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META-ANALYSIS

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Omega-3 fatty acids and endothelial function: A GRADE-assessed systematic review and meta-analysis

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Abstract

Introduction: N-3 polyunsaturated fatty acids (PUFAs) supplementation has been reported to have an impact on flow-mediated dilatation (FMD), a conventionally used clinical technique for estimating endothelial dysfunction. However, its proven effects on endothelial function are unclear. This systematic review and meta-analysis were conducted to evaluate the effects of n-3 PUFAs supplementation on FMD of the brachial artery.

Method: This study was performed following the PRISMA guidelines. To identify eligible RCTs, a systematic search was completed in PubMed/Medline, Scopus and Web of Science using relevant keywords. A fixed- or random-effects model was utilized to estimate the weighted mean difference (WMD) and 95% confidence interval (95% CI).

Results: Thirty-two studies (with 35 arms) were included in this meta-analysis, involving 2385 subjects with intervention duration ranging from 4 to 48 weeks. The pooled meta-analysis demonstrated a significant effect of omega-3 on FMD (WMD = 0.8%, 95% CI = 0.3–1.3, $p = .001$) and heterogeneity was significant ($I^2 = 82.5\%$, $p < .001$).

Conclusion: We found that n-3 PUFA supplementation improves endothelial function as estimated by flow-mediated dilatation of the brachial artery.

KEYWORDS

cardiovascular disease, endothelium, meta-analysis, omega-3 fatty acids

Seyyed Mostafa Arabi and Hossein Bahari should be considered as equal first author.

1 | INTRODUCTION

There is an increasing rate of cardiovascular diseases (CVDs) and associated mortality worldwide, which makes them the leading cause of death.[1,2] CVD initiation is affected by endothelial dysfunction, which is one of the premature steps in the pathological development of atherosclerotic diseases.[2] Endothelial dysfunction is associated with raised expression of inflammatory cytokines and adhesion molecules, factors that play a role in plaque formation and eventually plaque rupture.[3,4] Flow-mediated dilation (FMD) is a conventional, non-invasive gold standard for estimating endothelial dysfunction. FMD is defined as the percentage enlargement in artery diameter relative to baseline diameter.[5] For assessment of FMD, the baseline brachial artery diameter is documented via ultrasound, and a blood pressure cuff is set around the forearm and inflated for 5 min, impeding blood flow.[5] The cuff is deflated, inducing reactive hyperemia, and the vessel diameter is constantly recorded for 2 min post-cuff deflation. The production of endothelium-derived vasodilators, particularly nitric oxide (NO), is induced by shear stress, which dilates the brachial artery.

In addition, endothelium-derived hyperpolarizing factor (EDHF) and endothelium-independent vasodilation are also involved in the vasodilatory response of the brachial artery.[6] Previous studies have indicated that not only FMD but also nitroglycerine-induced vasodilation (NID), an index of endothelium-independent vasodilation, is affected in patients with cardiovascular risk factors or CVD.[7,8] Some studies have shown that brachial FMD can significantly predict cardiovascular events, but more studies are needed in this area.[9,10]

Numerous experimental and clinical studies have provided evidence that n-3 PUFAs can partly reduce the risk of CVD by enhancing vascular function. Alpha-linolenic acid (ALA, 18:3n-3), enriched in particular plants as well as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), enriched in fish oil, are three n-3 PUFAs that have vasoprotective effects. Decreased arterial plaque formation,[11] increased anti-inflammatory effects,[12] and improved endothelial-dependent vasodilation measured by FMD[13,14] are among the vasoprotective benefits. Moreover, it is unclear whether formulations containing only ALA, EPA, or DHA have similar benefits as a combination of EPA and DHA. Thus far, the evidence from large outcome trials has favoured the reduction of cardiovascular events by the preparations that only contained EPA.[15]

Therefore, we aimed to run a systematic review and meta-analysis of randomized controlled trials (RCTs) to evaluate the effect of n-3 PUFA supplementation on FMD.

2 | METHODS

This systematic review and meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[16]

2.1 | Search strategy

At first, a systematic search was done in the following databases without any restriction in time and language: MEDLINE, SciVerse, Scopus and Clarivate Analytics Web of Science databases. We used the following keywords and Medical Subject Heading (MeSH) to recover potentially pertinent articles[1]: omega-3 fatty acid (FA)[2]; endothelial function[3] randomized controlled trial (Table S1). We also searched the reference lists of included trials and relevant reviews for further qualified studies that were ignored by our electronic search.

2.2 | Study selection

First, we removed duplicates operating reference manager software. The remaining citations were then checked by two authors independently based on their titles and abstracts for relevance. In the next step, Full-text articles from potentially relevant publications were considered for inclusion in the review after initial screening. Inclusion criteria were human RCTs that measured the effects of omega-3 FA supplementation on flow-mediated dilation. Exclusion criteria included combined interventions (omega-3 plus other active ingredients), duplications, studies without outcomes of interest, and follow-up durations of less than 2 weeks. Two researchers (M. Ch and H. B) made study selection and screening separately. All controversies in the study selection procedure were resolved by agreement through discussion (SM. A).

2.3 | Data extraction

Information including the first author's name, year of publication, study location, study design, participant characteristics, sample size, intervention details including dose, duration, and type of omega-3 supplementation, and mean ± standard deviation (SD) and changes of outcomes including FMD levels before and after omega-3 intervention in both treatment and control groups were obtained from all studies by two independent authors (SM. A and H. B). If any studies supplied insufficient data for meta-analysis, the manuscript authors were contacted by E-mail. Disagreements were resolved by discussion between the authors or by a senior reviewer (A. S).

