

RESEARCH ARTICLE

Effect of Marine-Derived n-3 Polyunsaturated Fatty Acids on Major Eicosanoids: A Systematic Review and Meta-Analysis from 18 Randomized Controlled Trials

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Abstract

Background

Marine-derived n-3 polyunsaturated fatty acids (PUFA) may have a beneficial effect on inflammation via lowering pro-inflammatory eicosanoid concentrations. We aimed to assess the effect of marine-derived n-3 PUFA on prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), and leukotriene B₄ (LTB₄) through systematic review and meta-analysis of randomized controlled trials.

Method and Findings

A structured search strategy on PubMed, Web of Science and Cochrane up to November 2015 was undertaken in this meta-analysis. Standard mean difference was used to calculate the effect size of marine-derived n-3 PUFA on PGE₂, TXB₂ and LTB₄ in a random-effect model. A total of 18 RCTs with 826 subjects were included in this systematic review and meta-analysis. Supplementation of marine-derived n-3 PUFA significantly decreased concentrations of TXB₂ in serum/plasma in subjects with high risk of cardiovascular diseases (SMD: -1.26; 95% CI: -1.65, -0.86) and LTB₄ in neutrophils in unhealthy subjects (subjects with non-autoimmune chronic diseases or auto-immune diseases) (SMD: -0.59; 95% CI: -1.02, -0.16). Subgroup analyses showed a significant reduction of LTB₄ in subjects with rheumatoid arthritis (SMD: -0.83; 95% CI: -1.37, -0.29), but not in non-autoimmune chronic disease patients (SMD: -0.33; 95% CI: -0.97, 0.31). No significant publication bias was shown in the meta-analysis.

Conclusions

Marine-derived n-3 PUFA had a beneficial effect on reducing the concentration of TXB₂ in blood of subjects with high risk of CVD as well as LTB₄ in neutrophils in unhealthy subjects, and that subjects with RA showed lower LTB₄ content with supplementation of marine-derived n-3 PUFA.

Introduction

Previous studies have shown that inflammation plays a significant role in a number of widespread and destructive chronic diseases, including autoimmune diseases such as rheumatoid arthritis (RA) [1] and non-autoimmune chronic diseases including obesity and insulin resistance [2], cardiovascular disease (CVD) [3] and several neurodegenerative diseases such as Alzheimer's disease [4]. Several arachidonic acid (AA) -derived eicosanoids exert their significant influence on the inflammatory response. Prostaglandin E₂ (PGE₂) is involved in the classic signs of inflammation and possesses both pro-inflammatory and anti-inflammatory actions [5]; thromboxane A₂ (TXA₂) (precursor of TXB₂), formed by platelets, macrophages and polymorphonuclear leukocytes (PMNs), can induce vasoconstriction and promotes aggregation of platelets as well as adhesiveness of PMNs [6]; leukotriene B₄ (LTB₄) can not only increase vascular permeability and enhance local blood flow by stimulating neutrophil secretion [7], but also stimulate other inflammatory substances.

Previous studies have shown that increased intake of n-3 polyunsaturated fatty acids (PUFA), especially marine-derived n-3 PUFAs (eicosapentaenoic acid [EPA] docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]), are beneficial for coronary heart disease [8], metabolic syndrome [9] and Alzheimer's disease [10]. Previous evidence has suggested that the positive protective impact of n-3 PUFA on these diseases may be attributed to the anti-inflammatory function, including lowering of blood eicosanoids [11, 12]. However, the effects of n-3 PUFA on AA-derived major eicosanoids still remains controversial: several studies suggested that marine-derived n-3 PUFA resulted in decreased PGE₂ [13], TXB₂ [14] and LTB₄ [15], while increased PGE₂ [16] and TXB₂ [17] in response to n-3 PUFA were also found in some other studies. There has been no systematic review and meta-analysis conducted to summarize the available evidence of the effects of n-3 PUFA on major eicosanoids. Therefore, we conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) to assess the effect of marine-derived n-3 PUFA on AA-derived major eicosanoids (PGE₂, TXA₂/TXB₂ and LTB₄).

Materials and Methods

Search strategy and study selection

To identify randomized controlled studies involving the effects of marine-derived n-3 polyunsaturated fatty acids on major AA-derived eicosanoids (PGE₂, TXA₂/TXB₂, and LTB₄), we searched three electronic databases, PubMed, Web of Science and Cochrane Library, up to November 2015 for studies of humans published in all languages. We used the following key words for the literature search: ("fish oil" OR "seafood" OR "eicosapentaenoic acid" OR "docosahexaenoic acid" OR "docosapentaenoic acid" OR "n-3 PUFA") AND ("eicosanoids" OR "prostaglandin" OR "thromboxane" OR "leukotriene"). References of included studies were also reviewed to identify potential publications. We contacted authors for the detailed information of primary studies when possible.

