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Systematic Review

Fish Oil Supplementation and Circulating Lipoprotein (a) Levels: A Grade-Assessed Systematic Review and Dose-Response Meta-Analysis of 20 Randomized Controlled Trials

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Abstract

Objectives: Although fish oil plays an important role in the prevention of cardiovascular diseases, the results of clinical trial studies regarding its effect on lipoprotein (a) [Lp(a)] concentrations factor are inconsistent. Therefore, we performed a meta-analysis of available randomized controlled trials (RCTs) to elucidate the efficacy of fish oil on plasma Lp(a) concentrations.

Methods: Net changes in Lp(a) were used to calculate the effect size, which was reported as a weighted mean difference (WMD) and 95% confidence intervals (CIs). The inclusion criteria were adult participants and an intervention duration of ≥ 2 weeks. In the primary search, 340 articles were found, of which 37 were assessed in full text and 20 trials (1356 participants) were included in the meta-analysis.

Results: The meta-analysis indicated a significant reduction in plasma Lp(a) levels (WMD: -1.96 mg/L, 95% CI: -3.63 to -0.30; P = 0.021) following fish oil supplementation. In the sub-group analysis, a significant reduction was observed only at doses < 3 g/day (WMD: -7.35 mg/L, 95% CI: -13.80, -0.89, p = 0.026), and with treatment duration ≥ 12 weeks (WMD: -2.56 mg/L, 95% CI: -4.67, -0.45, p = 0.017). A dose-response analysis revealed that < 3 g/day of fish oil largely decreased Lp(a) levels (P-nonlinearity = 0.006).

Conclusion: The current evidence from RCTs showed that fish oil supplementation may significantly reduce Lp(a) levels at doses lower than 3 g/day when the supplementation lasts over 12 weeks. Further well-constructed randomized clinical trials are needed.

Keywords: *Fish Oil; Omega-3 Fatty Acid; Lipoprotein (a)*

Introduction

The atherogenicity of lipoprotein (a) [Lp(a)] has been shown in previous studies [1,2], and considerable evidence shows a relationship between high Lp(a) concentrations and increased risk of inflammation [3,4], stroke [5], coronary artery disease [6,7], myocardial

infarction [8], aortic valve calcification [9], and venous thromboembolism [10]. It has been shown that its levels are highly influenced by genetics [11] and vary among different populations with higher levels seen in blacks than in whites [12,13]. There are several mechanisms for the relationship between this lipoprotein and the increased risk of cardiovascular diseases including: binding to the extracellular matrix of vessels [14], the development of atherosclerosis plaques [15], and binding to oxidized phospholipids [16]. Unlike other plasma lipoproteins, the metabolism of this lipoprotein is not well known, and it also seems that due to genetic dependence, the effect of dietary interventions on it is insignificant. Furthermore, despite considerable research to find targeted therapies, the results have been disappointing [10]. Although Lp(a) levels are genetically regulated, pharmacological dose of niacin or nicotinic acid (1 - 3 g/day has been investigated as possible agent for the reduction of Lp(a) concentrations [17].

Although in epidemiological studies, an inverse relationship has been observed between the intake of a diet rich in fish and Lp(a) concentration [18], the mechanisms linking high fish intake and lower Lp(a) levels in plasma are unclear, and it seems that omega-3 fatty acids can reduce its the secretion and synthesis from the liver [19] as well as its catabolism or result in decreased assembly of apo(a) with LDL [20]. Nevertheless, available findings in humans are limited and inconclusive and there is an inconsistency between human clinical studies investigating the effects of fish-oil supplementation on Lp(a) levels [21,22]. Considering the controversial clinical findings, and due to the variable duration of studies, distinct study designs, and a diverse population with different sample sizes, it is difficult to draw an exact conclusion regarding the effect of fish oil on Lp(a) levels. To date, the current study is the first meta-analysis to assess this effect. In addition, in this meta-analysis, dose-response analysis was used to better and more clearly observe the effective dose and duration of fish oil intervention on Lp(a) levels. Hence, a systematic review and meta-analysis of available controlled trials seem appropriate to summarize the current data to assess the overall effect of fish oil intervention on Lp(a) levels in adults.

Methods

Search strategy

PRISMA guidelines were used to design the current meta-analysis [23]. PubMed, ISI Web of Sciences and SCOPUS databases were searched for reports of relevant RCTs published until 20th February 2022 that explored the influence of ginger on glucose control and insulin sensitivity using the following the MeSH terms and related keywords: ("fatty acids, omega 3"[MeSH Terms] OR "Fish Oils"[MeSH Terms] OR "fish-oil"[Title/Abstract] OR "fish- oil"[Title/Abstract] OR "omega-3 fatty acids"[Title/Abstract] OR "n-3 fatty acids"[Title/Abstract] OR "n-3 polyunsaturated fatty acids"[Title/Abstract] OR "polyunsaturated fatty acids"[Title/Abstract] OR "eicosapentaenoic acid"[Title/Abstract] OR "docosahexaenoic acid"[Title/Abstract] OR "EPA"[Title/Abstract] OR "DHA"[Title/Abstract]) AND ("lipoprotein a"[MeSH Terms] OR "lipoprotein a"[Title/Abstract] OR "lp a"[Title/Abstract] OR "lp a"[Title/Abstract]). References of all articles included in the meta-analysis, as well as all related meta-analysis and review articles, were hand-searched to identify other relevant studies. N.R, and S.R separately screened titles and abstracts to find potentially relevant studies using predetermined selection criteria to determine eligibility. In the case of any dissension M.MS was consulted.