2.4 | Quality assessment

Two researchers (M. Ch and LS. B) evaluated the quality of each included study independently. Disparities were consulted, and if consensus was not achieved, a third researcher was conferred (A.S). Quality was explicitly evaluated using the Cochrane Collaboration tool 1 (ROB 1), based on the following seven fields: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of the outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias. We ranked each part of the trials as low risk, unclear, or high risk.

2.5 | Statistical analysis

STATA 17 software (StataCorp, College Station) was used for statistical analysis. Differences were expressed as weighted mean difference (WMD) with a 95% confidence interval (CI). Mean and mean scores and their standard deviation (SD) were used to calculate WMDs. When SDs for baseline and final values are provided, SDs for net changes are imputed according to the Follmann method.[17] Because of the inherent variation between study characteristics, a random-effects meta-analysis was operated across all studies. Subgroup analysis and meta-regression were performed to find the possible source of heterogeneity based on the following indicators: baseline mean age, genders, study design, blinding, adjustment, omega-3 supplement type, dosage, study duration, participants' health condition and study quality. Heterogeneity across studies was calculated by using the I^2 statistic. $I^2 > 50\%$ demonstrated significant heterogeneity. In addition, the dose–response effects of n-3 PUFAs and FMD were examined. An influence analysis was used to check the impact of a single study on the overall effect size. Publication bias was estimated graphically via funnel plot asymmetry and statistically by Egger's regression test. $p < .05$ was assumed statistically significant.

3 | RESULTS

3.1 | Search results

In our primary search, we identified a total of 2107 studies. After excluding 552 duplicates and 1427 irrelevant studies based on title and abstract 128 studies remained for full-text reviewing by detail. Overall, 32 articles (with 35 arms) provided sufficient information and were considered eligible to be included in this dose–response meta-analysis (Figure 1). Characteristics of the 32 studies are listed in Table 1. The sample size of included

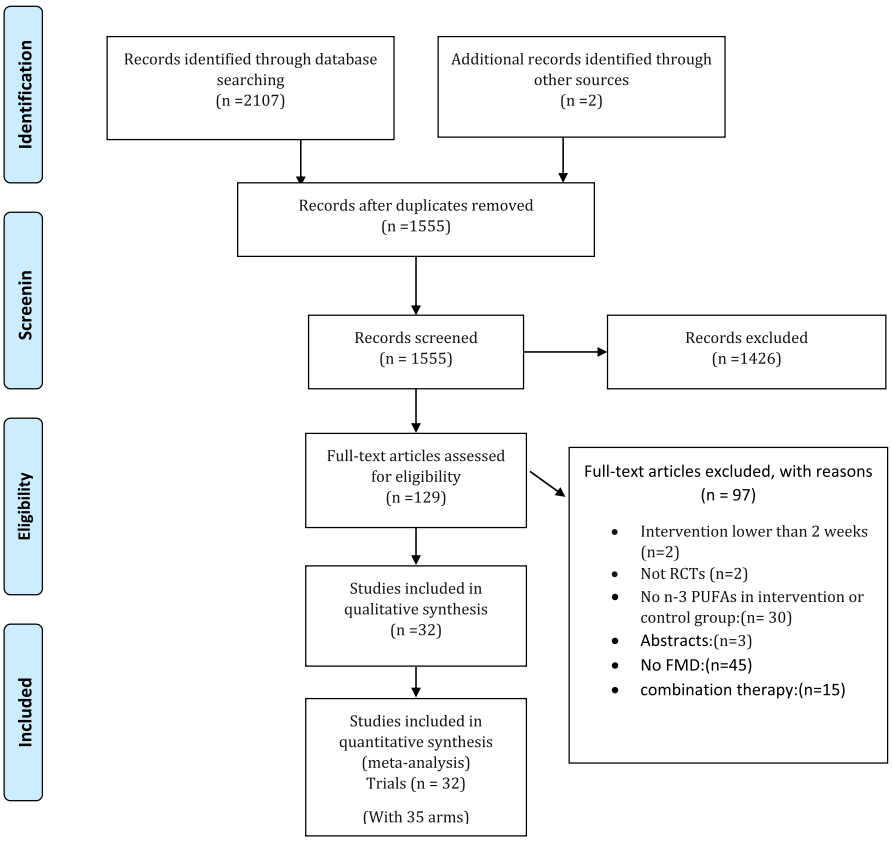

FIGURE 1 PRISMA flow diagram.

TABLE 1 Characteristic of included studies.

