Journal of the American Heart Association

ORIGINAL RESEARCH

Association Between Omega-3 Fatty Acid Intake and Dyslipidemia: A Continuous Dose–Response Meta-Analysis of Randomized Controlled Trials

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BACKGROUND: Previous results provide supportive but not conclusive evidence for the use of omega-3 fatty acids to reduce blood lipids and prevent events of atherosclerotic cardiovascular disease, but the strength and shape of dose–response relationships remain elusive.

METHODS AND RESULTS: This study included 90 randomized controlled trials, reported an overall sample size of 72 598 participants, and examined the association between omega-3 fatty acid (docosahexaenoic acid, eicosapentaenoic acid, or both) intake and blood lipid changes. Random-effects 1-stage cubic spline regression models were used to study the mean dose–response association between daily omega-3 fatty acid intake and changes in blood lipids. Nonlinear associations were found in general and in most subgroups, depicted as J-shaped dose–response curves for low-/high-density lipoprotein cholesterol. However, we found evidence of an approximately linear dose–response relationship for triglyceride and non-high-density lipoprotein cholesterol among the general population and more evidently in populations with hyperlipidemia and overweight/obesity who were given medium to high doses (>2 g/d).

CONCLUSIONS: This dose–response meta-analysis demonstrates that combined intake of omega-3 fatty acids near linearly lowers triglyceride and non-high-density lipoprotein cholesterol. Triglyceride-lowering effects might provide supportive evidence for omega-3 fatty acid intake to prevent cardiovascular events.

Key Words: 1-stage regression ■ hyperlipidemia ■ long-chain fatty acids ■ non-HDL cholesterol ■ triglyceride

Despite the enforced lipid-lowering measures over the past decade, global cardiovascular disease (CVD)-caused deaths rose by almost 20% from 2010 to 2020. Between 2015 and 2018, in the United States alone, dyslipidemia prevalence ranged from 17% to 38%, determined by either total cholesterol ≥200 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥130 mg/dL, triglyceride ≥150 mg/dL, or high-density lipoprotein cholesterol (HDL-C) <40 mg/dL.[1]

With the hope of protecting the population with hyperlipidemia from CVD events, high-intensity statin therapy targeting LDL-C was recommended for the treatment of blood cholesterol.[2,3] Another strategy is to lower the triglyceride level or triglyceride-rich lipoprotein.[4,5] Supplementation of omega-3 polyunsaturated fatty acids (ω3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is one of the lipid-lowering approaches.[6] Researchers have long

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This article was sent to Daniel Edmundowicz, MD, Guest Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.029512

For Sources of Funding and Disclosures, see page 12.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Intake of omega-3 fatty acids of more than 2 g/d appears to have a near-linear association with reductions in triglyceride and non-high-density lipoprotein cholesterol.
- Omega-3 polyunsaturated fatty acid supplementation at lower doses is associated with an increased level of low-density lipoprotein cholesterol.

What Are the Clinical Implications?

- A medium dose of omega-3 fatty acids is potentially needed for the management of dyslipidemia, and a higher dose may afford more benefits for people who are at high risk of developing cardiovascular diseases.
- The recommendation for omega-3 fatty acid supplementation to reduce cardiovascular disease risks could be supported in patients with a high level of triglyceride in the context of guideline-directed statin therapies.

Nonstandard Abbreviations and Acronyms

DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
ω3 PUFA	omega-3 polyunsaturated fatty acid

seen ω3 PUFA intake as a potential strategy to address vascular conditions, but there have also been concerns. ω3 PUFAs could reduce serum triglyceride concentration by approximately 15% to 30%[6–9] but could not affect or even increase LDL-C levels.[9–13] Previous systematic reviews and meta-analyses have been unable to reveal a significant dose–response relationship.[12,14] Some aggregated data have brought more uncertainty[6,9,13,15] rather than a solid conclusion. These past meta-analyses examined the dose–response relationship using pooled linear meta-regression[9,12,13,16] without taking into account the correlations among effects at different dose levels.[17]

Extrapolation of the causal relationship between ω3 PUFA intake and vascular risk remains controversial, both in large randomized controlled trials (RCTs) and in many extensive meta-analyses. ω3 PUFA intake has been associated with a reduced risk of major cardiovascular events, primarily in 2 trials: JELIS (Japan Eicosapentaenoic Acid Lipid Intervention Study)[18] and REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial).[19] However, many previous[20–23] and recently completed clinical studies[24,25] showed that ω3 PUFA supplementation did not offer significant favorable impacts on cardiovascular events. Moreover, JELIS was often challenged for its selection of patients with a relatively high background of fish consumption,[26] and REDUCE-IT was revisited for the use of mineral oil as a comparator,[27–29] respectively. A few meta-analyses found a statistically significant CVD risk reduction,[30,31] but more results showed insufficient evidence of a possible protective effect.[32–37] Neither linear assumption-driven meta-regressions[38–42] nor stratified dose analyses[42,43] have conclusively estimated the dose–response relationship between ω3 PUFA intake and relative risk reduction, raising the possibility of a nonlinear dose–response curve.[30]

This necessitates a rigorous examination of the dose–response effects of ω3 PUFAs on lipid changes among RCTs. We and others have used a 1-stage cubic spline regression model[17] to perform dose–response meta-analyses in 3 systematic reviews of blood pressure.[44–46] The 1-stage spline mixed model allows us to fully capture the nonlinear dose–response relationship and reflect heterogeneity in studies with <3 exposure levels.[17] Following a comprehensive review of the literature, this study aims to more precisely characterize the dose–response effect between ω3 PUFAs (DHA, EPA, or both) and lipid profile, including triglyceride, LDL-C, HDL-C, non-HDL-C, and apolipoprotein B (apoB), in the general population and relevant subgroups.

METHODS

The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines for the meta-analysis of randomized trials (Table S1). The data that support the findings of this study are available from the corresponding author upon reasonable request. This meta-analysis was carried out with data from previously published trials. Therefore, the approval of the ethics review or the institutional review board is not applicable.

