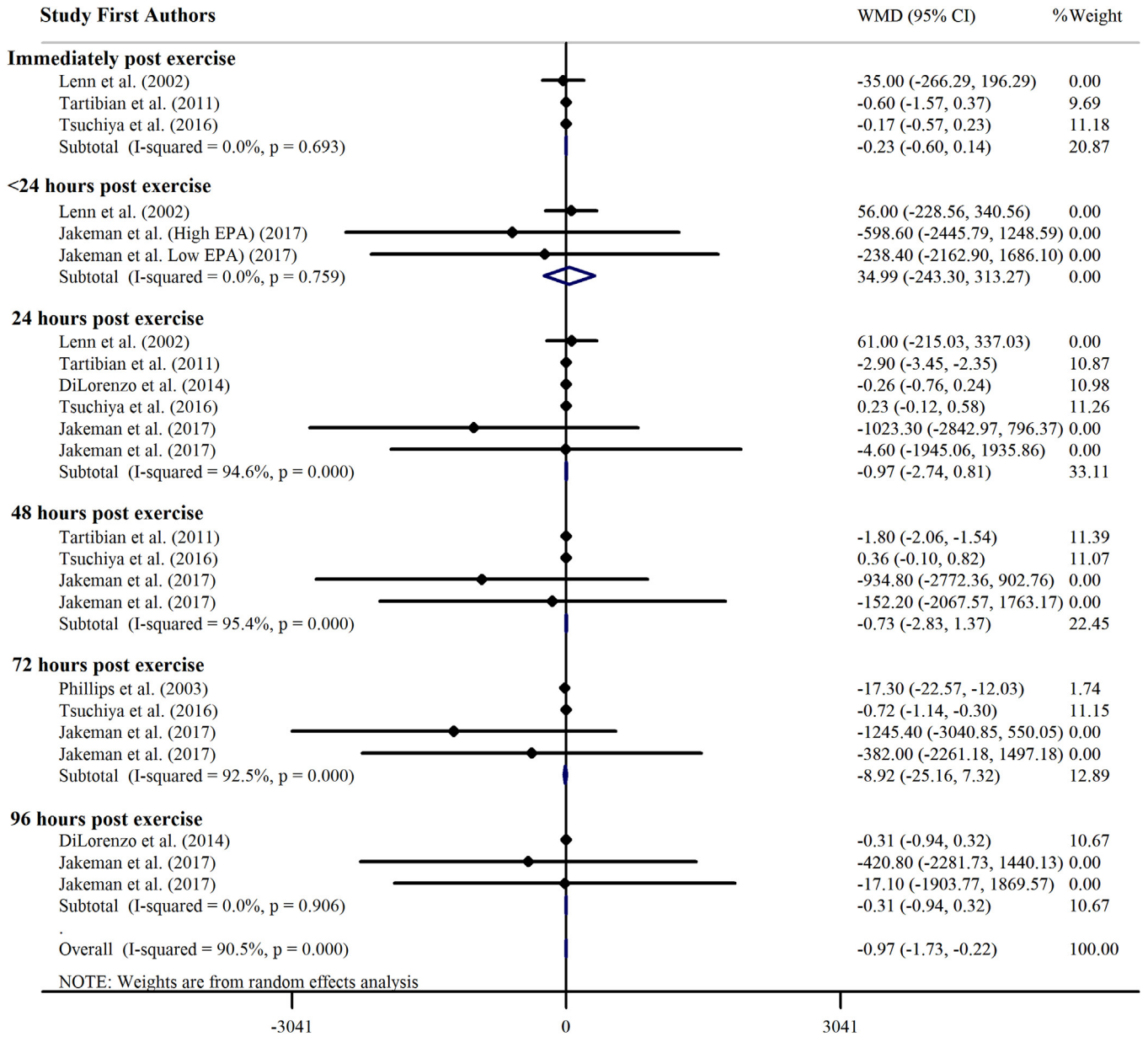

Fig. 2. Forest plot of the effect of omega-3 supplementation on IL-6 concentration. WMD: weighted mean difference; CI: confidence interval.

mentation on serum IL-6 levels differed according to follow-up after exercise, dose of omega-3, duration, time of supplementation, training status, and type of exercise (Table 3). Some subgroup analyses revealed that omega-3 supplementation resulted in a significant reduction in IL-6 concentrations in trials with 48 hours measurement of IL-6 after exercise, in trials with both lower and more than 2 g/day omega-3 consumption, lower and more than 1 month (acute supplementation was not significant), trials with before and

Table 3
Subgroup analysis to assess the effect of omega 3 on IL-6 concentration.