First author, Year, country	Design	Participants (*n*) Int/con	Health condition	Age means (year) Int/con	Treatment group	Control group	Dose (EPA/DHA)	Duration of intervention (weeks)	Treatment group	Control group	Adjustment
Oh/2014/Korea	RCT/SB	43/42	Hypertriglyceridemia	54/54	n-3 FA	Placebo	1000	8	1.55 ± 1.36	0.34 ± 1.8	Yes
							2000		1.77 ± 1.95	0.34 ± 1.8	Yes
							4000		2.64 ± 1.24	0.34 ± 1.8	
Singhal/2013/UK	RCT/DB	136/138	Healthy	28.2/27.6	DHA	Olive oil	1600	16	−0.5 ± 4.05	0.3 ± 3.78	Yes
Khorshidi/2022/Iran	RCT/DB	26/25	T1DM	13.8/12.9	Omega-3 capsules	Oral glycerin	600	12	3.10 ± 4.2	−0.6 ± 4	Yes
Kim/2021/USA	RCT/DB	17/16	COPD	67.5/66.2	n-3 PUFA	Corn oil	3000	24	−2.6 ± 6.2	−1.6 ± 6.02	No
O'Mahoney/2020/UK	RCT/DB	10/10	T1DM	32/36	n-3 PUFA	Corn oil	3300	36	−0.17 ± 1.48	−0.16 ± 1.49	Yes
Morishima/2020/Japan	RCT/DB	10/9	Healthy	20.4/21.2	Fish oil	Corn oil	2400	8	0.6 ± 1.05	−0.2 ± 1.15	Yes
Ramirez/2019/USA	RCT/DB	11/13	Peripheral artery disease	69/73	n-3 PUFA	Soy bean	4400	12	−1.5 ± 3.7	2.9 ± 3.6	No
Oikonomou/2019/Greece	RCT/crossover/DB	15/16	Ischemic HF	66/67	Omega-3	Olive oil	2000	8	0.99 ± 2.82	0.01 ± 4.5	No
Eide/2019/Norway	RCT/DB	52/54	Renal transplant	52.8/54.1	n-3 FA	Olive oil	3000	44	2 ± 3.8	0.5 ± 2.4	Yes
Siniarski/2018/Poland	RCT/DB	36/38	T2DM	64.4/66.7	n-3 PUFA	Juice	2000	12	0 ± 4.39	−0.5 ± 6.25	Yes
Sawada/2016/Japan	RCT/SB	54/53	CAD	67.8/68.9	EPA	Non-EPA	1800	24	1.6 ± 1.33	−0.1 ± 1.03	No
Zebrowska/2015/Poland	RCT/crossover	13/13	Endurance-trained athletes	23.1/23.1	Gold omega-3	Lactose	2600	3	4.9 ± 7.04	−0.3 ± 5.22	No
Grenon/2015/USA	RCT/DB	36/36	Peripheral Artery Disease	68/69	n-3 PUFA	Soy bean	4400	4	0.7 ± 1.8	0.6 ± 2.5	No
Tousoulis/2014/Greece	RCT/crossover/DB	15/14	Mets	44.31/44.31	n-3 PUFA	Placebo	2000	12	4.05 ± 4.09	−0.11 ± 2.75	No
Siasos/2013/Greece	RCT/crossover/DB	20/20	Healthy	27.63/27.63	n-3 PUFA	Placebo	2000	12	2.71 ± 4.74	−0.24 ± 1.76	No
Bozcali/2013/Turkey	RCT/DB	8/10	Cardiac syndrome X	48.7/47.3	Omega-3 fatty acid capsule	Placebo	1440	16	57 ± 42.89	−6 ± 62.92	No
Bello/2013/USA	RCT/DB	42/43	SLE	48.9/45.5	Omega-3	Corn starch	3000	12	−3.96 ± 11.73	−2.76 ± 9.68	Yes
Wright/2008/UK	RCT/DB	30/30	SLE	48.5/47.6	n-3 PUFA	Olive oil	3000	24	0 ± 0.55	−0.01 ± 0.54	No
Engler/2004/USA	RCT/crossover/DB	10/10	Familial hypercholesterolemia	14/14	DHA	Corn/soya oil	1200	6	2 ± 2.77	−0.9 ± 2.47	No
Mizia-Stec/2011/Poland	RCT/DB	19/19	MI	56/62	Omega-3	Placebo	1000	4	7.6 ± 9.41	−1.8 ± 7.64	Yes
Fahs/2010/USA	Crossover/DB	20/20	Healthy	25/25	Fish oil	Lactose capsules	1000	0.01	0.55 ± 2.86	−1.23 ± 2.3	No
Haberka/2011/Poland	RCT/DB	20/20	MI	58/62	n-3 PUFA	Placebo	1000	4	−8.1 ± 9.53	−2.2 ± 7.57	No
O. Hileman/2012/USA	RCT/DB	18/17	HIV-Infected	51/51	Omega-3-acid ethyl esters 1 capsule	Placebo	2000	24	−0.13 ± 2.60	1.47 ± 4.05	Yes
Koh/2012/Korea	RCT/DB	50/49	Hypertriglyceridemia	55/54	Omega-3 fatty acids	Placebo	2000	8	2.56 ± 2.25	0.09 ± 2.06	No
Schiano/2008/Italy	RCT/SB	16/16	Intermittent claudication	66/66	n-3 PUFA	Placebo	1000	12	3.3 ± 5.39	−0.3 ± 4.28	No
Wong/2010/China	RCT/DB	49/48	T2DM	61.2/59	Fish oil	Olive oil	4000	12	2 ± 5	1.6 ± 4.3	No
Stirban/2010/Germany	Crossover/DB	34/34	T2DM	56.8/56.8	n-3 PUFA	Olive oil	1000	6	−0.03 ± 3.52	−1.05 ± 3.09	No
Sanders/2011/UK	RCT/DB	71/81	Healthy	55/55	EPA + DHA	Olive oil	450	48	−0.2 ± 3.12	0.2 ± 3.17	Yes

TABLE 1 (Continued)