Literature Retrieval

The literature retrieval was performed for articles published before June 2022, using the PubMed and EMBASE databases (Table S2). Additional searches were carried out to screen the reference lists of relevant studies, reviews, and meta-analyses for more studies. Two authors (T.W. and N.Z.) independently reviewed each study and discrepancies were resolved through discussion. The prespecified eligibility criteria were parallel RCTs that examined the association between intake of DHA/EPA (combined or individual) and

lipid changes (triglyceride, LDL-C, HDL-C, non-HDL-C, and apoB) in adults (aged ≥18 years). The exclusion criteria are (1) concurrent controls were lacking; (2) the duration of the intervention was <4 weeks; (3) studies were carried out in pregnant and nursing women; and (4) trials with a small sample size (<20 in each arm), not providing statistical power greater than 70% to measure a reduction of 53.2 mg/dL (0.6 mmol/L) in triglyceride after treatment with fish oil compared with a control intervention, given the SD of 66.5 mg/dL (0.75 mmol/L) and the 2-tailed significance level at 0.05.[47,48]

Assessment of the methodological quality was performed independently using the Cochrane Risk-of-Bias tool RoB2.[49] Two authors (T.W. and X.L.) independently assessed the risk of bias in the domains of randomization (random sequence generation), blinding (allocation concealment, blinding of participants and personnel, and blinding of outcome assessors), missing outcome(s) (incomplete outcome data), measurement (method and measurement bias), and selection of results (reporting bias).

Data Extraction

Information from the included study was extracted independently by 2 authors (T.W. and X.Z.) and confirmed by the other 2 authors (Y.S. and B.L.) using a standardized form. The effects of each exposure dose were collected individually in our study. In experiments with multiple follow-up time points, only changes in lipid levels were extracted at the end of treatment versus before treatment. If the SD was not provided directly, we calculated it from SE, interquartile range, or CIs.[50]

Exposure and Outcome Assessment

Most studies used a combined supplementation of EPA and DHA. Exposure levels were expressed by combined DHA + EPA or DHA/EPA alone. In some cases, DHA/EPA dose was considered separately, even when mixed EPA + DHA formulation was administered. If possible, the achieved change in red blood cell (RBC) omega index, the percentage of EPA plus DHA of total fatty acid in the RBC membrane, was extracted. This index serves as a biomarker of absorbed and integrated fish oil and reflects long-term exposure levels.[51,52] We determined the net mean difference in lipid profile ($\Delta Lipid_{between}$) between the exposure levels of each RCT as the difference at the end of the intervention minus the corresponding pretreatment value ($\Delta Lipid_{intra\text{-}group}$). The numerical values of triglyceride, LDL-C, HDL-C, and non–HDL-C are given in mg/dL and mmol/L. To convert to mg/dL, the values in mmol/L for LDL-C, HDL-C, and non–HDL-C are multiplied by 38.6 and for triglyceride by 88.6.[2] Circulating non-HDL-C is used as an outcome to represent all atherogenic lipoproteins, such as cholesterol-containing LDL-C/intermediate-density lipoprotein and primarily triglyceride-containing very low-density lipoprotein. The non-HDL-C analysis includes only trials that reported non-HDL-C data. ApoB-containing lipoproteins, including very low-density lipoproteins, triglyceride-rich remnant particles, and LDL, are central causal factors in the progression of atherosclerotic plaque.[2,3] ApoB quantitation is performed as an outcome to predict the overall atherogenic lipid profile.

Publication Bias Assessment

Publication bias was examined visually using funnel plots to assess the SE as a function of effect size, along with Egger's regression test to examine small-study bias using R *metafor*.[53] We also used the trim-and-fill method to estimate the number of potential missing studies due to publication bias. A leave-one-out strategy was applied for sensitivity analyses, where we repeatedly ran the dose–response analysis to assess the missing study's influence on overall lipid changes.

Dose–Response Analysis

The control dose (0 g/d) was used as a reference for all analyses as described in our previous blood pressure analysis.[46] A 1-stage random-effects dose–response model[17] was established to predict the average dose–response relationship between DHA + EPA administration and changes in lipid levels. We tested the linearity assumption underlying the dose–response relationship by fitting a restricted cubic spline model with 3 knots (10^{th}, 50^{th}, and 90^{th} percentiles) of doses.[54] The included studies were pooled into a continuous dose–response curve, and then estimates of lipid changes were calculated at given doses (that is, 1, 2, 3, 4, and 5 g/d). Furthermore, subgroup analyses were performed by stratifying studies according to preexisting hyperlipidemia status (total cholesterol ≥200 mg/dL [5.2 mmol/L] or triglyceride ≥150 mg/dL [1.7 mmoL/L]), patients with hyperlipidemia taking lipid-lowering medications (yes versus no), baseline mean body mass index (≥25 or <25 kg/m^2), preexisting coronary heart disease (CHD) (yes versus no), mean age (≥50 or <50 years), duration of treatment (>13 or ≤13 weeks), and use of EPA/DHA only. The 1-stage cubic spline regression model was conducted using the *dosresmeta* R packages (https://github.com/alecri/dosresmeta).[17,55,56]

RESULTS

Study Characteristics

The systematic search retrieved 2385 relevant articles after removing duplicated 727 items. The title and abstract review further excluded 2086 articles.

A full-text examination of 299 articles yielded 90 eligible RCTs.[20,21,24,47,48,57–141] A PRISMA flow diagram of the literature screening can be seen in Figure 1. Study characteristics of included trials are shown in Table S3. These trials, published between 1990 and 2022, reported an overall sample size of 72 598 participants with a range of the mean age between 25.7 and 70.0 years and a range of the mean body mass index between 22.8 and 34.6 kg/m.[2] These trials were carried out in Europe (n=36, 40.0%), Asia (n=35, 38.9%), North America (n=18, 20.0%), and Oceania (n=1, 1.1%). Most trials (82/90) included both men and women, 8 included only men, and no trial included only women. Fifty-two (57.8%) trials were reported with hyperlipidemia, and 11 (12.2%) trials were restricted to participants without hyperlipidemia. Among those 52 trials with hyperlipidemia, patients were regularly treated with lipid-lowering medications (statins or fibrates) in 25 (48.1%) trials in addition to ω3 PUFA and in 17 (32.7%) trials with ω3 PUFA alone. Eighteen (20.0%) trials were conducted in participants with preexisting CHD and 46 (51.1%) trials in participants without CHD. The median duration of the intervention was 13.0 weeks (interquartile range, 8.5–26.0), and the duration was >13.0 weeks in 40 (44.4%) trials and <13.0 weeks in 50 (55.6%) trials. The most commonly used control/comparator was olive oil, along with the remainder consisting of various vegetable oils, such as safflower, sunflower, corn, soybean, and palm oils. Some controls were statin or fibrate alone or lipid-lowering medication plus olive oil. Sixty-three out of 90 trials reported the combined effects of DHA and EPA, with an average combined dose of 2.26 (interquartile range, 1.52–3.10, range, 0.30–6.90) g/d, DHA dose of 1.07 (interquartile range, 0.52–1.51, range, 0.12–3.68) g/d, and EPA dose of 1.48 (interquartile range, 0.82–1.83, range, 0.18–4.10) g/d (Figure S1); only 22 and 5 trials observed the effects of EPA or DHA alone, respectively.