Search strategy and study selection were undertaken independently by two investigators, with discrepancies resolved through group discussion. Inclusion criteria were: randomized controlled trial design including parallel and crossover design; the exposure of interest was marine-derived n-3 PUFA such as EPA and/or DHA on PGE₂ or TXB₂ or LTB₄; LTB₄ was determined in neutrophils by HPLC and PGE₂ and TXB₂ were measured by Radioimmunoassay or ELISA (enzyme-linked-immunosorbent assay). Studies were excluded if: subjects were diagnosed with acute disease or the duration was less than two weeks; there was dietary intervention such as marine fish or others; there were other additional interventions apart from n-3

PUFA such as exercise and anti-inflammatory drugs compared with control group; data indispensable for a meta-analysis were not reported and still unavailable after contacting authors; studies with a crossover design which did not report a wash-out period; and studies which did not have a placebo group or proper control group.

Data extraction and quality assessment

Extracted data from each article included the first author's name; year of publication; study population; study design and duration of intervention; number, age, gender or sex ratio and health status in subjects; method and dose of intervention; source of samples; data of mean changes and corresponding standard deviation for PGE₂, TXB₂ or LTB₄. Risk of bias table based on Cochrane Criteria was used to assess the quality of studies in the present meta-analysis, including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), outcome data (attrition bias) and outcome reporting (reporting bias) [18].

Statistical analysis

We conducted three independent meta-analysis for PGE₂, TXB₂ and LTB₄ and subgroup analysis was conducted to identify the source of heterogeneity. For parallel trials, mean changes and corresponding SDs from baseline to endpoint were collected for data analysis, data was estimated according to the previous method [19] if SDs of changes were not reported. For studies with a crossover design, mean changes between the levels of PGE₂, TXB₂ and LTB₄ and corresponding SDs at the end of two intervention periods were used in data analysis, as suggested by Cochrane handbook for Systematic Reviews of Intervention [18]. The meta-analysis was performed with Stata/SE 11.0 software (Stata Corporation, College Station, TX) by using the random effect model. Standard mean differences and 95% CIs were calculated for net changes in values with PGE₂, TXB₂ and LTB₄. Heterogeneity of treatment effects between studies was tested using the Chi-square method and $I^2 > 50\%$ was considered to be significant. To explore the possible influence of covariates on net changes, subgroup analyses and meta-regression were conducted to evaluate the effects of dose of total marine-derived n-3 PUFA, EPA and DHA respectively, ratio of EPA/DHA, duration and design of study, body mass index (BMI) and age of subjects and type of intervention and placebo on the overall effect size. We classified studies according to duration as short term (< 12 weeks) or long term (≥ 14 weeks). Region of subjects were mainly divided into four areas- Europe, America, Australia and Asia. For the following variables—age and BMI at baseline, dose of total marine-derived n-3 PUFA, EPA and DHA, ratio of EPA to DHA, we stratified studies according to their median. Meta-regression with restricted maximum likelihood estimation was performed to evaluate the potentially important covariates exerting substantial impact on heterogeneity between the trials. According to the Cochrane Handbook for Systemic Review, publication bias was examined by using funnel plots, Begg's test and Egger's regression test [20].

Results

Selection of relevant studies

The initial electronic search yielded 4107 records (1643 from PubMed, 2282 from Web of Science and 182 from Cochrane with 1770 duplicate records). Titles and abstracts were screened for 1751 citations, and 168 RCTs were obtained after eliminating other type designs. Of these, 50 RCTs were excluded because data indispensable for meta-analysis was still not available.