Study selection

Two independent investigators (M.A and S.R) reviewed the titles and abstracts of all identified studies based on PICO (population, intervention, comparator and outcome) to ascertain whether these studies are eligible for our meta-analysis based on inclusion criteria. Studies for the analysis were selected according to the following criteria: 1) a randomized controlled trial (RCT) with either parallel or crossover design in adults (age ≥ 18 years old); 2) the effects of the intervention on Lp(a) levels could be extracted from the article, i.e. presentation of sufficient information including standard deviation (SDs), standard error (SEs), or 95% CIs were available at baseline and at the end of the trial in both intervention and control group or any other information from which the calculation of mean and SD change was possible); and 3) having an intervention duration of at least 2 weeks. Studies were excluded if 1) we couldn't extract the net effect of the fish oil intervention (if fish oil was supplemented as an adjunct to another supplement, the control group containing that supplement); 2) fish oil consumption duration was < 2 wk; 3) non-RCTs including animal or observational studies with case-control,

cross-sectional, or cohort design; 4) the study lacked adequate information on the baseline or follow-up of Lp(a) levels, and 5) duplicate publication from the same study.

Data extraction

To increase accuracy in data extraction, the data of studies that fulfilled inclusion criteria were extracted separately (N.R, and S.R), according to a predefined extraction form as follows and any discrepancy was resolved by discussion and consensus and in the case in which they did not reach a definite conclusion M.MS was involved: The mean changes with corresponding SDs of the Lp(a) levels in the fish oil and control group. the first names of the authors, year of publication, the location of study, study method, duration of the study, health condition, sample size, the ginger dose utilized, type of fish oil and placebo, and other information including age, sex, and body mass index (BMI).

Quality assessment

The Cochrane collaboration's tool was used to assess for the risk of bias [24], which has several domains for assessment of the risk of bias in the included studies by two independent reviewers (F.T. and N. R.). These domains include the following: adequacy of random sequence generation, allocation concealment, blinding, blinding of outcome assessment, addressing of dropouts, selective outcome reporting, and other potential sources of bias. "Low," "High" or "Unclear" terms were applied to describe each domain. The robustness of evidence for each chosen outcome was graded according to the guidelines of the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) Working Group [25] and divided into 4 categories according to the corresponding evaluation criteria: high, moderate, low, and very low [26].

Quantitative data synthesis and statistical analysis

The mean change of Lp(a) was calculated as follows: measure at end of follow-up - measure at baseline. The mean change in Lp(a) between the intervention (fish oil intervention) and placebo or control groups used as the effect sizes in the meta-analysis. The effect size reported as Weighted mean differences (WMDs) and their corresponding SDs. The SD of the mean change was calculated using the following formula: [SD = square root [(SD pre-treatment)^2 + (SD post- treatment)^2 - (2R × SD pre-treatment × SD post-treatment)], in which a correlation coefficient (R) of 0.5 was assumed [27]. In studies that used SE instead of SD, the following formula was used to calculate SD, where n is the number of subjects: SD = SE × square root (n). The Hozo., *et al*. [28] method was used for the estimation of mean and SD values if outcomes of interest were reported in median and range. To calculate the heterogeneity between the studies, Cochran's Q- test and the I^2 test were used, values ≥ 50% (or $p < 0.05$) indicate the significance of the heterogeneity. The random effect model was used to report the results because there are often too many differences in the study designs, populations, comparison, etc. A sensitivity analysis was performed using the leave-one-out method [29]. The subgroup analyses were done for a dose of supplementation, intervention duration, and baseline Lp(a) levels, to explore the possible effect of these covariates on net effect size as well as for identifying the potential source of heterogeneity. Linear meta-regression was performed using the unrestricted maximum likelihood method to investigate the association between effect size and covariates such as dose and duration of intervention with fish oil. We explored the daily dose and duration of fish oil intervention for Lp(a) levels by dose-response analysis in non-linear manner. The overall quality of evidence for Lp(a) levels was assessed by the GRADE evidence profiles. Publication bias was evaluated using funnel plot asymmetry as well as Begg's rank correlation test in combination with Egger's weighted regression test [30]. The analyses were done using Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ) [31]. Dose response analysis was done with STATA software (version 17).

Results

Results of the literature search

The flowchart of the literature search is shown in figure 1. 344 articles were found in the initial comprehensive search. After removing duplicate articles, the remaining 180 articles were screened based on the title and abstract. After completing evaluations based on inclu-

sion criteria, 37 articles were eligible for full-text assessment. Among the full text articles evaluated, 17 studies were excluded for the following reasons: lack of control group (n = 2), not randomized placebo-controlled studies (n = 7), and not Lp(a) measurements performed (n = 8). Finally, 20 RCTs were included in the meta-analyses [20,22,32-42].