First author, Year, country	Design	Participants (*n*) Int/con	Health condition	Age means (year) Int/con	Treatment group	Control group	Dose (EPA/DHA)	Duration of intervention (weeks)	Treatment group	Control group	Adjustment
		71/80		55/55			900	48	0 ± 3.26	0.2 ± 3.17	
		71/80		55/55			1800	48	−0.4 ± 3.28	0.2 ± 3.17	
Miyoshi/2014/Japan	Crossover/SB	10/10	Healthy	31/31	Omega-3	Placebo	4000	4	−0.5 ± 1.2	−2 ± 0	No
Woodman/2003/Australia	RCT/DB	17/16	Hypertensive type 2 DM	61.2/61.5	EPA	Olive oil	4000	6	0.2 ± 2.59	3.5 ± 3.6	Yes
		18/16		60.9/61.5	DHA		4000	6	−1.9 ± 5	0.8 ± 3.3	
Dyerberg/2004/Denmark	RCT/DB	26/24	Healthy	39.2/37.6	Fish oil	Control oil	4000	8	−2 ± 3.19	−0.4 ± 2.33	No
Moertl/2011/Austria	RCT/DB	14/16	Chronic heart failure of nonischemic origin	58.6 ± 55.1	Omacar	Placebo	1000	12	1.9 ± 5.1	−1 ± 6.85	No
		13/16		61.9.55.1			4000	12	3.2 ± 6.46	−1 ± 6.85	
Rizza/2009/Italy	RCT/DB	26/24	T2DM	29.9/29.9	n-3 PUFA	Placebo	2000	12	3.8 ± 2.9	−0.2 ± 3.8	Yes

Abbreviations: CAD, Coronary artery disease; CKD, Chronic kidney disease; Con, control; DM, diabetes mellitus; ED, erectile dysfunction; F, female; HF, heart failure; HIV PIs, human immunodeficiency virus protease inhibitors; Int, intervention; M, male; RA, rheumatoid arthritis; RCT, randomized clinical trial.

RCTs varied from 18 to 274 participants, resulting in a total sample size of 2385 individuals. Twenty-five trials were designed as a parallel group and seven as crossover studies. Table 1 presents the main characteristics and baseline parameters of the included trials. These RCTs were performed in Korea,[18,19] UK,[20–23] Iran,[24] USA,[25–30] Greece,[31–33] Norway,[34] Poland,[35–38] Turkey,[39] Italy,[40,41] China,[42] German,[43,44] Denmark,[45] Australia[46] and Austria[47] between 2003 and 2022. Nine studies were crossover and others had a parallel design. The mean age of participants was between 12.9 and 69. The dosage of omega-3 varied from 600 to 18,000 mg/day and the duration of intervention ranged from less than 1 day to 48 weeks across selected RCTs.

3.2 | Quantitative data synthesis

Combining 35 effect sizes from 32 RCTs, including a total sample size of 2385 individuals, we found a significant effect of omega-3 FAs on FMD (WMD = 0.8%, 95% CI = 0.3–1.3, $p = .001$) and heterogeneity was significant ($I^2 = 82.5\%$, $p < .001$) (Figure 2). Subgroup analysis showed that study design, blinding type, age category, supplement type and quality of studies explained this heterogeneity (Table 2).

3.3 | Publication bias and influence analysis

The overall effect size of n-3 PUFA intervention on FMD levels was robust, and the result was not influenced by any single study omission. In the analysis of publication bias, the funnel plot and Egger's tests did not show any significant publication bias ($p = .4$) (Figure 3). Influence analysis was done to assess the potential impact of each trial on the pooled effect size. The effect size was not affected by any study and the results were not sensitive (Table S2).

3.4 | Meta-regression and dose–response analysis

Meta-regression analysis did not indicate a linear relationship between dosage ($p = .1$) and duration of the intervention ($p = 0.1$) with the changes in FMD level (Figure 4). Dose-dependent effect of omega-3 FAs supplementation on FMD is revealed in Figure 5. FMD level improved linearly and significantly ($P_{\text{non-linearity}} = .01$, $P_{\text{dose–response}} = .01$) up to n-3 PUFAs administration of 3 g/day ($MD_{3g/day}$: 0.6, 95% CI: 0.1, 1.2) (Table S3).