Subgrouped by	No. of trials	Effect size[a]	95% CI	*P* value	I^2 (%)
Follow-ups after exercise					
Immediately	3	−0.233	−0.602, 0.137	0.217	0.0
< 24 hours	3	34.987	−243.298, 313.272	0.805	0.0
24 hours	6	−0.966	−2.743, 0.811	0.287	94.6
48 hours	4	−1.441	−2.831, −0.031	0.041	95.4
72 hours	4	−8.923	−25.165, 7.319	0.282	92.5
96 hours	3	−0.310	−0.942, 0.322	0.336	0.0
Dose of omega 3					
< 2 g/day	7	−2.784	−4.262, −1.307	< 0.001	88.7
2 ≤ g/day	16	−0.630	−1.409, −0.148	0.013	30.8
Duration					
Acute (single dose in 1 day)	10	−520.256	−1125.281, 70.801	0.084	0.0
< 1 month	3	−2.582	−4.902, −0.263	0.029	95.0
1 month ≤	10	−0.798	−1.654, −0.058	0.048	95.0
Time of supplementation					
Before exercise	5	−0.279	−0.672, 0.113	0.163	0.0
After exercise	10	−520.256	−1154.941, 70.801	0.084	0.0
Before and after exercise	8	−1.223	−2.154, −0.293	0.010	96.8
Training status					
Trained	10	−520.256	−1257.542, 70.801	0.084	0.0
Untrained	13	−0.979	−1.740, −0.218	0.012	94.7
Type of exercise					
Anaerobic	20	−0.327	−0.891, 0.237	0.256	69.8
Anaerobic and aerobic	3	−1.841	−2.831, −0.851	< 0.001	90.1

CI: confidence interval.
[a] Calculated by random effects model.

after exercise time of supplementation, trials on untrained participants, and trials with combined anaerobic and aerobic exercise.

3.4. Effects of omega-3 supplementation on TNF-α concentration

The effect of the omega-3 supplementation on TNF-α concentration was evaluated in nine effect sizes of clinical trials, and pooled mean difference from inverse variance method showed a significant change in TNF-α concentration (WMD = −2.24 pg/mL; 95% CI: −3.15, −1.73; $P < 0.001$). There was significant heterogeneity among the studies (Cochran's Q test = 150.24, $P = 0.000$, $I^2 = 97.1\%$) (Fig. 3). Subgroup analysis was performed to assess whether the effect of omega-3 supplementation on TNF-α concentration differed according to follow-up after exercise, dose of omega-3, time of supplementation, and type of exercise (Table 4). The duration of supplementation in studies that reported TNF-α was only more than one month, and all participants were untrained. Some subgroup analyses revealed that omega-3 supplementation resulted in a significant reduction in TNF-α concentrations in trials with 24 and 48 hours measurement of TNF-α after exercise, lower and more than 2 g/day omega-3 consumption, before and after exercise supplementation, and trials with combined anaerobic and aerobic exercise.

3.5. Effects of omega-3 supplementation on CRP concentration

The effect of the omega-3 supplementation on CRP concentration was evaluated using four effect sizes of clinical trials, and the pooled mean difference from the inverse variance method represented a significant change in CRP concentration (WMD = −0.64 mg/L; 95% CI: −0.95, −0.33; $P < 0.001$). Additionally, there was significant heterogeneity among the studies (Cochran's Q test = 133.08, $P = 0.000$, $I^2 = 83.9\%$) (Fig. 4).

3.6. Sensitivity analysis and publication bias

Sensitivity analysis showed that none of the studies removed from the meta-analysis created any alterations in the outcomes of the meta-analysis on serum IL-6, TNF-α, and CRP concentrations. Funnel plots for IL-6 concentration were visually symmetrical (Fig. 5) and the results of Begg's test did not reveal any evidence of publication bias in studies that examined the effect of omega-3 consumption on IL-6 concentration (Begg's test, $P = 0.618$). The results of Egger's test did not reveal any evidence of publication bias in studies that examined the effect of omega-3 consumption on TNF-α and CRP concentrations (Egger's test, $P = 0.271$ and Egger's test, $P = 0.395$, respectively).