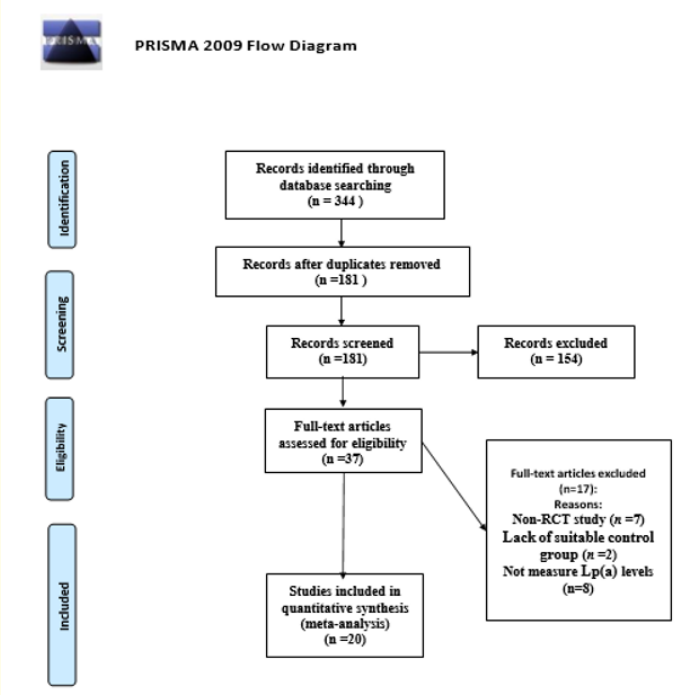


Figure 1: *Flow diagram of the study selection procedure showing the number of eligible randomized controlled trials for the meta-analysis of the effect of fish oil supplementation on Lp (a).*

Source: From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta- Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit www.prisma-statement.org.

Study characteristics

The characteristics of study that met all inclusion criteria of the meta-analysis are presented in table 1. In total, 1356 participants were randomized, of whom 691 were assigned to the fish oil supplementation group and 665 to the control group. The number of participants in these trials ranged from 16 to 549. Included studies were published between 1994 and 2020. A range of doses from 1 to 12 g of fish oil was administered in the included trials. Duration of fish oil supplementation ranged from 3 week to 24 weeks.

Data quality

Of 20 included studies, three trials had a low risk of bias, and from the 17 remaining articles, 11 trials were of low quality (high risk of bias) and eight RCTs had a moderate risk of bias. Details of the quality of bias assessment are shown in table 2. The overall quality of evidence Lp(a) is moderate by the GRADE evidence profiles.

Derosa	Conquer	Beavers	Beil	Bowden	**Study**
2009	1995	2009	1999	2009	**Year**
Italy	Canada	USA	Germany	Netherlands	**Country**
51.3/50.7	29.5/29.5	57/64	46/52	57.2/64.3	**Age (year) F/P**
Multi-center, case-control, placebo, randomized trial	A randomized double-blind study	Randomized double-blind study	Randomized double-blind study	Double-blind, permuted- randomized, and placebo- controlled experimental protocol	**Study Design**
326 F: 164 P: 162	20 F: 10 P: 10	33 18/15	30 F: 15 P: 15	33 F: 18 P: 15	**Sample size**
Caucasian patient with combined dyslipidemia (>18)	Healthy male (> 20y)	End-Stage Renal Disease	Patients with primary hypertriglyceridemia	End-Stage Renal Disease	**Health Status**
24	6	24	6	24	**Duration (Weeks)**
3g/d ethylic esters, EPA and DHA in the proportion of0.9 – 1.5), (3 time/d during meal)	4.3g omega3 (20 seal-oil capsule/ day: tota,1.3g EPA, 1.7g DHA, .8g DPA)	Fish-oil soft gels were packaged in a 1-g capsule that contained 160 mg of EPA and 100 mg of DHA, and 0.9 IU of d-a tocopherol as an antioxidant	3.15g n-3 fatty acids in 10.5g fish oil	2 soft-gel pills (1g each) of ω-3 supplements at each meal (3 times per 24 hours) for daily totals of 960 mg of EPA and 600 mg of DHA	**Intervention**
Sucrose, mannitol and mineral salt	Vegetable oil (evening primrose oil)	Corn oil	Oleic acid	Corn Oil	**Control**
91/93	209.2/162.8	360/320	208/183	288.8/312.3	**Baseline Lp(a) (mg/L)**
26.2/25.9	26.4/25	NR	27/26	NR	**BMI (kg/m^2)**