Overall Dose–Response Analysis for Lipid Changes

The calculated mean changes and SEs of the included trials were visualized by scatterplots (Figure S2). The model performance comparison indicated that the restricted cubic spline model fits the overall data better than the linear or quadratic model (Figure S3). Table 1 and Table S4 summarize the overall effects of the combined application of DHA+EPA on mean changes in lipid profile. An approximately linear relationship for both triglyceride and non-HDL-C suggests that increasing combined supplementation is associated

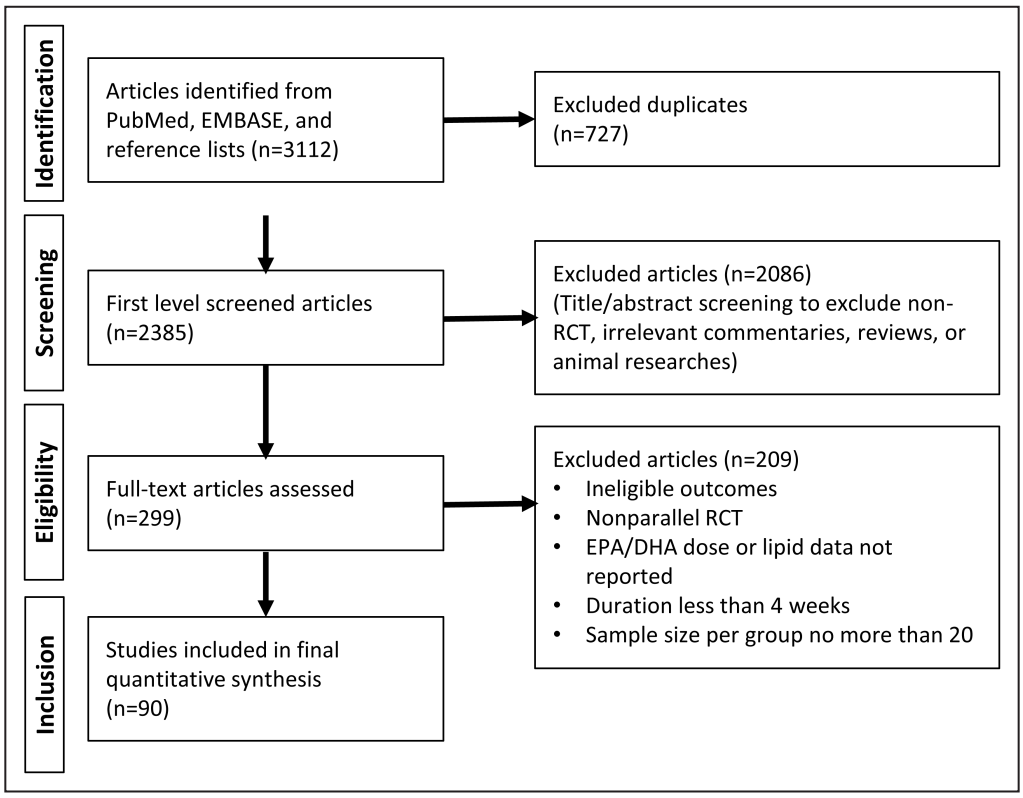

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of systematic literature search and screening for randomized controlled trials published through June 2022 that met the study inclusion and exclusion criteria.
DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; and RCT, randomized controlled trial.

with greater reductions in both instances compared with the control groups (combined application dose of 0 g/d), with a steeper gradient for triglyceride than for non-HDL-C across the entire dose range (Figure 2). The mean change in triglyceride was −42.61 (95% CI, −53.41 to −31.80) mg/dL for 2 g/d and −68.90 (95% CI, −98.40 to −39.40) mg/dL for 3 g/d of DHA + EPA. The mean change in non-HDL for 2 g/d of DHA + EPA was −4.13 (95% CI, −9.20 to 0.95) mg/dL and −8.31 (95% CI, −11.78 to −4.83) mg/dL at 3 g/d (Table 1). Significant nonlinear dose–response relationships were found between DHA + EPA intake and LDL-C or HDL-C changes. The J-shaped curve for LDL-C change peaked at 1.75 g/d intake with a moderate LDL-C increment of 2.91 (95% CI, 0.34–5.47) and HDL-C increment of 3.48 (95% CI, 1.09–5.86) mg/dL, respectively. The similar J-shaped curve for the HDL-C change indicated a limited increase (Figure 2). These findings provided strong evidence for the intake of DHA + EPA to reduce triglyceride and non-HDL-C levels, but not LDL-C levels, in a nearly linear manner in the overall population.[6,8,12]

The amount and ratio of EPA and DHA differ in various supplements, resulting in different bioavailability and absorption rates.[142–144] Therefore, we analyzed the lipid response to the achieved percentage change in the RBC omega index. Achieved change in the RBC omega index was negatively and almost linearly associated with changes in triglyceride and non-HDL-C but positively associated with changes in HDL-C, over a wide range of RBC omega index changes (0%–300%). These trends were not observed in the LDL-C change with marginally null effects throughout the entire exposure range (Table S5 and Figure S4).

Subgroup Analyses for Lipid Changes

In subgroup studies stratified by prespecified hyperlipidemic status at entry, the approximately linear trend for triglyceride change was found only in the population with hyperlipidemia but not in the population without hyperlipidemia, where the effect stably plateaued at roughly 40 mg/dL. The non-HDL-C change was almost the same as the overall effects because 21 out of 22 trials were among participants with hyperlipidemia (Table S4 and Figure 3).

Similar results for triglyceride were obtained in participants with hyperlipidemia, whether they were treated with lipid-lowering medication or not if they had ingested more than 2 g/d ω3 PUFAs. However, participants who received lipid-lowering medication had a much steeper curve in non-HDL-C than those who did not. ω3 PUFA increased LDL-C level significantly with a dose greater than 2 g/d, consistent with previous findings.[7,9,12,13] Moreover, this dose–response trend is independent of baseline LDL-C levels (≥130 versus <130 mg/dL, data not shown). Fatty acids combined with statins, compared with fatty acid monotherapy, could synergistically increase HDL-C levels at a dose greater than 2 g/d (Table S4 and Figure 4).

When we stratified according to baseline mean body mass index (<25 versus ≥25 kg/m^2), we found stronger triglyceride effects of ω3 PUFA monotherapy in participants with higher background body mass index, classified as overweight/obesity (Table S4 and Figure 5). Similar findings were also observed when stratified by preexisting CHD (yes versus no), where those with preexisting CHD saw greater reductions after the dose reached 2 g/d (Table S4 and Figure S5). Moreover, DHA + EPA supplementation demonstrated greater responses to lower triglyceride levels among patients with hyperlipidemia and CHD (Figure S6). This could warrant secondary prevention of EPA + DHA for CHD.[34,145] When we considered baseline mean age (<50 versus ≥50 years) and trial duration (4–13 weeks versus >13 weeks), the dose–response relationship demonstrated mild variations in triglyceride and non-HDL-C differences between age and trial duration and with little evidence to support other lipid-altering efficacy, compared with the overall effects (Figures S7 and S8).