4. Discussion

The aim of this investigation was to examine whether omega-3 consumption influenced the response of inflammatory markers to physical activity, followed by the start of a training program. The current meta-analysis results, performed on eight randomized controlled trials, showed the beneficial effects of omega-3 supplementation in decreasing inflammation markers during training protocols of different periods.

The correlation between inflammation markers and recovery and performance is complex and multifaceted. While acute inflammation is a natural response to exercise-induced stress and is essential for tissue repair and adaptation, chronic or excessive inflammation can lead to prolonged recovery times, decreased muscle function, and impaired performance. Managing inflammation through various strategies, including nutrition interventions like omega-3 supplementation, can help optimize recovery and performance outcomes. Omega-3 fatty acids have been shown to possess anti-inflammatory properties that can help modulate the inflammatory response to exercise-induced muscle damage. By reducing the production of pro-inflammatory cytokines and eicosanoids, omega-3 supplementation may attenuate the increase in inflammation markers like IL-6 and TNF-α, leading to faster recovery and improved performance outcomes [22,36,41].

EIMD and DOMS appear to be caused by two main mechanisms. The first is the mechanical damage that occurs, especially during intense exercise, and appears to be principally in the Z-disk of the sarcomere area in the musculotendon region [44]. The second mechanism, which is dependent on the first and subsequent mechanical damage, involves biochemical changes. The