Herrmann	Faghihi	Eritsland	Engstrom	Dawczynski
1995	2012	1995	2003	2009
Germany	Iran	Norway	Sweden	Germany
53.9/53.9	31.1/36.6	59.9	39/42	57·9
Double-blind trial	Randomized, double-blind, placebo- controlled, within-subject trial	Randomized, controlled study	A randomized double-blind repeated measures experiment	Double-blind, placebo- controlled cross-over study
53 F:35 P:18	41 F: 20 P: 21	549 F: 280 P: 269	16 F: 8 P: 8	21 F: 21 P; 21
Ischemic heart disease	Schizophreni a, bipolar I (18-60y)	Patients with coronary artery disease, undergoing bypass operations	Healthy, nonsmoking subjects (26 - 65y)	Rheumatoid arthritis
4	6	24	3	12
12g/d (4capsule 3time/d) 8.5 N-3 fatty acid	< 1 g/day EPA/DHA	4 g of an n-3 PUFA concentrate (containing > 85% of long chain n-3 PUFAs)	18% consist of eicosapentaenoic acid (20:5, EPA) and 12% consist of docosahexaenoic acid	0.7g EPA, 0.1g DPA and 0.4g DHA, amounted to 2.4g
Olive oil		Not described just mentioned	Ordinary caviar paste	Commercial dairy products with comparable fat contents were used as a placebo
449.7/438.8	466.4/453.5	80/77	317/170	416/380
NR	25.95/26.28	NR	NR	NR

Sanders	Christensen JH	Qin	Prisco	Marckmann	Kooshki
1997	2004	2015	1994	1997	2011
England	Denmark	China	Italy	Denmark	Iran
23	60/64	46.0/44.3	32/32	NR	50/ 50
Randomized cross-over study	Randomized, controlled study	Double-blind, randomized clinical trial	Double-blind study	Parallel, randomized, and double-blind	Double-blind, placebo-controlled trial
F: 26 P: 26	58 F: 28 P: 30	70 F:36 P:34	20 F: 10 P: 10	47 F: 24 P: 23	34 F: 17 P:17
Healthy male	Patients with chronic renal failure	Nonalcoholic fatty liver disease	Healthy male (27- 41y)	Healthy Men	Hemodialysis Patients
3	8	12	16	4	10
1.5% of energy from polyunsaturated fatty acids was supplied as eicosapentaenoic (EPA; 20:5n-5) and docosahexaenoic (DHA; 22:6n-3) acid (approximately 5 g/day)	2.4g of n-3 PUFAs (4 capsules of fish oil)	4g of fish oil capsules, containing a total of 728 mg of EPA and 516 mg of DHA	4 g/d EPA and DHA ethyl stress (total dose was 2.04g EPA and 1.4g DHA)	Fish oil (4 g daily)	2080 mg marine omega-3 fatty acids as four capsules (310 mg eicosapentaenoic acid and 210 mg docosahexaenoic acid)
Linoleic acid (approximately 5 g/ day)	Olive oil	Corn oil capsules	Olive oil	Sunflower oil	Medium chain triglycerides oil
109/99	340/260	168/162	102/110	36/76	302/196
22.8	28/28	26.4/ 26.0	NR	NR	19.5/20

Ying	Yuan	Wohl	Svensson	Svensson	Shearer
2022	2020	2005	2008	2004	2012
Australia	China	USA	Denmark	Denmark	USA
60	58.5/64.8	42/45	66/	60/58	44/45
A randomized, crossover trial	a randomized, controlled trial	A randomized, controlled trial	Double-blind randomized placebo-controlled design	Randomized, placebo- controlled cross-over study	Double-blind randomized placebo- controlled design
20 20/20	60 F: 30 C: 30	52 F: 26 P: 26	206 F: 103 P: 103	58 F: 29 P: 29	60 F: 17 P: 15
Familial hypercholesterolemia	Patients with acute myocardial infarction	Hypertriglyceridemia	Patients treated with chronic hemodialysis	Patients with chronic renal failure	Metabolic syndrome
8	12	16	12	8	16
4 g/day ω3FA supplementation (Omacor® 46% EPA and 38% DHA in ethyl ester form	1g ω-3 PUFA (180-200 mg/g EPA, 178 120-140 mg/g DHA	Omega-3 fatty acid supplementation consisting of 1750 mg of eicosapentaenoic acid and 1150 mg of docosahexaenoic acid	1.7g of n-3 PUFA	2.4g of n-3 PUFAs (4 capsules of fish oil)	4.3g omega3 fatty acid
No ω-3FA treatment	Guideline-adjusted therapy alone	Without fish oil supplementation	Olive oil	Olive oil	Placebo
420/440	848.3/508.7	110/120	174/254	340/260	49/73
27/27	25.2/25.2	27.4/27.2	24.7/24	28/28	34/30

Table 1: *Demographic characteristics of the included studies.*

Group	Number of studies	Net Change (95% CI)	p	P-heterogeneity	I^2 (%)
Total	13	-2.91, 95% CI: -5.52 to - 0.30	0.028	< 0.001	86.45
Baseline Lp(a)	-	-	-	-	-
≥ 300 mg/L	6	-1.23, 95%CI: -1.87 to -0.58	p < 0.001	< 0.001	92.37
< 300 mg/L	7	0.05, 95% CI: -0.40, 0.52	p = 0.816	0.98	0.00
Intervention duration					
≥ 12 weeks	7	0.07, 95% CI: -0.31, -0.46	p = 0.713	0.97	0.00
<12 weeks	6	-8.36, 95% CI: -9.98, -6.74	p < 0.001	< 0.001	90.97