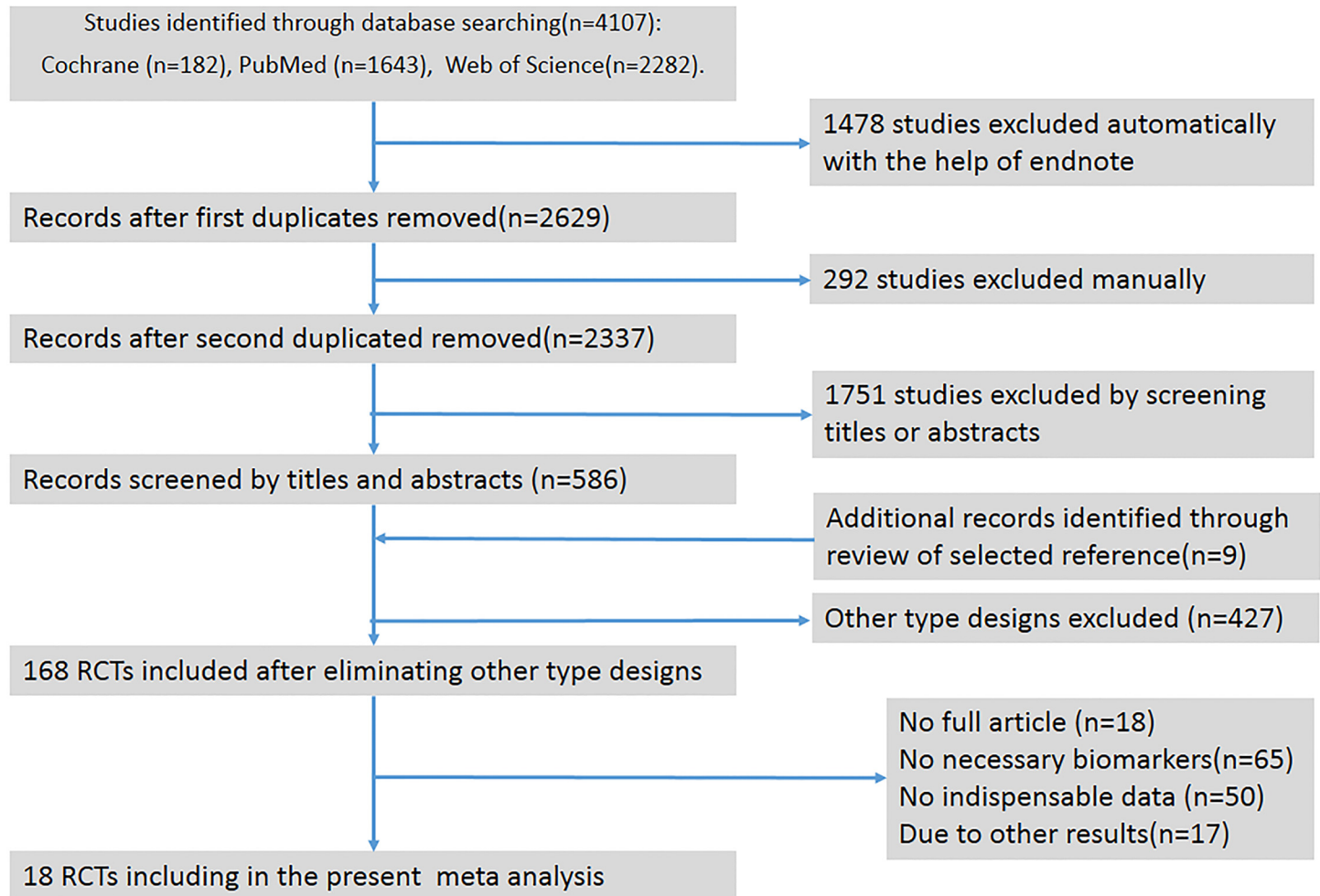


Fig 1. Flow diagram of studies evaluated in the review. RCT, randomized controlled trial.

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Four studies were excluded because they only provided figures without definite information, and two studies had insufficient information (no data at baseline or endpoint). We contacted 15 authors for data and only received replies from 4 of them, but the required data was not available. Finally, 18 studies of RCTs, including 27 study arms with 826 subjects were included in this systematic review and meta-analysis. Detailed processes of the study selection are shown in [Fig 1](#). PRISMA Flow Diagram PRISMA and checklist required for meta-analysis are shown in [S1 Fig](#) and [S1 PRISMA Checklist](#).

Characteristics of the studies

Primary characteristics of the 18 studies including 27 study arms are shown in [Table 1](#). A total of 826 subjects were randomly assigned to intervention or control groups with a completion rate of 57.23%. Of the 20 included randomized controlled trials, subjects in most studies were from Europe (n = 7), and others were from the United States (n = 4), Australia (n = 3), Brazil (n = 1), Korea (n = 1), Iran (n = 1) and Canada (n = 1).

For studies that reported values of PGE₂, TXB₂ or LTB₄ at more than 2 time points, only changes from baseline to endpoint were used to calculate the overall effect size [21]. For studies that included more than one intervention group, to avoid the duplication of subjects for

Table 1. Characteristics of included studies for systematic review and meta-analysis.