There is an apparent need to differentiate the role of DHA and EPA in conferring lipid and vascular impacts.[11,146–148] Our classification of the retrieved experiments using DHA/EPA as individual fatty acids revealed that the magnitude of triglyceride decrease is similar in treatment with DHA and EPA alone (Table S4 and Figure S9). The effects of DHA on HDL-C appeared to reach the plateau after a dose of 2 g/d. DHA is more likely to be associated with an increase in LDL-C compared with EPA alone (Table S4 and Figure S9). When the dosage of DHA/EPA intake was considered separately, as shown in Figure S10, there was still an approximately linear relationship in triglyceride reduction, though the slope became gradual. The dose–response effects of non-HDL-C stabilized after the separate DHA/EPA dose of more than 2 g/d. In multiple subgroup analyses, separate EPA seemed to show weaker lipid-lowering effects than separate DHA, which exerted greater triglyceride-lowering effects in participants with hyperlipidemia, overweight/obesity, and CHD across the entire dose range (Figures S11-14). These disparities between separate DHA and EPA were not evident for responses of non-HDL-C (Figures S11-14). With the removal of all EPA/DHA monotherapies (Figure S15), the dose responses are consistent with the previous data (Figure 2). Collectively, combined supplementation of DHA and EPA appeared to exert a robust effect on triglyceride reduction but not other serum lipids.

Lastly, except for a nearly linear dose–response association between apoB and the RBC omega index, J-shaped curvilinear trends are commonly seen in general or in various subgroup responses (Figures S16 and S17).

Table 1. Estimated Average Dose–Response Relationship Between DHA+EPA Consumption (g/d) and Lipid Reduction (mg/dL)‡

Lipid	Participants	N*	1.0 g/d		2.0 g/d		3.0 g/d	
			MD	(95% CI)	MD	(95% CI)	MD	(95% CI)
Triglyceride	All	86	−19.21	(−32.01 to 6.41)	−42.61	(−53.41 to 31.80)	−68.90	(−98.40 to 39.40)
LDL-C	All	80	2.91	(0.34 to 5.47)	3.48	(1.09 to 5.86)	2.43	(−0.36 to 5.22)
HDL-C	All	87	1.36	(0.47 to 2.25)	1.69	(0.78 to 2.61)	1.32	(−0.97 to 3.60)
Non-HDL-C	All	22	−1.18	(−6.24 to 3.89)	−4.13	(−9.20 to 0.95)	−8.31	(−11.78 to 4.83)
Hyperlipidemia status								
Triglyceride	Yes	49	−23.05	(−43.59 to 2.51)	−49.89	(−63.28 to 36.49)	−80.58	(−150.43 to 10.74)
	No	11	−17.24	(−31.01 to 3.48)	−27.36	(−45.82 to 8.89)	−32.58	(−50.72 to 14.43)
LDL-C	Yes	48	2.82	(−1.25 to 6.90)	4.17	(0.09 to 8.24)	4.01	(0.50 to 7.51)
	No	10	7.79	(1.83 to 13.75)	7.64	(1.15 to 14.14)	2.48	(−6.33 to 11.29)
HDL-C	Yes	51	1.96	(0.59 to 3.34)	2.38	(0.62 to 4.13)	1.15	(0.05 to 2.26)
	No	10	3.43	(1.22 to 5.63)	2.92	(−0.84 to 6.69)	−0.30	(−10.56 to 9.96)
Non-HDL-C†	Yes	21	−0.89	(−6.37 to 4.58)	−3.74	(−9.57 to 2.09)	−8.24	(−11.80 to 4.68)
Participants with hyperlipidemia taking lipid-lowering medication								
Triglyceride	Yes	22	1.93	(−15.04 to 18.90)	−27.96	(−44.08 to 11.84)	−98.23	(−201.25 to 4.79)
	No	17	−18.97	(−46.12 to 8.19)	−52.75	(−71.38 to 34.12)	−100.71	(−160.80 to 40.61)
LDL-C	Yes	24	1.21	(−1.49 to 3.92)	1.06	(−2.79 to 4.91)	−0.83	(−3.84 to 2.17)
	No	15	−0.41	(−3.77 to 2.95)	3.02	(−0.07 to 6.12)	10.13	(5.57 to 14.70)
HDL-C	Yes	24	−0.56	(−2.92 to 1.79)	0.64	(−1.41 to 2.69)	4.09	(−9.20 to 17.38)
	No	17	4.15	(0.63 to 7.66)	4.98	(0.64 to 9.32)	2.65	(0.01 to 5.28)
Non-HDL-C	Yes	13	1.44	(−7.38 to 10.27)	−1.90	(−11.46 to 7.67)	−9.59	(−13.90 to 5.27)
	No	3	−1.87	(−7.72 to 3.98)	−3.52	(−11.49 to 4.46)	−4.88	(−10.31 to 0.55)
Baseline mean body mass index								
Triglyceride	≥25 kg/m^2	53	−25.54	(−42.03 to 9.04)	−46.86	(−58.64 to 35.08)	−65.27	(−91.38 to 39.17)
	<25 kg/m^2	22	−5.76	(−24.62 to 13.10)	−9.23	(−23.58 to 5.12)	−11.47	(−82.65 to 59.72)
LDL-C	≥25 kg/m^2	52	4.15	(0.41 to 7.89)	5.00	(1.74 to 8.27)	3.56	(0.34 to 6.79)
	<25 kg/m^2	20	1.00	(−2.62 to 4.62)	−1.42	(−3.50 to 0.67)	−5.83	(−13.76 to 2.10)
HDL-C	≥25 kg/m^2	55	1.56	(0.76 to 2.36)	1.78	(0.82 to 2.75)	1.08	(0.15 to 2.01)
	<25 kg/m^2	21	1.76	(−5.20 to 8.73)	4.69	(−1.47 to 10.85)	8.20	(−12.01 to 28.41)
Non-HDL-C†	≥25 kg/m^2	18	1.19	(−5.32 to 7.69)	−1.78	(−8.32 to 4.76)	−7.61	(−11.31 to 3.90)

DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; and non-HDL-C, non-high-density lipoprotein cholesterol.

*Numbers may not sum to group totals due to missing data or unspecified subgroups in the trials.

†Due to the unavailability of data, only 1 subgroup estimate was performed in the absence or presence of hyperlipidemia, overweight/obesity (≥25 kg/m^2), and preexisting coronary heart disease.