Table 2: *Pooled estimates of effects on Lp(a) within various subgroups.*

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of out-come assessment	Incomplete outcome data	Selective outcome reporting	Other potential threats to validity	General risk of bias
Beavers, 2009	L	H	L	H	L	L	H	H
Beil, 1991	U	H	L	H	L	L	H	H
Bowden, 2015	L	U	L	H	L	L	H	M
Dawczynski, 2009	U	H	L	H	L	L	L	M
Derosa, 2009	L	U	L	H	L	L	U	L
Eristland, 1995	U	H	H	H	L	L	H	H
Faghihi, 2012	L	H	L	H	L	L	U	M
Herrmann, 1995	U	H	L	H	L	L	H	H
Engstorm, 2003	L	H	L	H	L	L	U	M
Kooshki, 2011	U	H	L	H	L	L	L	M
Marckmann, 1997	U	H	L	H	L	L	H	H
Svensson, 2004	U	H	H	H	L	L	H	H
Prisco, 1994	U	H	L	H	L	L	H	H
Qin, 2015	L	U	L	H	L	L	U	L
Sanders, 1997	U	H	H	H	H	L	U	H
Shearer, 2012	L	U	L	H	L	L	H	M
Svensson, 2008	L	L	L	U	L	L	H	L
Swahn, 1998	L	H	L	H	L	L	L	M
Ying, 2022	U	H	H	H	H	L	H	H
Yuan, 2020	L	U	H	H	L	L	U	M

Table 3*. Quality assessment of clinical trials (according to the Cochrane guideline) investigating the associations between fish oil and Lp (a).*

L: low risk of bias; H: high risk of bias; U: unclear risk of bias

Effect of fish oil on circulating Lp(a) concentrations

Combined results from 20 studies with 22 treatment arms (691 cases and 665 controls), indicated a significant decrease in Lp(a) after fish oil supplementation (WMD: -1.96 mg/L, 95% CI: -3.63 to -0.30; P = 0.021) (Figure 2, upper plot). The results were reported using the random-effect model because significant heterogeneity was found (I^2 = 55.48%, p = 0.001). To evaluate each study's effect on the overall effect size we conducted a leave-one-out sensitivity analysis by removing each trial from the analysis step by step (Figure 2A). The estimated effect size for the fish oil's effect on plasma Lp(a) concentrations was robust, suggesting that removing each single trial did not change the results of the analysis (Figure 2B).