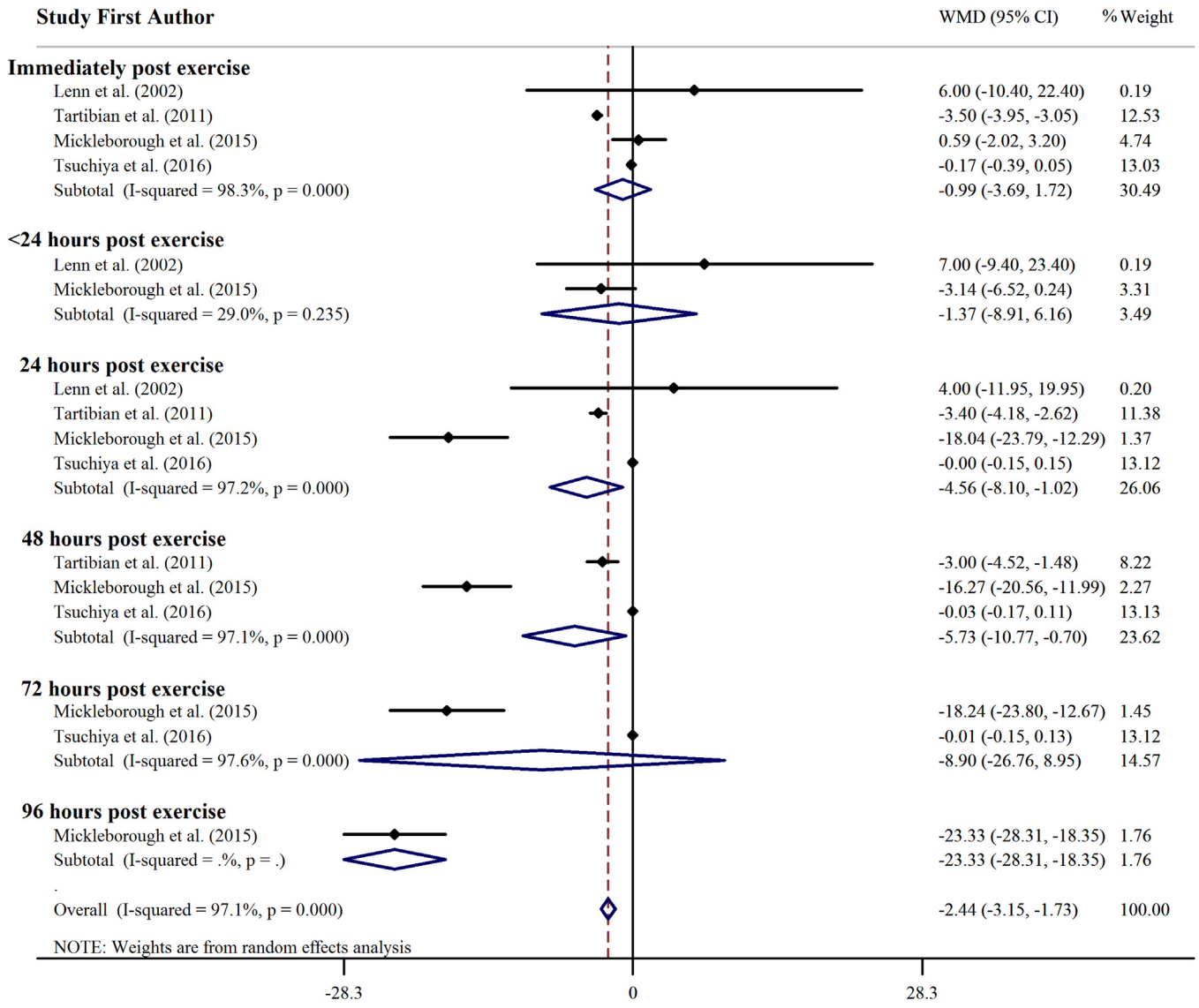

Fig. 3. Forest plot of the effect of omega-3 supplementation on TNF-α concentration. WMD: weighted mean difference; CI: confidence interval.

Table 4
Subgroup analysis to assess the effect of omega 3 on TNF- α concentration.

Subgrouped by	No. of trials	Effect size[a]	95% CI	*P* value	I^2 (%)
Follow-ups after exercise					
Immediately	4	−0.986	−3.687, 1.716	0.474	98.3
< 24 hours	2	−1.374	−8.905, 6.156	0.721	29.0
24 hours	4	−4.564	−8.104, −1.025	0.011	97.2
48 hours	3	−5.733	−10.767, −0.698	0.026	97.1
72 hours	2	−8.901	−26.757, 8.954	0.329	97.6
96 hours	1	−23.333	−28.312, −18.355	–	–
Dose of omega 3					
< 2 g/day	12	−7.474	−9.797, −5.150	< 0.001	93.1
2 ≤ g/day	4	−0.533	−1.110, −0.044	0.041	0.0
Time of supplementation					
Before exercise	3	5.635	−3.743, 15.014	0.239	0.0
Before and after exercise	13	−2.485	−3.198, −1.771	< 0.001	97.7
Type of exercise					
Anaerobic	7	−0.033	−0.109, 0.044	0.403	0.0
Anaerobic and aerobic	9	−8.211	−10.598, −5.825	< 0.001	94.9

CI: confidence interval.
[a] Calculated by random effects model.