‡The complete dose–response outcomes are presented in Table S4.

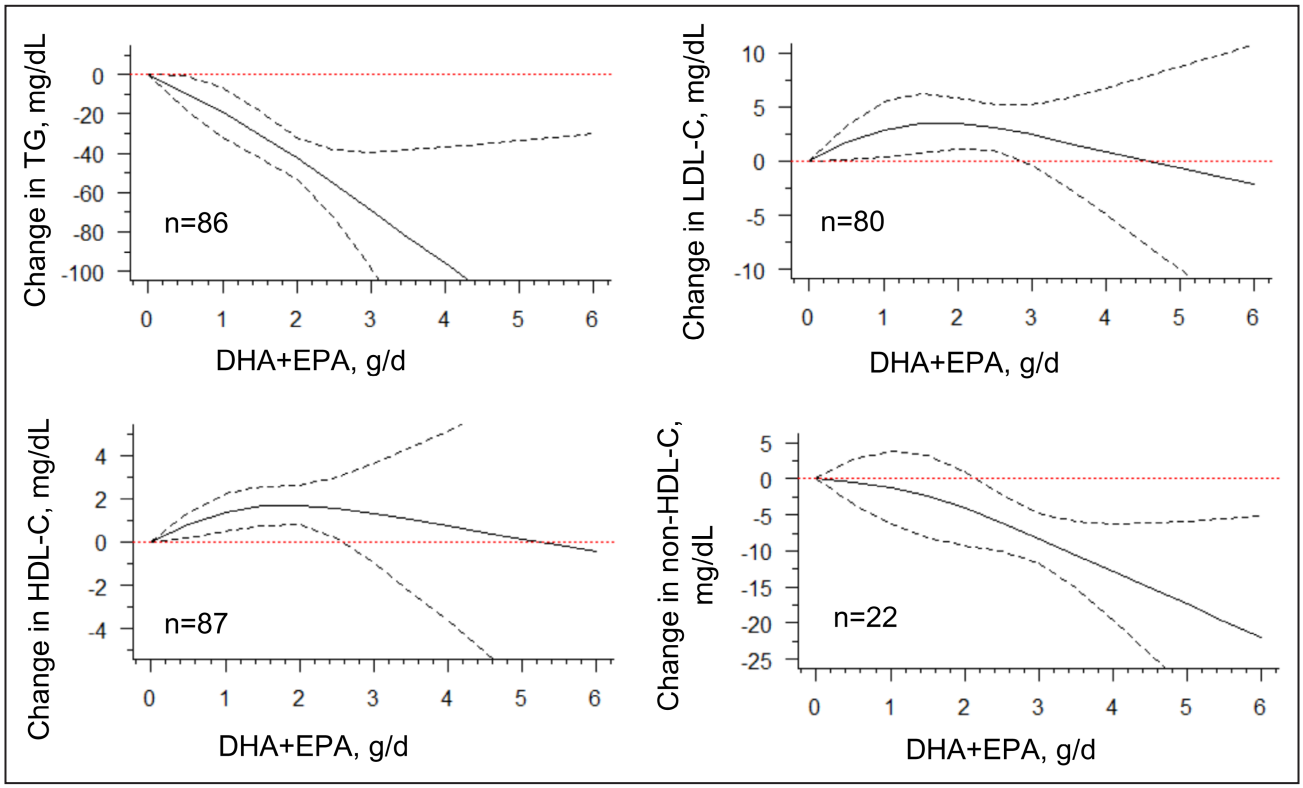

Figure 2. Dose–response relationship between changes in lipids and combined intake of DHA+EPA.
Marginal average dose–response curve (solid line) with 95% point-wise CIs (dashed lines) estimated by a 1-stage random-effects restricted cubic spline model, using 0 g/d as the reference. Studies included n=86 for TG, n=80 for LDL-C, n=87 for HDL-C, and n=22 for non-HDL-C. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and TG, triglyceride.

Risk of Study Bias and Publication Bias

After evaluating all trials included in the lipid profile study, 3 trials were classified as high and another 3 as moderate risk of bias, and the remaining trials were classified as low risk of bias (Table S6). The exclusion of moderate- and high-risk biased trials did not appreciably change the shape of the dose–response curve (results not shown). The funnel plot and Egger's regression test indicated asymmetry only in the overall triglyceride models (z=−3.37, P<0.001) but not in the pooled HDL-C, LDL-C, and non-HDL-C models (Figure S18). This suggests that publication bias, if present due to the effects of the small study, did not strongly affect our overall findings. Leave-one-out sensitivity analyses in 1-stage regression models proved that overall effects were not driven by a small number of specific trials but reflected the global effect of all included trials (Figure S19).

DISCUSSION

In this dose–response meta-analysis using a 1-stage method, we examined the strength and shape of the lipid-lowering effects of DHA+EPA supplementation with up-to-date literature. We found evidence of an approximately linear dose–response relationship for triglyceride and non-HDL-C reduction among the general population and especially in populations with hyperlipidemia and overweight/obesity. These inverse correlations were more prominent in participants receiving basal lipid-lowering medications or with preexisting CHD, given the intake dose was higher than 2 g/d.

The current meta-analysis differs from others in the statistical methodology used and the consideration of a nonlinear relationship. Previous dose–response models using pooled meta-regression method were conducted based on the assumption that a linear causal relationship existed,[9,12,13,16] without taking into account the correlations at different dose levels. The current 1-stage model is more flexibly capable of estimating nonlinear dose–response curves based on aggregated data with <3 exposure levels.[17] Moreover, 1-stage dose–response meta-analysis does not assume a particular shape for the relationship, allowing for nonlinear relations between exposure and outcome, which includes linear, U-shape, and J-shape curvilinear models. Therefore,