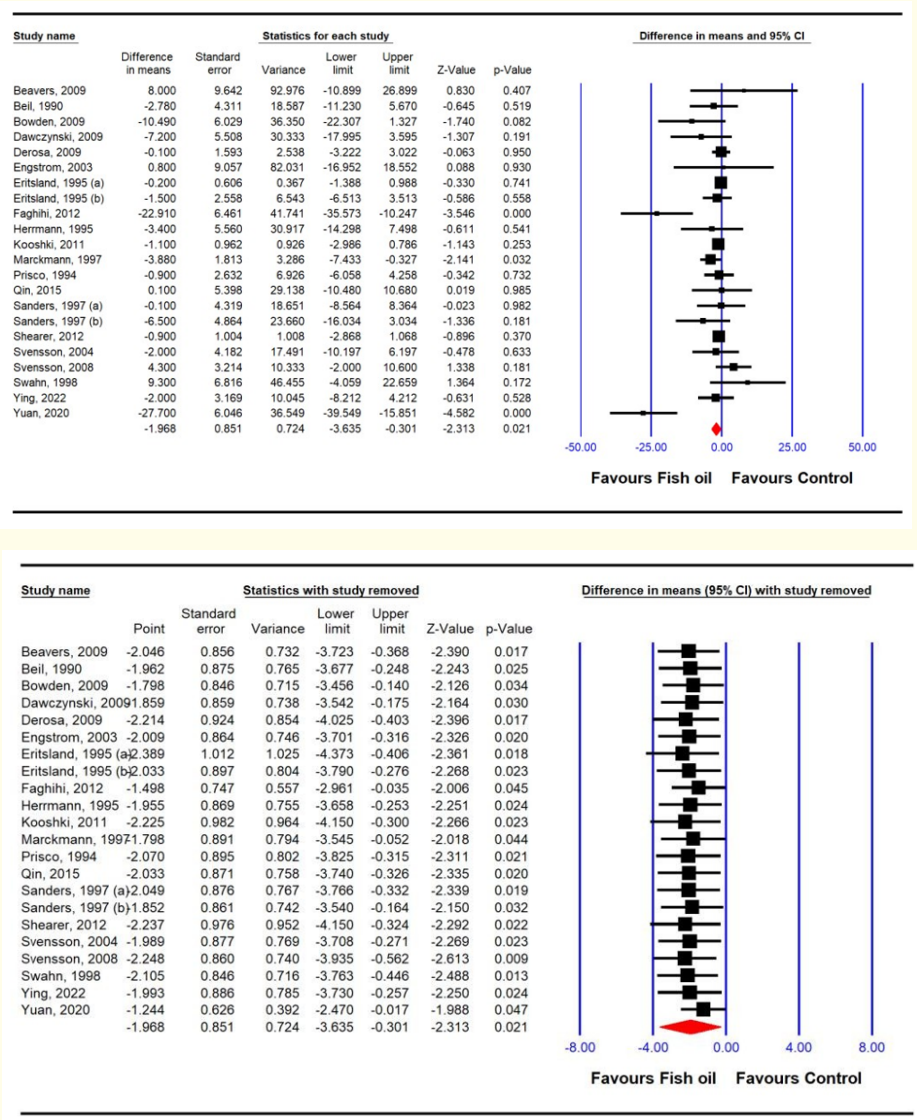


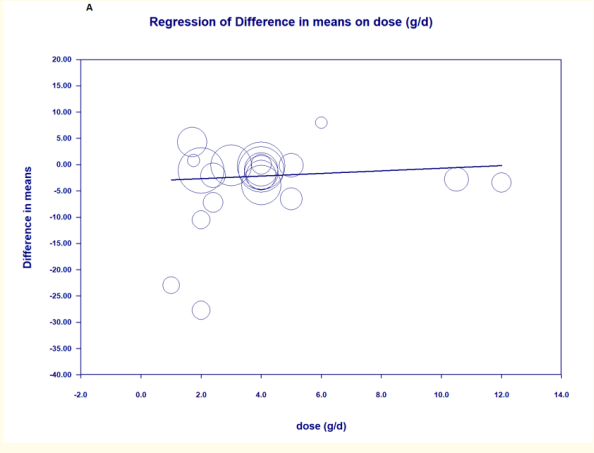
Figure 2: *Forest plot displaying weight mean difference and 95% confidence intervals for the impact of fish oil supplementation on Lp (a) concentrations (upper plot). Lower plot shows leave-one-out sensitivity analysis (lower plot).*

Subgroup analysis

When the studies were stratified according to the dose of fish oil supplementation, as either a high dose (≥ 3 g/d) or a low dose (< 3 g/d), significant reductions in Lp(a) were observed in subsets of studies with < 3 g/d of fish oil (WMD: -7.35 mg/L, 95% CI: -13.80, -0.89, p = 0.026), but not in those with ≥ 3 g/day (WMD: -0.68 mg/L, 95% CI: -1.55, 0.18, p = 0.123). There was also a greater reduction in plasma Lp(a) concentrations in the subset of trials with baseline Lp(a) values ≥ 300 mg/L (WMD: -1.23 mg/L, 95%CI: -1.87 to -0.58, p < 0.001) compared with the subset with baseline values < 300 mg/L (WMD: 0.05 mg/dL, 95% CI: -0.40, 0.52, p = 0.816). Subgroup analysis was also performed by dividing the duration of Lp(a) intervention (≥ 12 weeks versus < 12 weeks). Subgroup analysis indicated that fish oil consumption has a significant effect on reducing Lp(a) in long term (≥12 weeks) interventions (WMD: -3.05 mg/L, 95% CI: -5.56, - 0.55, p = 0.017) but not in those supplementing Lp(a) for <12 weeks (WMD: -1.26 mg/L, 95% CI: -3.59, 1.06, p = 0.286).

Meta-regression analysis

Weighted unrestricted maximum likelihood meta-regression analysis was performed to assess the impact of potential moderators on the pooled effect size. Potential associations between the Lp(a)-lowering effects of fish oil with dose, duration of supplementation and Lp(a) baseline were evaluated using meta-regression analysis. A significant invers association was found between the Lp(a)-lowering effect of fish oil and baseline Lp(a) values (slope: - 0.035; - 0.06 to -0.01; p = 0.004) (Figure 3A). The results also suggested that the pooled estimate is independent of fish oil dose (slope: 1.03; 95% CI: - 0.64, 2.72; p = 0.226), and duration of treatment (slope: 0.34; 95% CI: -0.15, 0.85; p = 0.178) (Figure 3B and 3C).



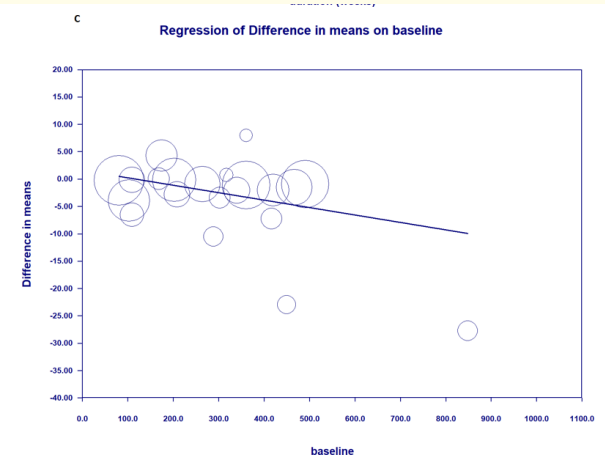


Figure 3: *Meta-regression plots of the association between mean changes in plasma Lp (a) concentrations with dose of supplementation, duration of supplementation and changes in baseline Lp (a) concentrations.*

Non-linear dose-response

The results of the non-linear dose-response analysis (Figure 4) showed a significant association between the fish oil dose with Lp(a) (P non-linearity = 0.006). Considering the dosage subgroup analysis and dose-response curve, a fish oil dose of lower than 3 g/day has better efficacy in improving Lp(a) levels. In addition, there were no significant non-linear associations between the duration of supplementation and Lp(a) change (P non-linearity = 0.244).