Author	Studies			No	Country	Subjects			Treatments				T
	Sample type	Biomarkers	Duration (weeks)			Healthy status	BMI (kg/m ²)	Age (yr)	n-3 (g)	EPA (g)	DHA (g)	EPA /DHA	
Andrade et al.2007	Plasma	PGE ₂	6	20	Brazil	Health	23.5	27.5	1.45	0.95	0.5	1.9	Fish oil
Tempel et al.1990	Neutrophils	LTB ₄	12	16	Netherlands	RA	UN	53	3.36	2.04	1.32	1.54	Fish oil
Ve'ricel et al.1999	Platelet	TXB ₂	6	20	France	Healthy	UN	76.5	0.18	0.03	0.15	0.2	Fish oil
Park et al.2013	Serum	PGE ₂	16	81	Korea	RA	22.4	48.4	3.255	2.09	1.165	1.79	Fish oil
Peck et al.1996 (O)	Plasma	PGE ₂	8	13	USA	Hemodialysis	25.4	50.2	UN	UN	UN	UN	Fish oil
Peck et al.1996 (S)	Plasma	PGE ₂	8	12	USA	Hemodialysis	24.4	52.2	UN	UN	UN	UN	Fish oil
Pirich et al.1999	Serum	TXB ₂	6	26	Australia	Hypercholesterolemia	UN	57	0.356	0.216	0.14	1.54	Fish oil
Sørensen et al.1992	Serum	TXB ₂	7	34	Denmark	Pregnant	UN	29.6	2.7	UN	UN	UN	Fish oil
Singer et al.2004	Plasma	TXB ₂	24	65	Germany	Cardiac arrhythmias	25.9	43.5	0.9	0.54	0.36	1.5	Fish oil
Turini et al.1994	Neutrophils	LTB ₄	6	20	Canada	Healthy	25.3	26.5	4.5	3.3	1.2	2.75	Fish oil
Tartibian et al.2011	Plasma	PGE ₂	4	30	Iran	Healthy	24.9	30.2	0.54	0.324	0.216	1.5	Fish oil
Thusgaard et al.2009	Neutrophils	LTB ₄	12	48	Denmark	HIV-infect	24.7	45	3.36	1.84	1.52	1.21	Fish oil
Maaloe et al.2011	Neutrophils	LTB ₄	8	56	Denmark	Chronic kidney diseases	UN	59	1.44	0.864	0.576	1.5	Fish oil
Knapp et al.1989 (L)	Serum	TXB ₂	4	16	USA	Hypertension	UN	UN	3	1.8	1.2	1.5	Fish oil
Knapp et al.1989 (H)	Serum	TXB ₂	4	16	USA	Hypertension	UN	UN	15	9	6	1.5	Fish oil
Mori et al.1992	Neutrophils	LTB ₄	4	29	Australia	Peripheral vascular disease	25.4	61.9	4.6	2.8	1.8	1.56	Fish oil
Phang et al.2013	Plasma	TXB ₂	4	47	Australia	Healthy	24.6	39.6	1.2	1	0.2	5	Fish oil
Phang et al.2013 (H)	Plasma	TXB ₂	4	47	Australia	Healthy	24.6	39.6	1.2	0.2	1	0.2	Fish oil
Rees et al.2006 (YL)	MNCs	PGE ₂	12	31	UK	Healthy	24.8	24.3	1.65	1.35	0.3	4.5	EPA
Rees et al.2006 (OL)	MNCs	PGE ₂	12	22	UK	Healthy	27.6	61	1.65	1.35	0.3	4.5	EPA
Rees et al.2006 (YM)	MNCs	PGE ₂	12	31	UK	Healthy	23.5	24.8	3.3	2.7	0.6	4.5	EPA
Rees et al.2006 (OM)	MNCs	PGE ₂	12	20	UK	Healthy	28.1	60.9	3.3	2.7	0.6	4.5	EPA
Rees et al.2006 (YH)	MNCs	PGE ₂	12	31	UK	Healthy	24	24	4.95	4.05	0.9	4.5	EPA

(Continued)

Table 1. (Continued)

Author	Studies			Subjects					Treatments				
	Sample type	Biomarkers	Duration (weeks)	No	Country	Healthy status	BMI (kg/m ²)	Age (yr)	n-3 (g)	EPA (g)	DHA (g)	EPA /DHA	T
Rees et al.2006 (OH)	MNCs	PGE ₂	12	20	UK	Healthy	27	60.1	4.95	4.05	0.9	4.5	EPA
Kremer et al.1990 (L)	Neutrophils	LTB ₄	24	26	USA	RA	UN	58.8	UN	UN	UN	UN	EPA
Kremer et al.1990 (H)	Neutrophils	LTB ₄	24	23	USA	RA	UN	58	UN	UN	UN	UN	EPA
Kremer et al.1987	Neutrophils	LTB ₄	14	26	USA	RA	UN	56.8	4.5	2.7	1.8	1.5	EPA

T, intervention in treatment group; UN, unclear; RA, rheumatoid arthritis; MNCs, mononuclear cells; PGE₂, prostaglandin E₂; TXB₂, thromboxane B₂; LTB₄, leukotriene B₄; L, low, H, high; YL, young and low, OL, old and low, YM, young and middle, OM, old and middle, YH, young and high, OH, old and high.

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analysis, ‘shared’ control group was split into two or more control groups with smaller sample size, which included two or more (reasonably independent) comparisons.

Knapp et al [22] compared a high dose and low dose of fish oil with safflower oil and mixed oils. The high dose arm was compared with mixed oil arm labelled Knapp L, whereas the low dose arm was compared with safflower oil arm labelled Knapp H. Kremer et al [23] included low dose, high dose and control, the control was isolated into two units and labeled Kremer L and Kremer H. Phang et al [24] compared EPA group and DHA group with Placebo group. The EPA arm was compared with Placebo arm labelled Phang E, whereas DHA arm was compared with Placebo arm labelled Phang D. Peck et al [16] compared fish oil with various control oils including olive oil and safflower oil, the fish oil arm was separated into two small arms labelled Peck O and Peck S respectively. There were eight groups in the study reported by Rees et al [25], six study arms were labelled Rees (YL), Rees (OL), Rees (YM), Rees (OM), Rees (YH), Rees (OH) respectively according to age (Y-young, O-old) and dose (L-low, M-moderate, H-high).

Quality of studies

None of the included trials reported any significant differences in characteristics of subjects in the treatment or control arm. Allocation concealment was adequate in 5 studies and unclear in others. Sixteen studies were double-blind design, one study [22] only masked participants, and five studies were unclear. Risk-of-bias graph and risk of bias summary are shown in Figs 2 and 3.

Effect of marine-derived n-3 PUFA supplementation on PGE₂

Five studies reported the effect of marine-derived n-3 PUFA on PGE₂ [16, 25–28], among which two studies [26, 28] included in the meta-analysis showed a significant reduction on PGE₂ in plasma of healthy subjects with the supplementation of marine-derived n-3 PUFA compared with placebo supplementation (SMD:-1.27; 95% CI: -1.90,-0.63).