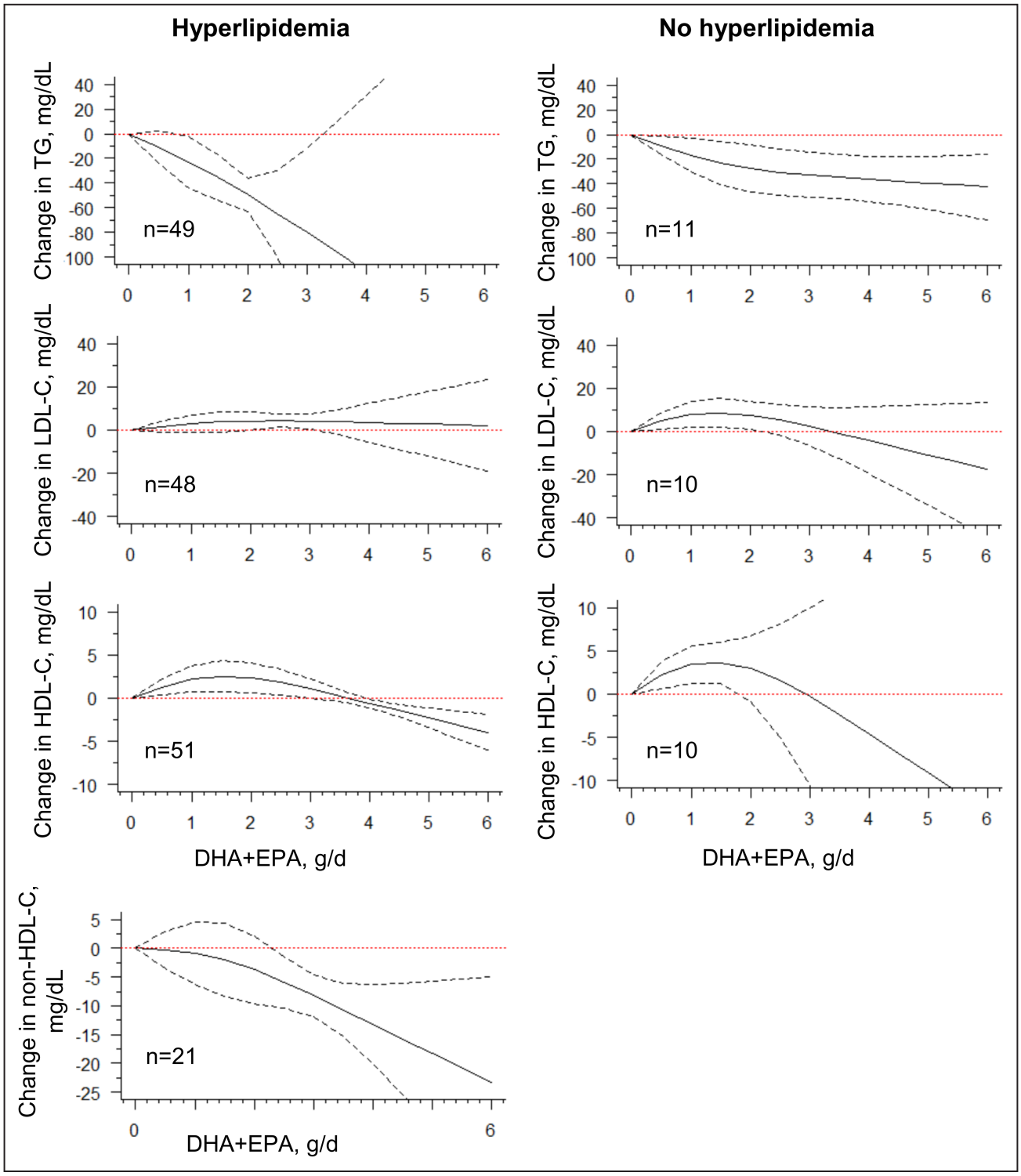

Figure 3. Dose–response relationship between changes in lipids and combined intake of DHA+EPA of studies stratified by hyperlipidemia status.

Marginal average dose–response curve (solid line) with 95% point-wise CIs (dashed lines) estimated by a 1-stage random-effects restricted cubic spline model, using 0 g/day as reference, in participants with or without hyperlipidemia. n=the number of the included study. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and TG, triglyceride.

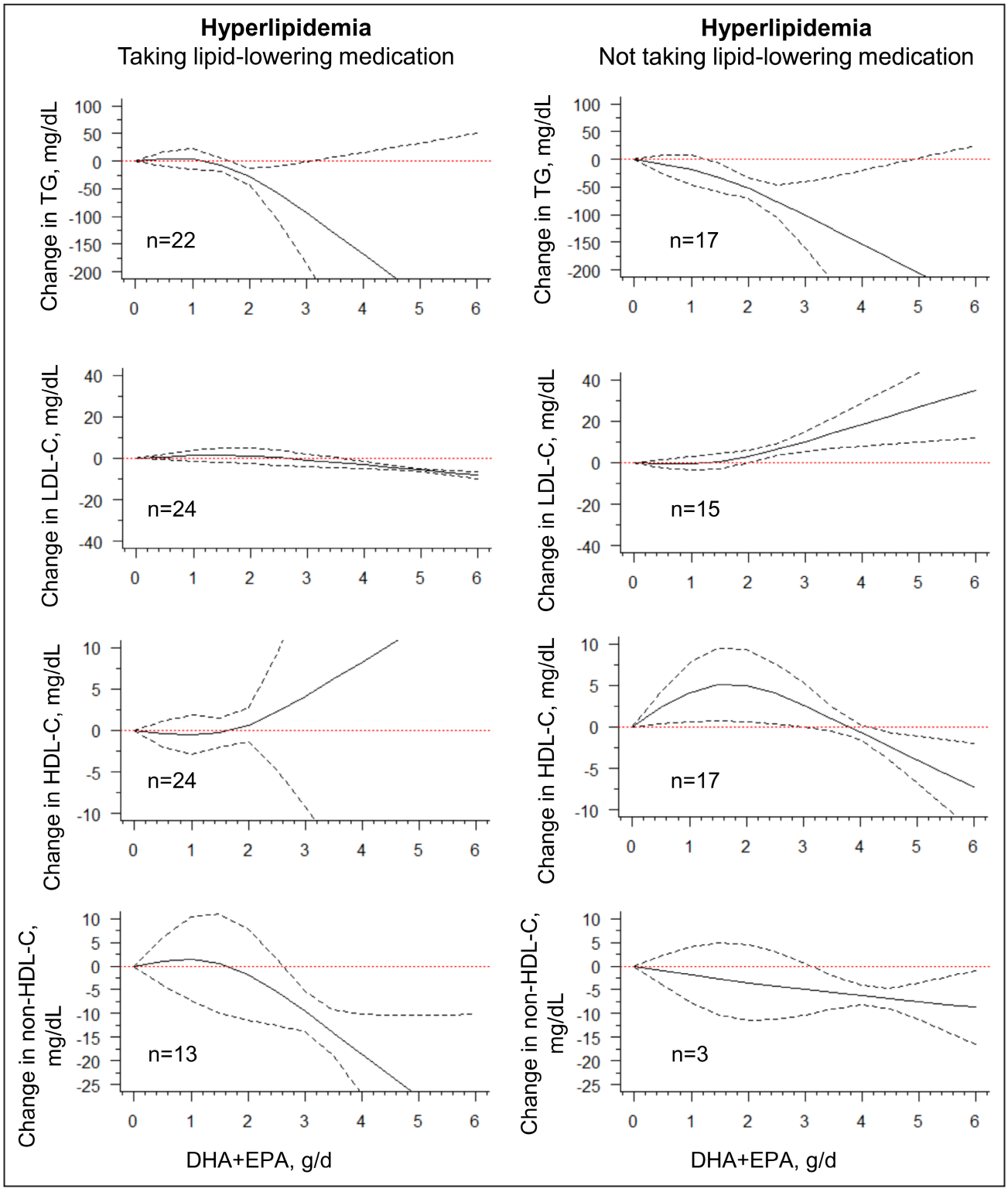

Figure 4. Subgroup analysis for changes in lipids and combined intake of DHA + EPA among hyperlipidemic participants. Marginal average dose–response curve (solid line) with 95% point-wise CIs (dashed lines) estimated by a 1-stage random-effects restricted cubic spline model, using 0 g/day as reference, in participants taking or not taking lipid-lowering medications. n=the number of the included study. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and TG, triglyceride.