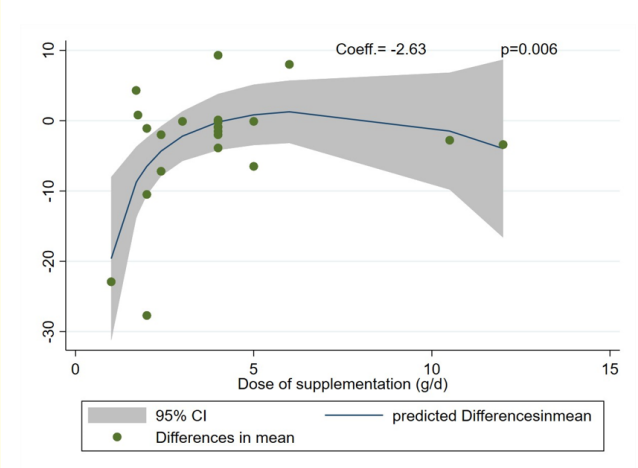


Figure 4: *The results of the non-linear dose-response analysis association between mean changes in plasma Lp (a) concentrations with dose of supplementation, and duration of supplementation.*

Publication bias

Visual inspection of funnel plot didn't suggest a significant potential publication bias in the meta-analysis of fish oil effect on plasma Lp(a) concentrations (Figure 5). This observation was confirmed by the results of Begg's rank correlation test (Kendall's Tau with continuity correction = -0.199, z = 1.29, two-tailed p-value = 0.194), and Egger's linear regression test (intercept = -0.80, standard error = 0.43; 95% CI = -1.71, 0.10, t = 1.84, df = 20, two-tailed p = 0.079). An attempt was made to adjust the effect size by imputing potentially missing studies using "trim and fill" correction. This approach led to the imputation of 1 missing trials on the left side of funnel plot, yielding a corrected effect size of -2.16 mg/L (95% CI: -3.86 to -0.47). The "fail-safe N" test showed that 52 studies would be needed to bring the effect size down to a non- significant (p > 0.05) value.

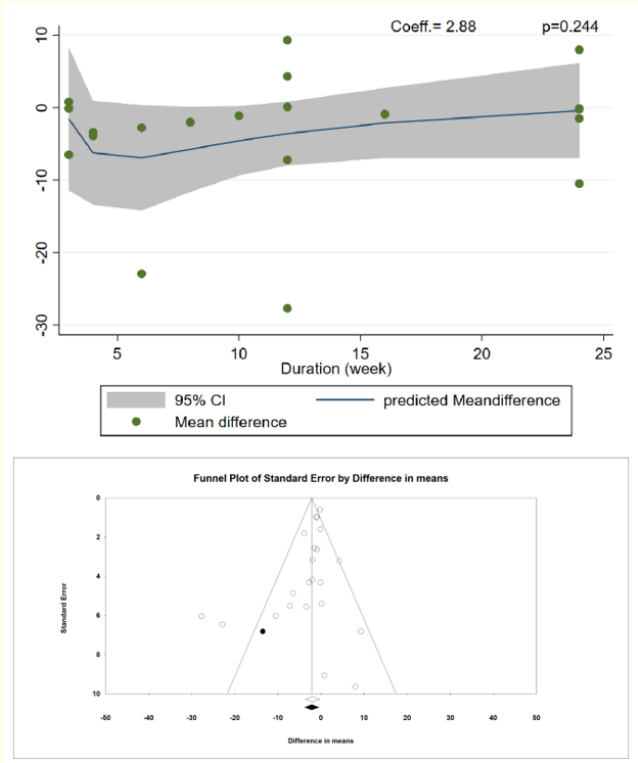


Figure 5: *Funnel plot displaying publication bias in the studies reporting the impact of fish oil supplementation on Lp (a) concentrations.*

Discussion

In the current meta-analysis, which for the first time summarized the effect of existing clinical trials regarding the effect of fish oil supplementation on plasma Lp(a) concentrations, a significant lowering effect of fish oil supplementation on plasma Lp(a) levels was observed. Considering the dosage subgroup analysis and dose-response analysis, a fish oil dose of lower than 3 g/day has better efficacy in improving Lp(a) levels. In addition, sub-group analyses revealed that Lp(a) levels were reduced in trials lasting ≥ 12 weeks, and among participants with baseline Lp (a) levels ≥ 300 mg/dL.