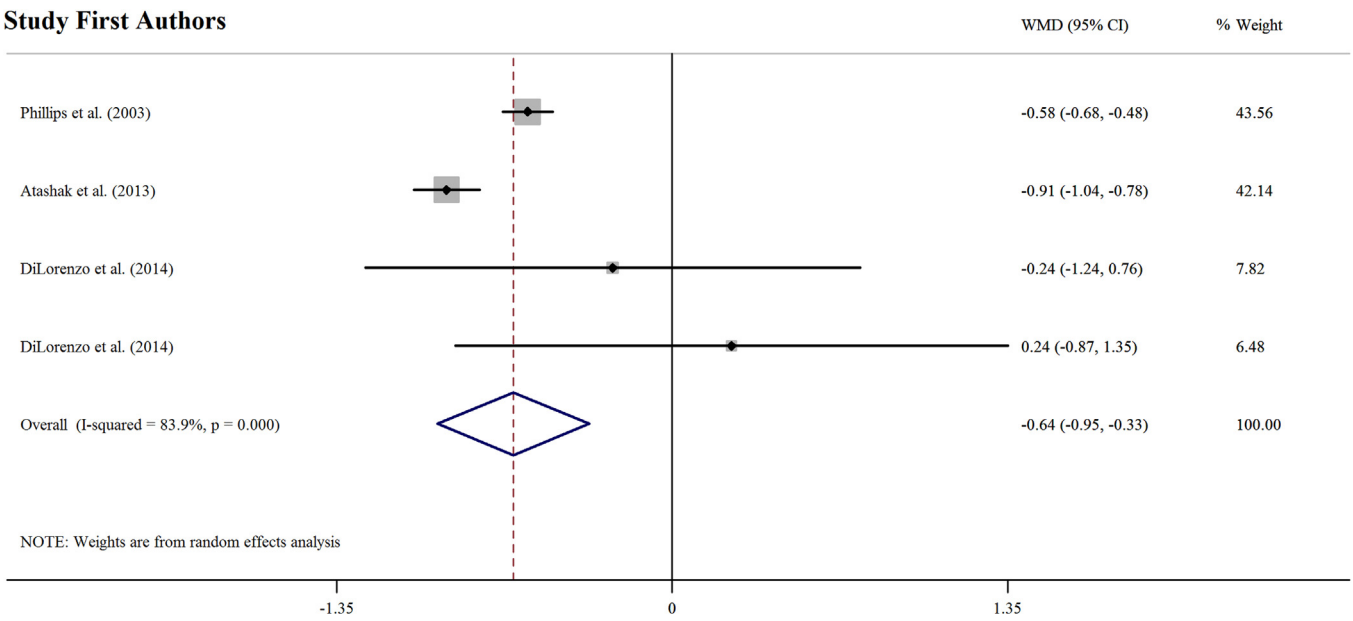

Fig. 4. Forest plot of the effect of omega-3 supplementation on CRP concentration. WMD: weighted mean difference; CI: confidence interval.

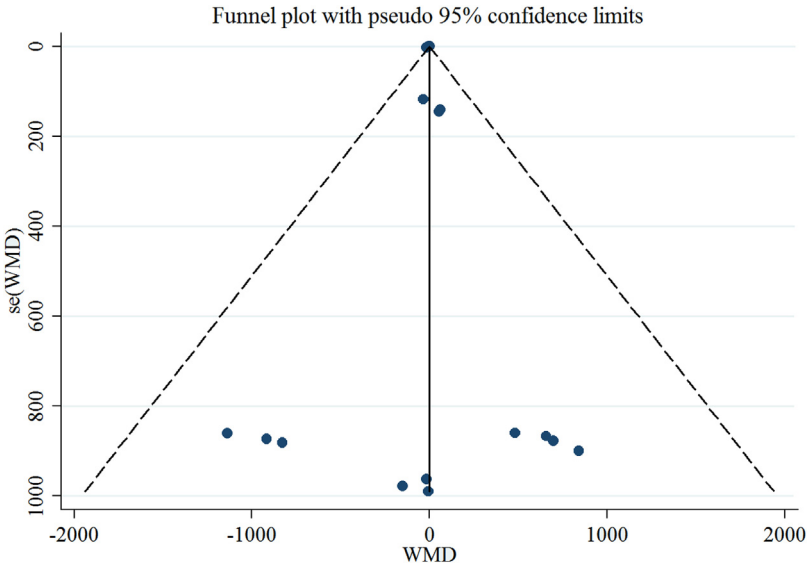

Fig. 5. Funnel plot for evaluating publication bias in IL-6 concentration measurements.

second mechanism is not well understood, but pro-inflammatory substance release and free radical-initiated damage have been implicated. Muscle damage seems to initiate an inflammatory response, resulting in cytokine release, localized edema and increased blood flow and permeability of the muscle tissue [45–47]. Therefore, inflammation may be responsible for the initiation, reinforcement, and resolution of skeletal muscle injury. Previous studies have demonstrated that higher cellular omega-3 fatty acids content decreases inflammatory factor generation by altering the COX2 and LOX pathways and via the nuclear binding of PPAR and NF-KB [48,49]. In addition, high LC-PUFA consumption of omega-3 fatty acids, mainly EPA and DHA, has been shown to result in the incorporation of these fatty acids into the cellular membrane phospholipid bilayer [50,51].

In the current meta-analysis, subgroup analysis based on follow-up times indicated that the effect of omega-3 supplementation on attenuating serum IL-6 and TNF-α levels was significant at 48 h after exercise. In addition, this effect was significant 24 h after exercise. IL-6 has been suggested as an inflammation marker, with peak levels evident at 2 days post exercise damage and a return to baseline values 7 days after exercise [52]. Usually, muscle damage responses after exercise tend to be somewhat more delayed and peak at 1 and 2 days follow-ups post exercise [53]. This delay could be due to oxidative stress induced by lipid peroxidation, which may result in membrane permeability and allow muscle enzymes, such as CK, LDH, and Mb, to escape [54]. The anti-inflammatory effects of omega-3 as well as its protective effects on membranes [55] may have prevented the increase in IL-6 and TNF-α serum levels after 24 and 48 hours follow-ups after exercise compared to placebo groups, and consequently reduced CK leakage and other enzymes from muscle cell membranes in peak follow-ups after exercise more efficiently [56].