Effect of marine-derived n-3 PUFA supplementation on TXB₂

Six studies [21, 22, 24, 29–31] were included in this systematic review and meta-analysis, and data from three studies [22, 30–31] with four arms were pooled to demonstrate that marine-

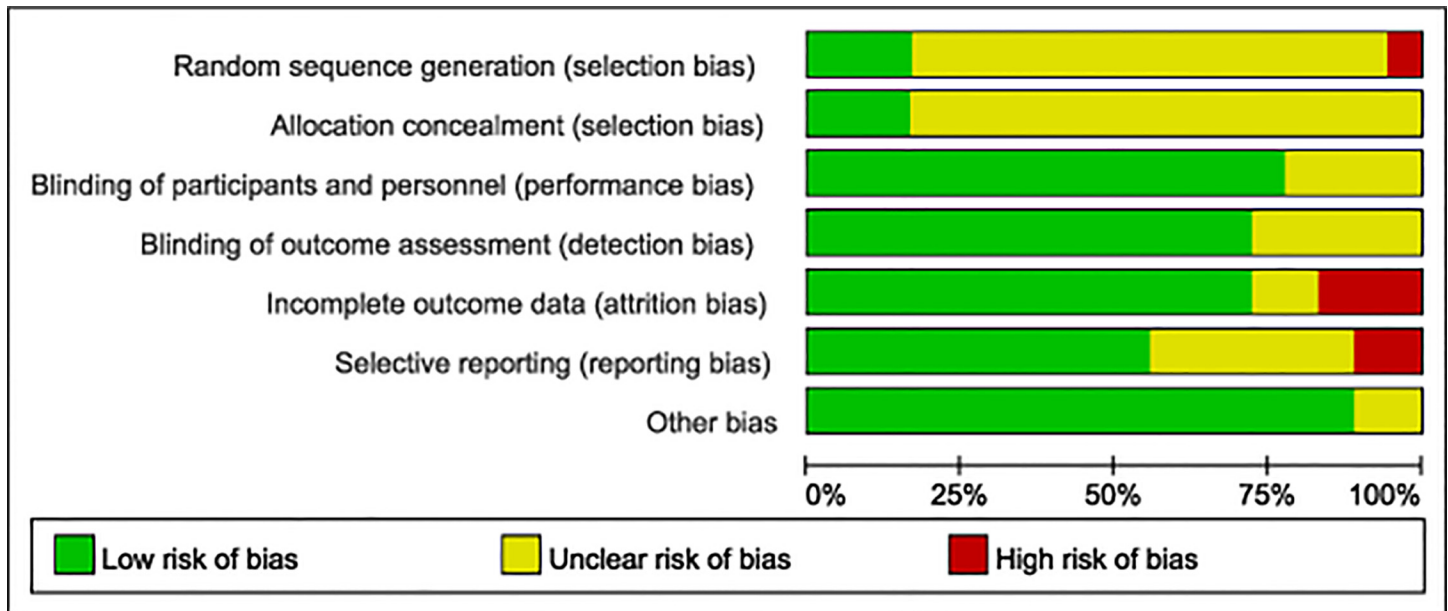


Fig 2. Risk-of-bias graph. The review of judgments of authors about each risk-of-bias item is presented as percentages across all included studies.

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derived n-3 PUFA showed a significant difference in reducing TXB₂ in subjects with high risk of CVD compared with placebo (SMD:-1.26; 95% CI: -1.65, -0.86; I² = 0.0%) (Fig 4).

Effect of marine-derived n-3 PUFA supplementation on LTB₄

Six studies [23, 32–36] with seven study arms reported the effect of marine-derived n-3 PUFA on LTB₄ in unhealthy subjects and the other one [37] reported in healthy subjects. Marine-derived n-3 PUFA was more effective in reducing the concentration of LTB₄ in neutrophils of unhealthy subjects compared with placebo (SMD:-0.59; 95% CI: -1.02, -0.16; I² = 62.6%) (Fig 5).

Subgroup analysis and meta-regression

Subgroup analyses and meta-regression analyses were conducted to evaluate the effects of the confounding factors on primary outcomes of marine-derived n-3 PUFA on LTB₄ for each potential variable including dose of total marine-derived n-3 PUFA, EPA and DHA, ratio of EPA to DHA, duration and design of study, body mass index (BMI) and age of subjects and type of intervention and placebo. Full details are shown in Table 2. Subgroup analyses showed a more significant reduction on LTB₄ in RA patients with supplementation of marine-derived n-3 PUFA (SMD: -0.83; 95% CI: -1.37, -0.29), but not in non-autoimmune chronic disease patients (SMD: -0.33; 95% CI: -0.97, 0.31) (Fig 5). No significant reduction on LTB₄ was found when the duration of intervention with marine-derived n-3 PUFA was less than 14 weeks (SMD: -0.34; 95% CI: -0.81, 0.13) (Table 2). There was no significant difference in serum (SMD: -1.05; 95% CI: -1.61, -0.49) and plasma (SMD: -1.46; 95% CI: -2.00, -0.91) concerning the effect of marine-derived n-3 PUFA on TXB₂. The use of anti-inflammatories failed to significantly change the results for the effect of marine-derived n-3 PUFA on LTB₄ (P = 0.286).