High plasma level of Lp(a) have been recognized as an independent risk factor for coronary heart disease [43]. Although Lp(a) level is considered to be largely under genetic control, reduction of the its concentration would be of great clinical interest. Lp(a) serum levels may also be influenced by some exogenous factors including fish oil consumption [21]. Several studies have investigated the effect of fish oil supplementation on Lp(a) levels, and the results of these studies are contradictory; some of these studies have observed slight or no effect [44-46], and others reporting significant effect [35,47] (as in the current study). Given the contradictions in the literature, when we reviewing the relevant study designs, believe that such inconsistencies may be related to different dosages, duration of treatment (varied from 3 weeks to 6 months), populations assessed, variability in its measurements as well as large changes in its baseline levels (varied from 80 mg/L to 466.4 mg/L in the fish oil group), as we observed considerable heterogeneity across studies.

Although our findings concur with a results from double-blind controlled trial [36], there is stronger evidence in epidemiological studies in relation to the consumption of a diet rich in fish and Lp(a). In the study of Marcinova., *et al*. [18] a genetically controlled population based study, The researchers investigated the lipoprotein levels in two populations consuming a diet containing fish (n = 622), and a vegetarian diet (n = 686) in two populations in Tanzania. The hypothesis of the researchers of this study was based on that the any observed difference in Lp(a) level between the two groups must be due to the differences in diet composition, because they are very similar in terms of genetic and environmental characteristics except for dietary intake. The result showed a 48% difference between groups in plasma Lp(a) levels ([27 mg/dL] in the vegetarian group, and [14 mg/dL] in the fish diet group; P < 0001). Since the change in apo(a) size influence Lp(a) levels, both groups were matched in terms of apo(a) phenotypes; however Lp(a) values were still found to be 40% lower in the fish population than in the vegetarian population. This well-designed study, which controlled genetic variation in Lp(a) levels strongly proposed that diets high in fish can be beneficial in Lp(a) reduction.

The mechanisms by which omega-3 fatty acids exert their effect on Lp(a) are not clear, but according to previous studies some biological plausibility exists for the potential Lp(a)-lowering role of omega-3 fatty acids may affect Lp(a) expression [21] and can reduce the formation linkage between apolipoprotein (a) and LDL- C particle. Furthermore, decrease in triglyceride levels [48], direct entry of omega-3 fatty acids in the portal vein system, decreasing the secretion and synthesis Lp (a) from the liver [19] and increasing Lp(a) catabolism [20] also been suggested as a mechanisms of fish oil-induced decrease in lipoprotein(a). In addition, it has been shown that inflammation process increases circulating levels of Lp(a) [49,50]. An important pro- inflammatory cytokine, interlukin-6 (IL-6), has a causal relevance with the regulation of Lp(a) metabolism. Therefore, inhibition of IL-6 signaling decreases expression, synthesis and serum levels of Lp(a) [51]. In fact fish oil has an anti-inflammatory effect [52,53], probably the decrease in Lp(a) level can be attributed to the anti-inflammatory effects of omega-3 fatty acids. This explanation remains however elusive and need specific demonstration.

In concur with our finding, Schmidt E. Berg., *et al*. [45] showed that subjects with higher initial Lp(a) values, significantly have a better response to omega-3 fatty acids. This observation is of interest and should be tested in larger groups of persons and high-quality trials. In addition, it has been reported that the oxidized low-density lipoprotein (oxLDL) concentration is positively correlated with the Lp(a) concentration [54], and modest doses of fish-oil is more favorable on LDL cholesterol or LDL apolipoprotein B levels than large dose ones [55]. Fish oil consumption in large however, can elevate LDL-cholesterol and apoB levels [56,57]. It should be noted that according to the Food and Drug Administration, consumption of more than 3 g/day of omega-3 fatty acids is not safe [58].

The present study also has several weaknesses, and the findings should be interpreted with caution. The qualified RCTs generally had small or modest populations (six study lower than 50 patients). In addition, the included studies were heterogeneous concerning the baseline Lp(a), sample size, fish oil dose administration, duration of treatment and Lp (a) baseline. Due to the limitation of the data, we also were not able to perform further analysis, especially the relationship between the levels of omega-3 fatty acids and Lp (a) levels. Furthermore, the quality of the included studies varied from low to high, with only three studies found to be of high quality (low risk of bias). We also could not find any study, exploring the effect of fish oil supplementation among different genders; therefore, the difference

between genders about the magnitude of fish oil supplementation could not be addressed in the current meta-analysis and should be considered in future studies.

Conclusion

In conclusion, this meta-analysis of available randomized controlled trials suggested a significant benefit of fish oil supplementation in reducing plasma Lp (a) levels that is more evident with increasing Lp(a) values. Finally, the causal conclusion remains to be re-evaluated by more high- quality, large scale and well-designed RCTs, and the reliability of this finding should be considered with caution.

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The authors' responsibilities were as follows: H.S conceived the study. SH.B carried out the literature search. S.R and F.T carried out data extraction and independent reviewing. F.T and S.R conducted the quality of included studies. M.MS conducted data analysis. Sh.B and M.A and NB wrote the manuscript. N.B conducted critical revision. All authors approved the final manuscript.

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Conflict of Interests

The author declares no competing interests.

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