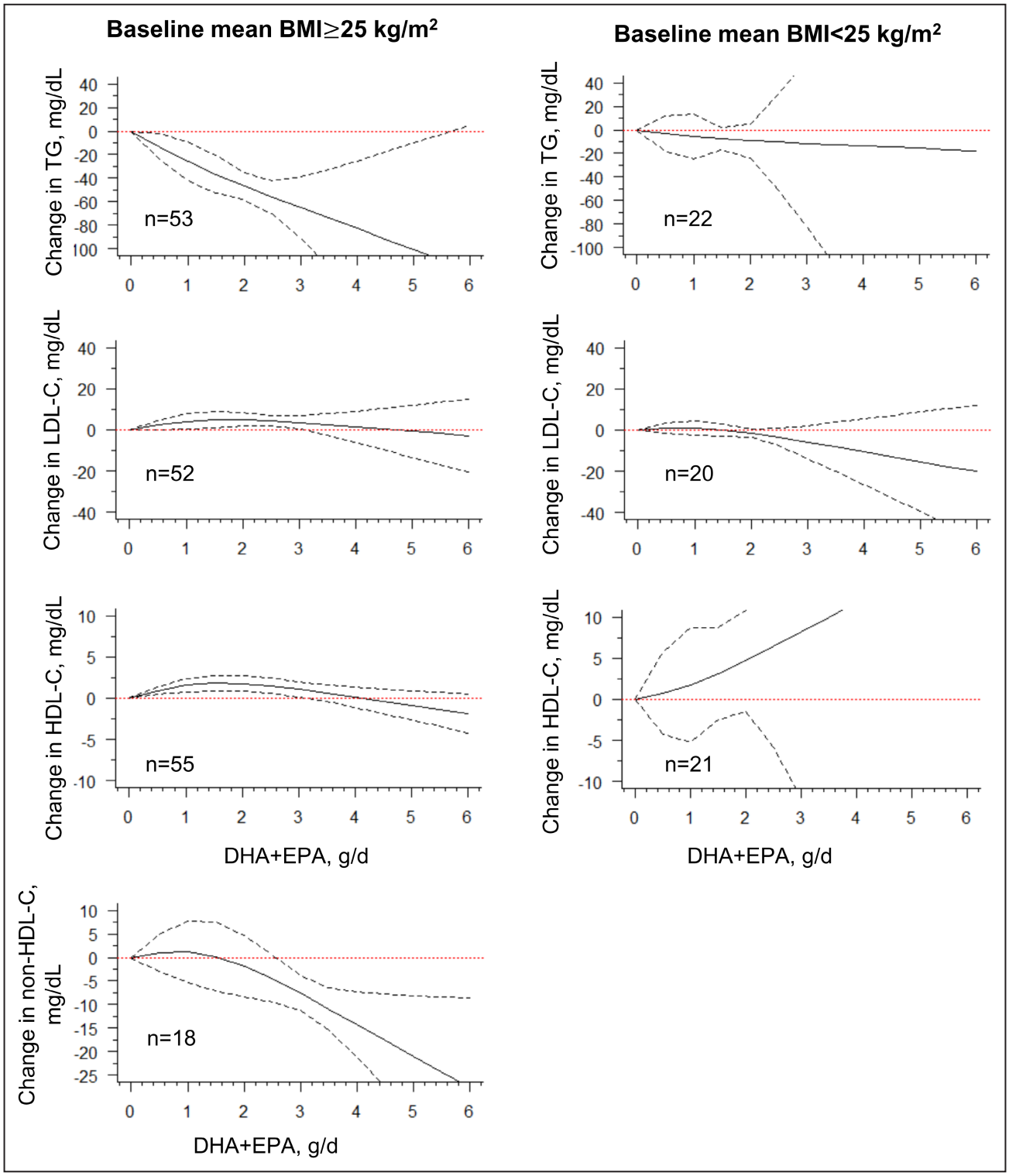

Figure 5. Dose–response relationship between changes in lipids and combined intake of DHA + EPA of the studies stratified by overweight/obesity classified by the baseline mean of body mass index (BMI).
Marginal average dose–response curve (solid line) with 95% point-wise CIs (dashed lines) estimated by a 1-stage random-effects restricted cubic spline model, using 0 g/day as reference, among participants with a mean BMI ≥25 or <25 kg/m². n=the number of the included study. BMI indicates body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and TG, triglyceride.

we think that either a near-linear or a nonlinear relationship is entirely driven by the data instead of an assumption given by the investigators. We have now provided a pictorial presentation to illustrate that triglyceride and non-HDL-C reduction confers biological plausibility in a dose-dependent manner in an atherosclerosis setting and other cardiometabolic complications.

Though LDL is widely recognized as the dominant atherogenic factor,[149] the current analysis suggested that LDL-C, a surrogate of LDL particle concentration, did not appear to be targeted by EPA and DHA in the treatment of dyslipidemia, an outcome in agreement with many published meta-analysis results.[4,17–19] Previous meta-regression analyses assumed a linear relationship between ω3 PUFA intake and triglyceride changes among RCT studies.[9,13] Without any assumption, our current 1-stage dose–response analyses coincided with a nearly linear association between triglyceride reduction and ω3 PUFA intake. Using the continuous dose–response curve, we have estimated the optimal dose for triglyceride reduction in various subgroup analyses. For example, medium to high doses (>2 g/d) were predicted to exert significant triglyceride-lowering effects among hyperlipidemic participants. These dose predictions cannot be performed in previous meta-analyses that failed to reveal a significant dose–response relationship[12,14] and brought uncertainty.[6,9,13,15] Moreover, the triglyceride-lowering potency was proportionally mirrored in non-HDL-C reduction with moderate gradients. To our best knowledge, this is the first dose–response meta-analysis of the relationship between ω3 PUFA intake and non-HDL-C changes, an indicator of the cholesterol content of all atherogenic lipoproteins.[100,150,151]