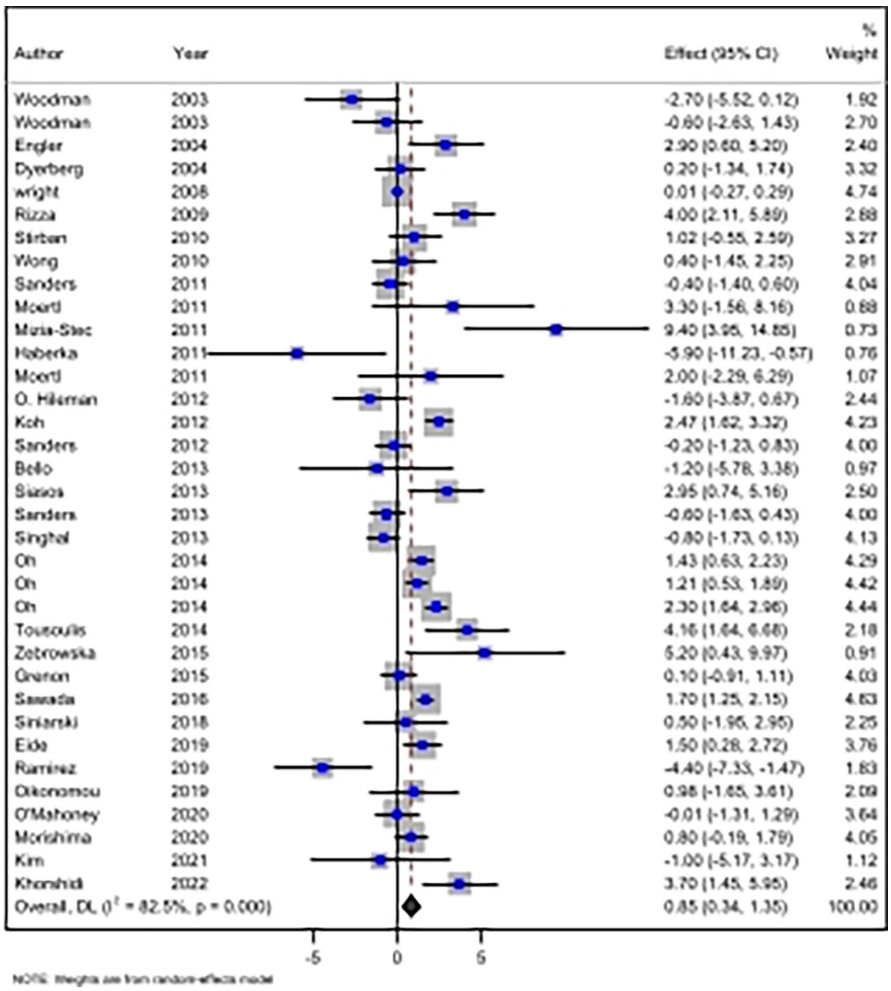

FIGURE 2 Forest plot representing weighted mean difference and 95% confidence intervals (CIs) for the effect of n-3 PUFAs supplementation on the FMD levels.

3.5 | Risk of bias

Based on Cochrane Cooperation's tool 10 studies were good,[22,24,27,28,34,35,42,44,47,48] 16 studies were fair[20,21,23,26,30–33,35,37,41,45,46,49–52] and other studies poor quality.[19,25,36,38,47,53] All studies had a low source of bias in random sequence generation and selective reporting. Regarding allocation concealment, 15 studies were highly biased[18,19,25,28,30,32,33,36–38,41,47,50–53] and 16 of them had other sources of bias.[18,19,21,25–27,29,31–33,36,38,44,45,51,53] Four studies had a high risk of bias for blinding participants and personnel,[36,38,52,53] and nine studies for incomplete outcome data.[18–21,23,25,30,45,46] The findings of the Cochrane Cooperation's tool quality assessment of the included studies were indicated in Table 3.

3.6 | Grading the evidence

Based on the grading analysis, the certainty of studies was evaluated and due to the high-quality of studies, the certainty of studies was determined high because the inconsistency, indirectness and publication bias were highly graded and just imprecision was downgraded (Table 4).

4 | DISCUSSION

In the present study, the result of 32 trials demonstrated that n-3 PUFAs supplementation significantly improved endothelial function by FMD, at a dose ranging from 0.6 to 4.9 g/day, and between 3 and 48 weeks. Also, n-3 PUFAs administration significantly increased FMD for participants who had diseases with mean age ≤55 years. old and those who received omega-3 FAs supplements with a dose of more than 1 g/day for ≤12 weeks in subgroup analysis. Moreover, our finding based on GRADE evaluation is of high importance and it was robust in an influence analysis.

Flow-mediated dilation is a non-invasive measurement of endothelial function that reflects the ability of blood vessels to dilate in response to increased blood flow. Endothelial dysfunction, as indicated by impaired FMD, is an early sign of atherosclerosis and is related

TABLE 2 Subgroup analyses of n-3 PUFAs supplementation in adults.

	Number of studies	WMD (95% CI)	*p*-value	Heterogeneity	
				$p_{heterogeneity}$	I^2 (%)
Subgroup analyses of omega-3 supplementation on FMD					
Overall effect	35	0.9 (0.4, 1.4)	<.001	<.001	82.8
Study design					
Parallel	29	0.5 (0.05, 1.1)	.03	<.001	84.1
Cross-over	6	2.4 (1.2, 3.6)	<.001	.342	34.3
Blinding					
D	29	0.6 (0.1, 1.2)	.01	<.001	76.8
S	4	1.6 (1.2, 2.1)	<.001	.1	47
Health status					
Healthy	8	0.1 (−0.5, 0.9)	.6	.009	62.7
Non-healthy	27	1.04 (0.4, 1.6)	.001	<.001	83.8
Gender					
B	29	1.05 (0.4, 1.6)	<.001	<.001	83.8
M	5	−0.4 (−2.2, 1.2)	.5	.005	73.1
Trial duration (weeks)					
>12	11	−0.1 (−0.8, 0.5)	.6	<.001	85.6
≤12	24	1.4 (0.8, 2.1)	<.001	<.001	69.8
Mean age (years)					
>55	14	0.2 (−0.8, 1.2)	.7	<.001	75.0
≤55	21	1.1 (0.5, 1.7)	<.001	<.001	85.3
Age category					
Adolescents	2	3.3 (1.7, 4.9)	<.001	<.001	82.6
Adults	33	0.7 (0.2, 1.2)	.006	.6	0
Adjustment					
Yes	19	0.6 (−0.02, 1.3)	.05	<.001	81.9
No	16	0.9 (0.08, 1.7)	.03	<.001	81.2
Risk of bias					
Some concerns	17	0.5 (−0.2,1.2)	.1	<.001	86.1
Low risk of bias	11	0.8 (0.2,1.4)	.006	.205	25.1
High risk of bias	7	1.6 (0.8,2.4)	<.001	.003	70.1
Supplement type					
EPA	2	0.7 (−1.4, 2.9)	.4	.03	78.7
DHA	3	−0.1 (−2.8, 2.5)	.9	.004	82.0
Fish oil	3	0.5 (−0.1, 1.3)	.1	.535	0
n-3 PUFA	27	1.01 (0.4, 1.6)	.001	<.001	83.1
Omega dosage (mg)					
≥1000	27	0.8 (0.2, 1.3)	.002	.005	83.9
≤1000	8	1.01 (−0.2, 2.2)	.05	.1	78.6