Sensitivity analysis

We also conducted sensitivity analyses to explore the source of heterogeneity. Sensitivity analysis was performed by studying whether effect size changes significantly after excluding special

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Andrade et al.2007	?	?	?	?	+	+	+
Knapp et al.1989	?	+	+	?	+	+	+
Kremer et al.1987	?	?	+	+	-	?	+
Kremer et al.1990	?	?	+	+	?	?	+
Maaløe et al.2011	?	?	+	+	+	+	?
Mori et al.1992	?	?	+	+	-	-	+
Park et al.2013	+	+	+	+	+	?	+
Peck et al.1996	?	?	+	+	+	?	+
Phang et al.2013	+	?	+	+	+	+	+
Pirich et al.1999	?	?	?	?	+	?	+
Rees et al,2005	?	?	+	+	+	+	+
Singer et al.2004	?	?	+	+	+	+	+
Sørensen et al.1993	?	+	+	+	+	?	+
Tartibian et al.2011	?	?	+	+	+	+	+
Tempel et al.1989	?	?	+	+	+	-	?
Thusgaard et al.2009	+	?	+	+	?	+	+
Turini et al.1994	-	?	?	?	-	+	+
Véricel et al.1999	?	?	?	?	+	+	+

Fig 3. Risk of bias summary. Review author’s judgements about each risk of bias item for each included study.

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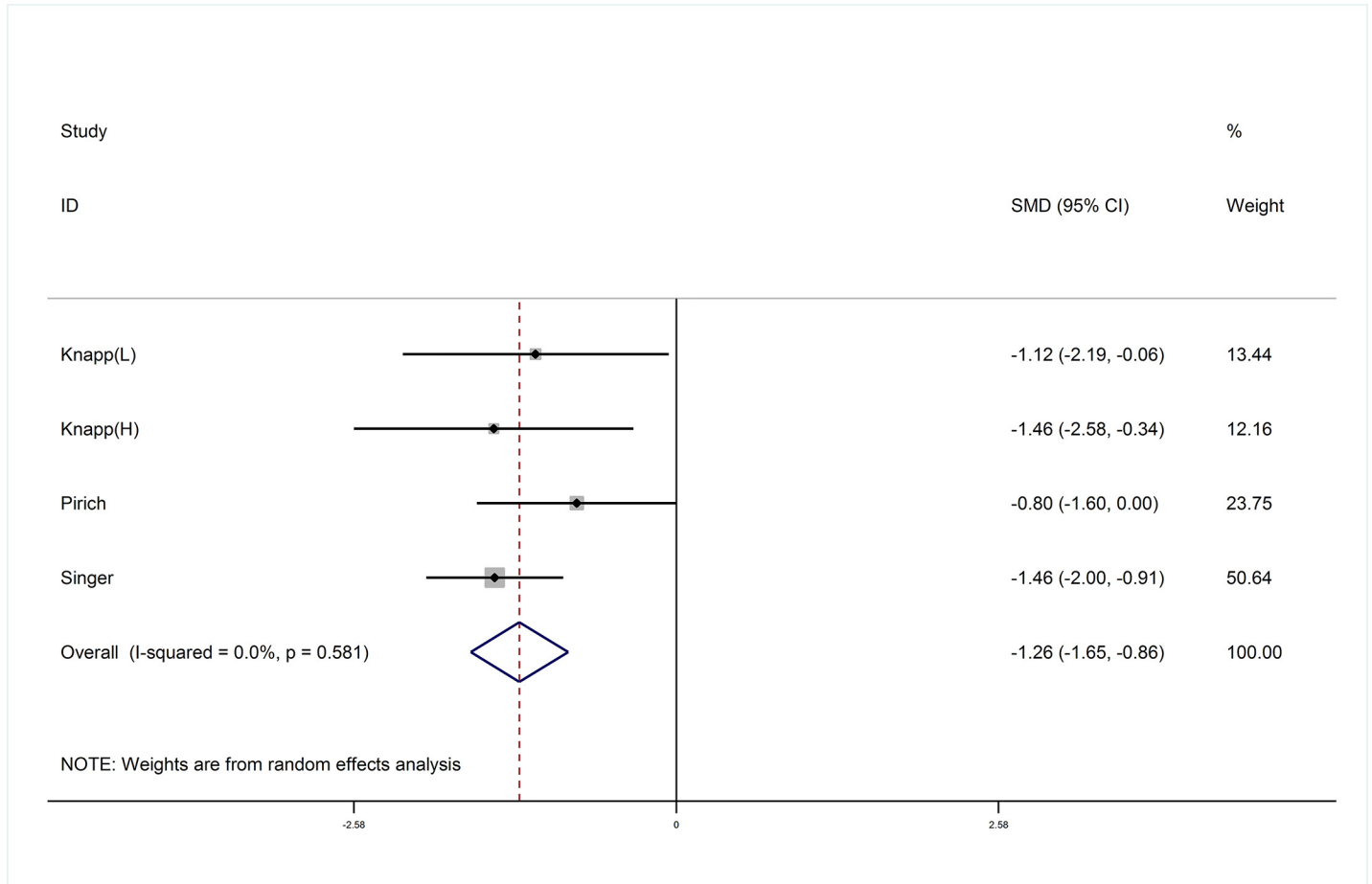


Fig 4. Pooled effect size of marine-derived PUFA supplementation on TXB₂ in subjects with high risk of CVD. CVD, cardiovascular disease. SMD, standard mean difference.