Moreover, omega-3 supplementation for more than a month significantly lowered the IL-6 levels. However, acute supplementation (single dose in one day) had no significant effect on lowering IL-6 levels. In addition, subgroup analysis revealed that omega-3

supplementation both before and after exercise resulted in a significant decline in IL-6 and TNF-α concentrations. Considering the subgroup analysis based on dosage and the significant effect of both doses of lower and higher than 2 g/day omega-3 on IL-6 and TNF-α, it can be speculated that the duration of supplementation is more important than the dose of omega-3 supplementation. Therefore, skeletal muscle fiber disruption, especially in the basal lamina sheath, is the main feature of muscle damage without cell necrosis. Mechanical stimuli, particularly anaerobic exercise, can increase micro damage in muscle fibers imposed via contractions, and based on the intensity, length, and volume, inflammation, degree of damage, and muscle soreness may be combined over time and persist chronically [57,58]. For these reasons, omega-3 can affect muscle damage more efficiently before and after the exercise supplementation protocol and for several days of consumption. Taken together, more than 200 mg/day of DHA and 400 mg/day of EPA may be required to exert beneficial effects on inflammation and EIMD. However, the amount of DHA and EPA is limited to 3 g/day for safety based on a comprehensive database of natural medicines [41].

In addition, lower serum inflammation marker levels might depend on the early site of EIMD, training status of the participants, or familiarity with the exercise applied and exercise type [59]. In this regard, subgroup analysis indicated that trials with untrained participants showed a significant decrease in IL-6 concentrations with omega-3 supplementation. Thus, omega-3 supplementation was more effective in the untrained participants. In addition, subgroup analysis indicated significantly lower serum IL-6 and TNF-α levels in participants who performed combined anaerobic and aerobic exercise with omega-3 consumption compared to anaerobic exercise only. The levels of inflammatory markers seemed to depend on the type of exercise involved. Brief bouts of maximal exercise [23,46] or moderate exercise [57] did not appear to alter the levels of inflammatory markers, whereas endurance types combined with aerobic exercise or prolonged exercise and strenuous resistance eccentric exercise of larger muscle mass have generally shown significant increases in IL-6 and TNF-α serum levels, and omega-3 supplementation is more effective for these types of exercise.

The main limitation of this study was its high heterogeneity, influenced by factors such as dose of omega-3, duration of trials, time of supplementation, exercise type, and the small number of female participants in the articles. Sex had a significant influence in other articles in serum CK activity [60] and we mentioned the small numbers of women in the studies. Moreover, some participants may be less or more sensitive to changes in myocyte membrane permeability or may have dissimilar biomarker clearance rates because of their different responses to training [61,62]. As a result, the interpretation of the study results should be done with caution.

5. Conclusion

In summary, the results of the current meta-analysis indicate that omega-3 supplementation appears to be effective in alleviating inflammation that occurs after exercise-induced muscle damage. Further research with diverse dosages of omega-3 and different exercise protocols is needed to assess the optimal dose and number of repetitions per day for optimized recovery. Finally, we focused on inflammatory markers related to recovery post exercise, including IL-6, TNF-α, and CRP levels. Other key correlates of exercise recovery, such as muscle damage markers, muscle performance, and neuromuscular function, are known to change after EIMD. Additional reviews are necessary to evaluate these outcome measures following different therapeutic recovery strategies.

Human and animal rights

The authors declare that the work described has not involved experimentation on humans or animals.

Informed consent and patient details

The authors declare that the work described does not involve patients or volunteers.

Disclosure of interest

The authors declare that they have no competing interest.

Funding

This work did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Mohammad Hossein Rahimi: conceptualization, validation, writing – original draft, supervision, project administration; Yasaman Nasir: conceptualization, formal analysis, investigation, writing – original draft, writing – review and editing, supervision, funding acquisition.

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