Abbreviations: B, both; CI, confidence interval; D, double blind; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; M, male; S, single blind; WMD, weighted mean differences.

to an increased risk of cardiovascular disease (CVD) events.[9] Some studies have indicated that small improvements in FMD levels were associated with a decreased risk of cardiac events.[9,54] Based on a study conducted by Green et al., each 1% improvement in FMD level was related to a 17% reduction in cardiovascular events.[54] FMD and glyceryl trinitrate-mediated dilation (GTNMD) are two methods to assess endothelial-dependent and

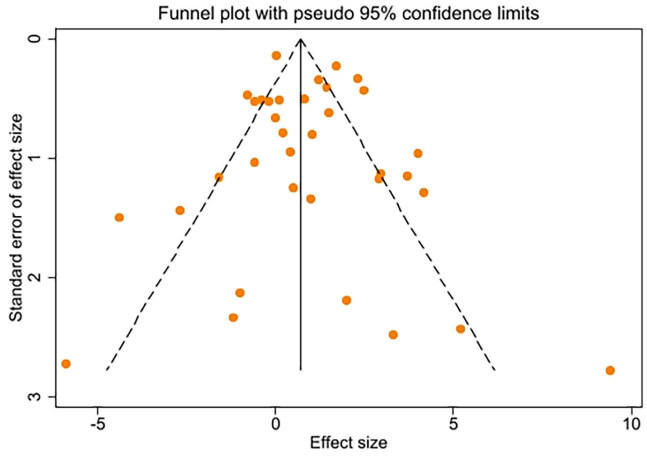

FIGURE 3 Funnel plot of the effects of n-3 PUFAs supplementation on FMD levels.

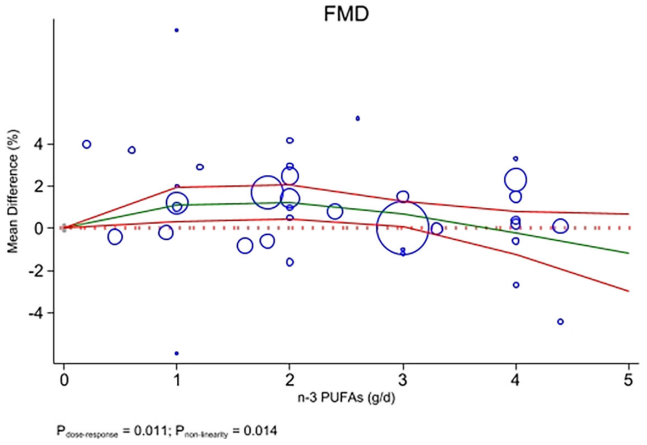

FIGURE 5 Dose-dependent effect of n-3 PUFAs supplementation treatment on FMD.

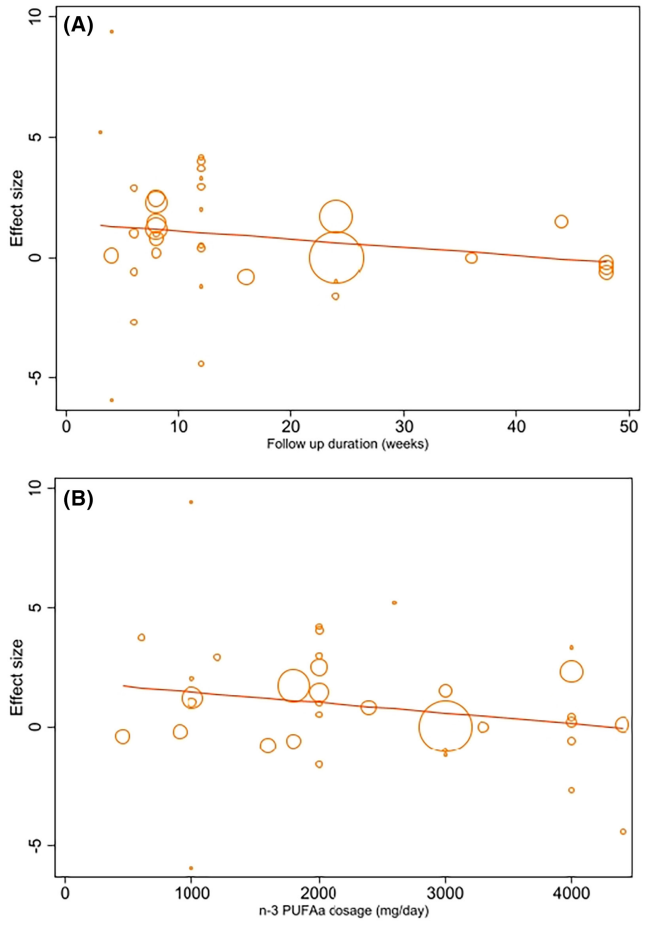

FIGURE 4 Random-effects meta-regression plots of the association between WMD of FMD following n-3 PUFAs administration on FMD (A. Follow-up duration, and B. n-3 PUFA dosage).