The association between triglyceride-lowering and ω3 PUFA intake could causally lead to the reduction of cardiovascular risks in patients with high triglyceride, as previously reported in trials[18,19] and meta-analyses.[38] Furthermore, our findings indicate that statin and fish oil synergistically offer benefits in reducing non-HDL-C compared with fish oil alone. High-dose eicosapentaenoic ethyl ester combined with baseline statins can lead to a remarkable decline in first and recurrent events in high-risk patients with hypertriglyceridemia.[18,19] However, patients with hyperlipidemia without baseline lipid-lowering medication may suffer from increased serum LDL-C levels and decreased HDL-C levels. A possible explanation for this synergy is that people who qualify for triglyceride-lowering trials, despite statin therapy, have hypertriglyceridemia that is differently affected by fish oil.[134] Further subgroup analyses demonstrated greater responses in patients with preexisting CHD and overweight/obesity when treated with ω3 PUFA, indicating fish oil's potential benefits in secondary prevention. However, the most recently completed RESPECT-EPA (Randomized Trial for Evaluation in Secondary Prevention Efficacy of Combination Therapy–Statin and Eicosapentaenoic Acid)[152] showed that among Japanese patients with chronic coronary artery disease treated with statin therapy, additional EPA may be associated with a minimal reduction in adverse cardiovascular outcomes (10.9% of the icosapent ethyl group versus 14.9% of the control group, P=0.055) after 6 years of follow-up.[153] DHA is biochemically and pharmacologically different from EPA in membrane incorporation, lipoprotein oxidation, and generation of specialized pro-resolving lipid mediators.[146,148] Although DHA/EPA as individual fatty acids revealed a similar magnitude of decrease in triglyceride in our analysis, increases in LDL-C were significantly greater in participants treated with DHA alone than in those treated with EPA alone, which was consistent with previous synthesized results.[11] This may explain why EPA + DHA combination treatment in various trials did not demonstrate an effect on reducing cardiovascular risk.[23–25,133] However, the available studies of DHA and EPA monotherapy, especially for DHA (n=5), are limited, with many studies at an EPA dose of 1.8 g/d or below. The wide CIs in higher dose ranges lead to unstable models, which would warrant more high-dose monotherapy studies in the future.

Our current dose–response analyses recommend taking more than 2 g/d ω3 PUFA from a pharmacokinetic perspective, securing the active substances absorbed to reach the systemic circulation or tissues, such as the cell membrane. Clinical trials revealed that ω3 PUFA supplementation of <1 g/d resulted in a very limited reduction in atherosclerotic CVD risk of major vascular events and CVD-caused deaths.[22,23,133,154] Conversely, patients with hypertriglyceridemia treated with a medium-to-high dose of icosapent ethyl were less likely to develop ischemic events, including CVD death.[18,19] However, taking into account the selection of the target population with a higher level of ω3 PUFA in JELIS[26] or the use of mineral oil as a comparator in REDUCE-IT,[27] we still need more conclusive evidence from well-designed trials to examine the potency of ω3 PUFA supplementation to prevent cardiovascular events.

Exposure and outcome measurements play a critical role in the estimation of valid causal relationships. We used a prestandardized protocol for dose intake (exposure level) in our data extraction process, excluding trials of DHA/EPA supplementation through diet, where the exposure level was hardly determined by the accurate fraction of pure DHA/EPA amount over the food consumed daily. Exposure levels were examined from 3 different perspectives: total combined doses of DHA + EPA, individual use of DHA/EPA (monotherapy), and separate doses. To precisely reflect the exposure level, we further included the achieved omega-3 index change in the RBC membrane. The outcome measurement was also taken into account in our risk of bias assessment. All included trials have

demonstrated detailed measurement protocols (such as automatic biochemistry measurement and standardized staff training, etc.) to obtain stable lipid profile readouts, though some of these studies were not designed to test the effect on lipids as the primary outcome. Intrinsically significant variations among original trials, such as the device for lipid measurement and the year of study (conducted 1990–2022), are likely to bring some uncertainty to our results and potentially weaken the conclusion. Although we attempted to examine the influence of these factors on our overall findings in subgroup analyses, we acknowledge that it is not possible to account for this heterogeneity directly in our analyses. The overall risk of bias did not divert from our expectations.

There are several limitations. First, the current study was carried out with study-level data but not individual data. This weakness may be compensated for by 1-stage methods that allow for the estimation of a nonlinear trend that accounts for the correlation between studies. Another effort was made by subgrouping strategies, considering the status of hyperlipidemia (with or without lipid-lowering medications), overweight/obesity, CHD, age, and duration. Second, we did not consider the influence of diabetes and metabolic syndrome on the lipid profile as possible cofounders. Unlike meta-regression analysis, the 1-stage dose–response could not handle multivariate or network analyses. Third, our current study was limited to the dose–response relationship between DHA/EPA supplementation and serum lipid changes. We did not perform further analyses to reveal whether changes in lipid profiles would result in a reduction in end point risk. We did not explain why comparable associations were evident for EPA- and DHA-only to lower serum triglyceride, but purified high-dose EPA had generally shown more robust benefits compared with mixed EPA + DHA in cardiovascular event trials. The mechanisms for end point prevention appear to be attributed to the pleiotropic effects in addition to serum lipid regulation.[38,148] Fourth, intrinsically significant data sparsity in the original trials might bring some uncertainty to our results and potentially weaken the conclusion. For example, because of a limited number of studies, a wider CI in a higher dose range is very evident, and the discrepancy between EPA and DHA is still unclear. Future well-designed studies with an appropriate comparator/placebo and population selection examining DHA/EPA-only effects should further investigate these issues.

CONCLUSIONS

The use of the new model reveals a nearly linear response at doses greater than 2 g/d of DHA + EPA supplementation in overall and subgroup analyses in the performance of triglyceride and non-HDL-C reduction. Individuals who are at high risk for developing CVD, such as those with hyperlipidemia and overweight/obesity, may be more responsive to the beneficial impacts of ω3 PUFA. This research helps improve our understanding of the moderate effects of omega-3 fatty acids on lipid reduction and CVD prevention.

ARTICLE INFORMATION

Received January 14, 2023; accepted April 3, 2023.

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Acknowledgments

The authors sincerely thank Edward Li for his diligent proofreading of this article.

Sources of Funding

This work was supported by the Macau Science and Technology Development Fund (FDCT) (0123/2020/A and 0053/2021/A1) and the Faculty Research Grants of Macau University of Science and Technology (FRG-21-038-SP).

Disclosures

None.

Supplemental Material

Data S1

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