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trials one by one. Some trials were not consistent with others in terms of intervention method, measurement method, samples and volunteers. Rees et al [25] detected PGE₂ concentration in mononuclear cell; special volunteers were recruited such as elite swimmers [28] and pregnant women [21]; Radioimmunoassay [16, 22, 30] and ELISA [24–29, 31] were used to determine the concentrations of PGE₂ and LTB₄. Moreover, the studies by Mori et al [33], Pirich et al [30] and Turini et al [37] had low quality and high risk bias. However, the results of the sensitivity analysis on PGE₂, TXB₂ and LTB₄ (Fig 6) showed that the pooled effects remained non-significant when analyses were limited to high-quality trials and conducted after excluding special trials.

In the study by Knapp [22], the high dose arm was compared with mixed oil arm labelled Knapp L, whereas the low dose arm was compared with safflower oil arm labelled Knapp H. The pooled effects remained non-significant when we compared the high dose arm with safflower oil and low dose arm with mixed oil arm.

Publication bias

On the basis of funnel plots (Figs 7 and 8) and Egger’s test, no significant publication bias was shown in the meta-analysis of TXB₂ in serum or plasma of subjects with high risk of CVD (P = 0.59) and LTB₄ in neutrophils of unhealthy subjects (P = 0.60) with supplementation of marine-derived n-3 PUFA.