endothelial-independent functions, respectively. But there was no study to investigate the effects of n-3 PUFAs on GTNMD and a study by Freestone showed that as the number of cardiovascular risk factors increase, there is a rise in smooth muscle dysfunction which leads to decreasing responsiveness to GTN.[55] This decrease in GTN response happens separately from any endothelial dysfunction and may have contributed to the conditions of both the studied cases and controls. Hence, we studied FMD as a more specific factor in endothelial function. Studies have indicated that omega-3 may contribute to improving endothelial function by modifying or improving several parameters.[56] It is possible that by receiving n-3 PUFAs, the ratio of omega-6 to omega-3 in cellular phospholipids will change and increase the fluidity of the membrane of endothelial cells.[57] Linoleic acid by converting to arachidonic acid in the phospholipids of the cell membrane can lead to an increase in inflammatory mediators. Omega-3 FAs exert their anti-inflammatory effects by reducing arachidonic acid contents and increasing resolvins.[58–60] Exclusively, both EPA and DHA diminished TAG and inflammatory markers, but only DHA diminished HR, BP, and several small thick LDL particles, and EPA impacts on FMD are endothelial cell-dependent, whereas those of DHA appear to be endothelial cell-independent.[61]

The relationship between increased CRP levels and vascular dysfunction was observed in some studies. Supplementing with omega-3 FAs can be effective in reducing inflammation by reducing the C-reactive protein concentrations, which shows the positive effects of omega-3 FA on vascular function.[62,63] Moreover, markers of inflammation related to endothelial functions such as E-selection and hs-CRP concentrations considerably declined following high fatty fish intake in overweight and obese participants.[64]

Clinical and experimental studies have shown that n-3 PUFAs, through the induction of nitric oxide (NO) in the endothelial cells and by reducing adhesive molecules such as vascular cell adhesion molecule-1 (VCAM-1) and inter-cellular adhesion molecule-1 (ICAM-1) in the

TABLE 3 Quality of trials included in the meta-analysis of omega-3 supplementation and FMD.

Studies	Random sequence generation	Allocation concealment	Selective reporting	Other sources of bias	Blinding (participants and personnel)	Blinding (outcome assessment)	Incomplete outcome data	General risk of bias
Khorshidi et al., 2022	L	L	L	L	L	L	L	Low risk of bias
Kim et al., 2021	L	H	L	H	L	L	H	High risk of bias
O'Mahoney et al., 2020	L	L	L	L	L	L	L	Low risk of bias
Morishima et al., 2020	L	L	L	L	L	L	L	Low risk of bias
Ramirez et al., 2019	L	L	L	H	L	L	L	Some concerns
Oikonomou et al., 2019	L	L	L	H	L	L	L	Some concerns
Eide et al., 2019	L	L	L	L	L	L	L	Low risk of bias
Siniarski et al., 2018	L	L	L	L	L	L	L	Low risk of bias
Sawada et al., 2016	L	L	L	L	L	H	L	Some concerns
Zebrowska et al., 2015	L	H	L	H	H	H	L	High risk of bias
Grenon et al., 2015	L	L	L	H	L	L	L	Low risk of bias
Tousoulis et al., 2014	L	H	L	H	L	L	L	Some concerns
Oh et al., 2014	L	H	L	H	L	L	H	High risk of bias
Miyoshi et al., 2014	L	H	L	H	H	L	L	High risk of bias
Sanders et al., 2011	L	L	L	L	L	L	H	Some concerns
Singhal et al., 2013	L	L	L	L	L	L	H	Some concerns
Siasos et al., 2013	L	H	L	H	L	L	L	Some concerns
O. Hilman et al., 2012	L	H	L	L	L	L	H	Some concerns
Bello et al., 2013	L	H	L	L	L	L	L	Low risk of bias
Koh et al., 2012	L	H	L	H	L	H	H	High risk of bias
Haberka et al., 2011	L	H	L	H	H	H	L	High risk of bias
Mizia-stec et al., 2011	L	H	L	L	L	L	L	Some concerns
Moertl et al., 2011	L	L	L	L	L	L	L	Low risk of bias
Wong et al., 2010	L	L	L	L	L	L	L	Low risk of bias
Stirban et al., 2010	L	L	L	H	L	L	L	Low risk of bias
Engler et al., 2004	L	H	L	H	L	L	L	Some concerns
Dyerberg et al., 2004	L	L	L	H	L	L	H	Some concerns
Rizza et al, 2009	L	H	L	L	L	L	L	Some concerns
Woodman et al., 2003	L	L	L	L	L	L	H	Some concerns
Wright et al, 2008	L	L	L	H	L	L	H	Some concerns

TABLE 4 GRADE evidence table for n-3 PUFAs compared to placebo for endothelial functions.

Certainty assessment							No. of patients		Effect			
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	N-3 PUFA	Placebo	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
FMD												
35	Randomized trials	Not serious	Not serious[a]	Not serious	Serious[b]	Dose response gradient	1191	1214	–	MD 0.8% higher (0.3 higher to 1.3 higher)	⨁⨁⨁⨁ High	Important

Abbreviations: CI, confidence interval; MD, mean difference.

[a]Serious inconsistency since $I^2 = 82.5\%$. However, the value of I was <50% in the subgroup of trials with Cross-over design, and the significance, direction and magnitude of the effect remained unchanged (MD: 2.4 (95% CI; 1.2, 3.6), $N = 6$, $I^2 = 34.3\%$), not downgrade).

[b]Serious imprecision since the point estimate was smaller than MCID for FMD (MCID = 1.11%), Downgrade.

endothelium cells, reduce inflammation and platelets aggregations and inhibit the growth of vascular wall smooth muscle, which ultimately increases endothelial function[65,66] that NO does this by through activating AMP-activated protein kinase. Also, omega-3 FAs increase the responsiveness of the endothelial cells to NO by reducing reactive oxygen species (ROS).[67,68] Another mechanism by which omega-3 FAs may lead to an improvement of endothelial function is its triglyceride-lowering effects due to the reduction in chylomicron and VLDL remnants. Several studies have shown that omega-3 can increase the oxidation of triglycerides in liver cells through the activation of peroxisome activator alpha receptors. In addition, omega-3 FAs can effectively reduce the production of triglycerides by inhibiting acyl-CoA transferase and acyl-CoA 1 enzymes. Also, omega-3 FAs reduce the level of triglycerides by stimulating liver receptors such as hepatic nuclear factor alpha 4, farnesol X receptor, and hepatic X receptor.[69–72] Corresponding to these findings, Goodfellow's study indicated that omega-3 FAs treatment significantly improved endothelial function in hypercholesterolemia subjects.[14]

According to the investigated mechanisms, the effects of omega-3 FAs on improving endothelial function through FMD were justified in our study in line with our results omega-3 FA can improve endothelial function through postprandial FMD. Also, a subsequent subgroup analysis revealed that n-3 PUFA FAs supplementation in patients compared to healthy subjects has significant effects on endothelial function, which had been observed in previous studies.[73] Patients with inflammatory conditions, dyslipidemia, or related risk factors may show more significant effects than healthy participants with no added cardiovascular risk factors after being treated with omega-3 FAs and improving these conditions.[20,74] The results obtained in our study were confirmed by the study of Wang et al.[73]

Subgroup analysis demonstrated that participants with ≥1 g/day omega-3 FAs had a more favourable effect on FMD. Furthermore, we detected a dose–response effect of omega-3 treatment on FMD. We observed that the greatest effect of treatment with omega-3 FAs was produced at a dose of 2 g/day (MD: 1.2%; CI 0.4–2.06). Dose–response analysis showed that by increasing the dose of n-3 PUFA FAs to 3 g/day, positive clinical effects are produced, but the changes made in doses of 4–6 g/day were not significant. In general, low to moderate omega-3 FA doses have been shown to be beneficial for endothelial function, and high doses of omega-3 may act as a pro-inflammatory agent and not be beneficial.[75,76] On the other hand, the effects of omega-3 FAs treatment may change with age. We detected this treatment was a significant effect on FMD in participants with ≤55 years old. Because endothelial

function decreases with aging and may show less effectiveness following n-3 PUFAs intervention.[77] Although positive results were observed in subgroups, high heterogeneity is still an important limitation that should be considered. Based on the results of the subgroup analysis, the study design and the type of omega-3 supplement intervention were identified as sources of heterogeneity.

We identified a significant effect following supplementation with omega-3 FAs and improving endothelial function by FMD index, but this effect size is lower compared to the minimum clinically important difference (MCID) changes, which should be considered by clinicians in practice. In the subgroup analysis, we found that the effect size in participants with a mean age ≤55 years, especially in the adolescent group, in trials with duration ≤12 weeks, cross-over design, and in high risk of bias trials were higher than MCID.[55]

Finally, one of the primary limitations of our study was the presence of significant heterogeneity, which must be interpreted with caution. To address this issue, we conducted a subgroup and meta-regression analysis in order to strengthen the reliability of our findings. The strengths of our study were the non-change of the results after performing the sensitivity analysis, high validity following GRADE evaluation, examination of the dose–response relationship, and lack of publication bias, which can give acceptable validity to these results, and evidence for clinical efficacy is strong enough to make recommendations concerning to a specific dose or the durations of intakes for different populations.

5 | CONCLUSION

The implications of the present study for primary and secondary prevention of CVD are significant. The study suggests that n-3 PUFA supplementation may have a beneficial effect on FMD, which is a key predictor of future cardiovascular events. Thus, incorporating interventions that improve endothelial function, such as lifestyle modifications or pharmacological therapy, may be an important component of CVD prevention strategies. The current findings add to the growing body of evidence supporting the use of n-3 PUFAs in cardiovascular health and confirm their potential benefits in reducing the risk of CVD events. Improvement in FMD could be a plausible explanation for the observed benefit in cardiovascular outcomes following n-3 PUFA supplementation.

AUTHOR CONTRIBUTIONS

Seyyed Mostafa Arabi, Amirhossein Sahebkar, and Gerald F. Watts conceived the study. Hossein Bahari, Leila Sadat Bahrami, and Mahla Chambari contributed to the literature search, screening articles, data extraction, and quality assessment. Hossein Bahari and Seyyed Mostafa Arabi contributed to the literature search and manuscript drafting. Seyyed Mostafa Arabi, Mohammaed Ibrahim Mohaildeen Gubari analysed and interpreted data. Amirhossein Sahebkar and Gerald F. Watts critically revised the statistical analysis and final version of the manuscript. Amirhossein Sahebkar supervised the study. The final form of the manuscript was read and accepted by all authors.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

REGISTRATION

The systematic review and meta-analysis protocol was registered to the PROSPERO Web site (www.crd.york.ac.UK/PROSPERO) (Registration number CRD42022377867, date: 03/12/2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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