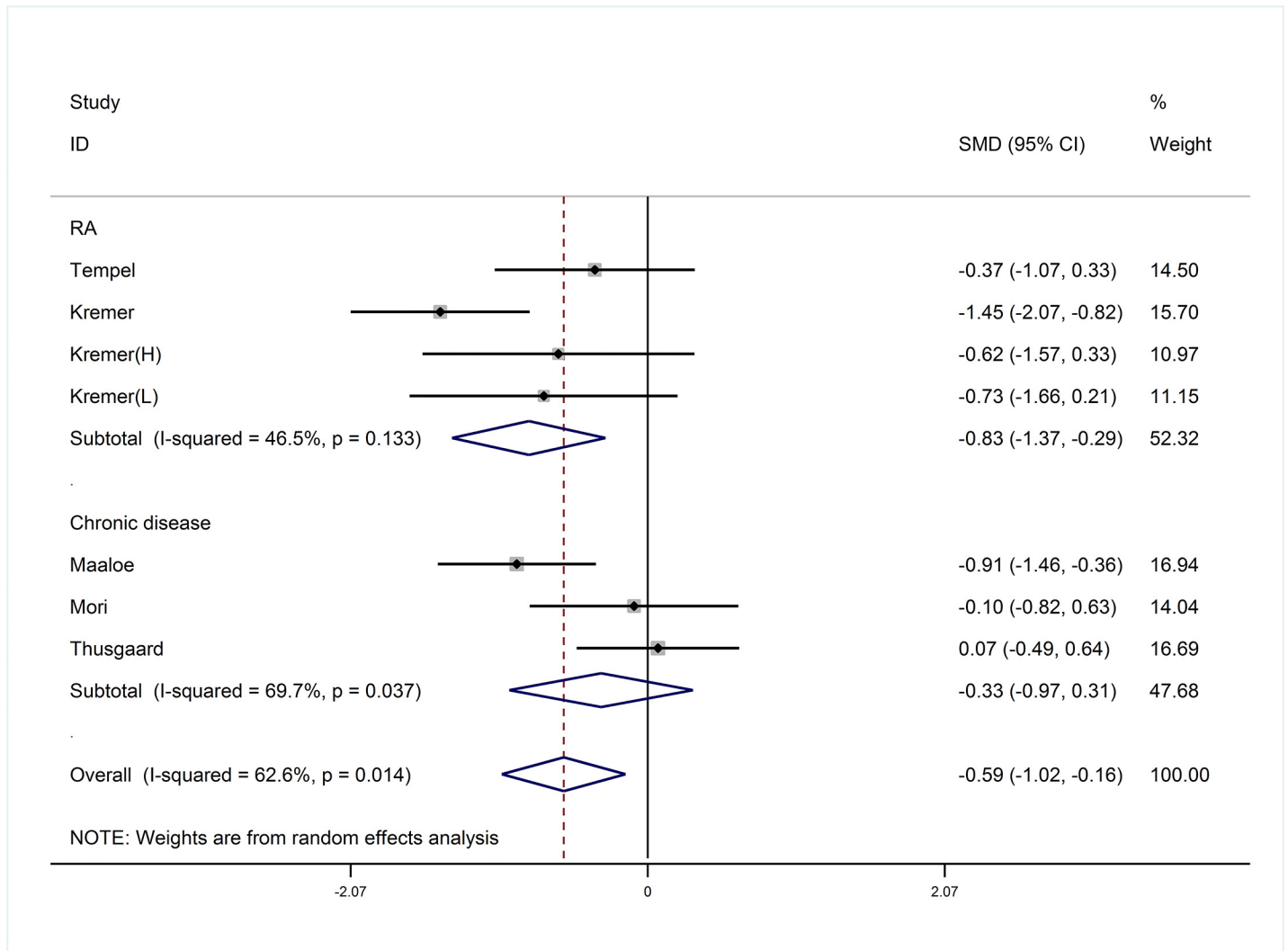


Fig 5. Pooled effect size of marine-derived n-3 PUFA supplementation on LTB₄ in unhealthy subjects including RA and Chronic diseases patients. RA, rheumatoid arthritis. SMD, standard mean difference.

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Discussion

The results of this meta-analysis supported the effect of marine-derived n-3 PUFA on suppressing TXB₂ in subjects with high risk of CVD and LTB₄ in neutrophils in unhealthy subjects. TXB₂ can induce vasoconstriction and promote aggregation of platelets as well as adhesiveness of PMNs, and LTB₄ is involved in modulating the intensity and duration of inflammatory responses. On one hand, n-3 PUFAs, especially marine-derived n-3 PUFAs, directly inhibit AA metabolism to produce eicosanoids by replacing AA as the substrate, and indirectly alter the expression of inflammatory genes through effects on transcription factor genes [12]. On the other hand, marine-derived n-3 PUFAs, especially EPA, can also act as a substrate catalyzed by 5-lipoxygenase enzymes and cyclooxygenase enzymes to generate several eicosanoids. These eicosanoids, such as LTB₅ and LTE₅, have slightly different structures and less potent pro-inflammatory function compared with those formed from AA [38]. Furthermore, EPA and DHA can also generate a novel genus of mediators, termed specialized pro-resolving mediators.

Table 2. Subgroup analysis for the effect of marine-derived n-3 PUFAs on LTB₄ in unhealthy subjects (subjects with non-autoimmune chronic diseases or auto-immune diseases).

Subgroup analysis	N	SMD (95%CI)	LTB ₄		P ₂
			Heterogeneity		
			I ² (%)	P ₁	
Overall	7	-0.59 (-1.12, -0.25)	62.6	0.014	
Sex ratio(male/female)					0.286
<0.5	3	-0.33 (-0.97, 0.31)	69.7	0.037	
> = 0.5	4	-0.83 (-1.37, -0.29)	46.5	0.133	
Age					0.965
<median	3	-0.58 (-1.50, 0.34)	84.4	0.002	
> = median	4	-0.63 (-1.01, -0.25)	3.80	0.374	
Health status					0.163
Chronic diseases	3	-0.33 (-0.97, 0.31)	69.7	0.037	
Rheumatoid arthritis	4	-0.83 (-1.37, -0.29)	46.5	0.133	
Duration					0.138
<14weeks	4	-0.34 (-0.81, 0.13)	54.6	0.086	
> = 14 weeks	3	-1.04 (-1.59, -0.48)	27.8	0.250	
Daily dose of total n-3 PUFA(g)					0.987
<median	2	-0.69 (-1.21, -0.16)	30.0	0.232	
> = median	3	-0.49 (-1.46, 0.48)	85.6	0.001	
NR	2	-0.68 (-1.34, -0.06)	0	0.880	
daily dose of EPA(g)					0.679
<median	2	-0.42 (-1.39, 0.54)	83.2	0.015	
> = median	3	-0.65 (-1.49, 0.18)	77.8	0.011	
NR	2	-0.68 (-1.34, -0.01)	0	0.880	
daily dose of DHA(g)					0.987
<median	2	-0.69 (-1.21, -0.16)	30.0	0.232	
> = median	3	-0.49 (-1.46, 0.48)	85.6	0.001	
NR	2	-0.68 (-1.34, -0.01)	0	0.880	
Ratio of EPA/DHA					0.765
< 1.55	3	-0.76 (-1.62, 0.10)	84.8	0.001	
> = 1.55	2	-0.24 (-0.74, 0.27)	0	0.594	
NR	2	-0.68 (-1.34, -0.01)	0	0.880	
Region					0.927
Europe	3	-0.41 (-1.01, -0.19)	66.6	0.500	
America	3	-1.04 (-1.59, -0.48)	27.8	0.250	
Australia	1	-0.10 (-0.82, 0.63)	NA	NA	

N, number of included studies (or comparisons); NR, not reported; NA, not associated with this item; P₁ value for heterogeneity within each subgroup; P₂ value for heterogeneity between subgroups with meta-regression analysis.

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Subgroup analysis results showed a significant effect on LTB₄ in RA patients in neutrophils with supplementation of marine-derived n-3 PUFA, Park et al [27] also found that n-3 PUFA supplementation significantly decreased LTB₄ levels in serum of RA patients who weighed more than 55kg. For subjects with chronic diseases, the function of marine-derived n-3 PUFA did not reach statistical significance. However, PGE₂ was significantly increased in hemodialysis patients compared with control group following supplementation of marine-derived n-3

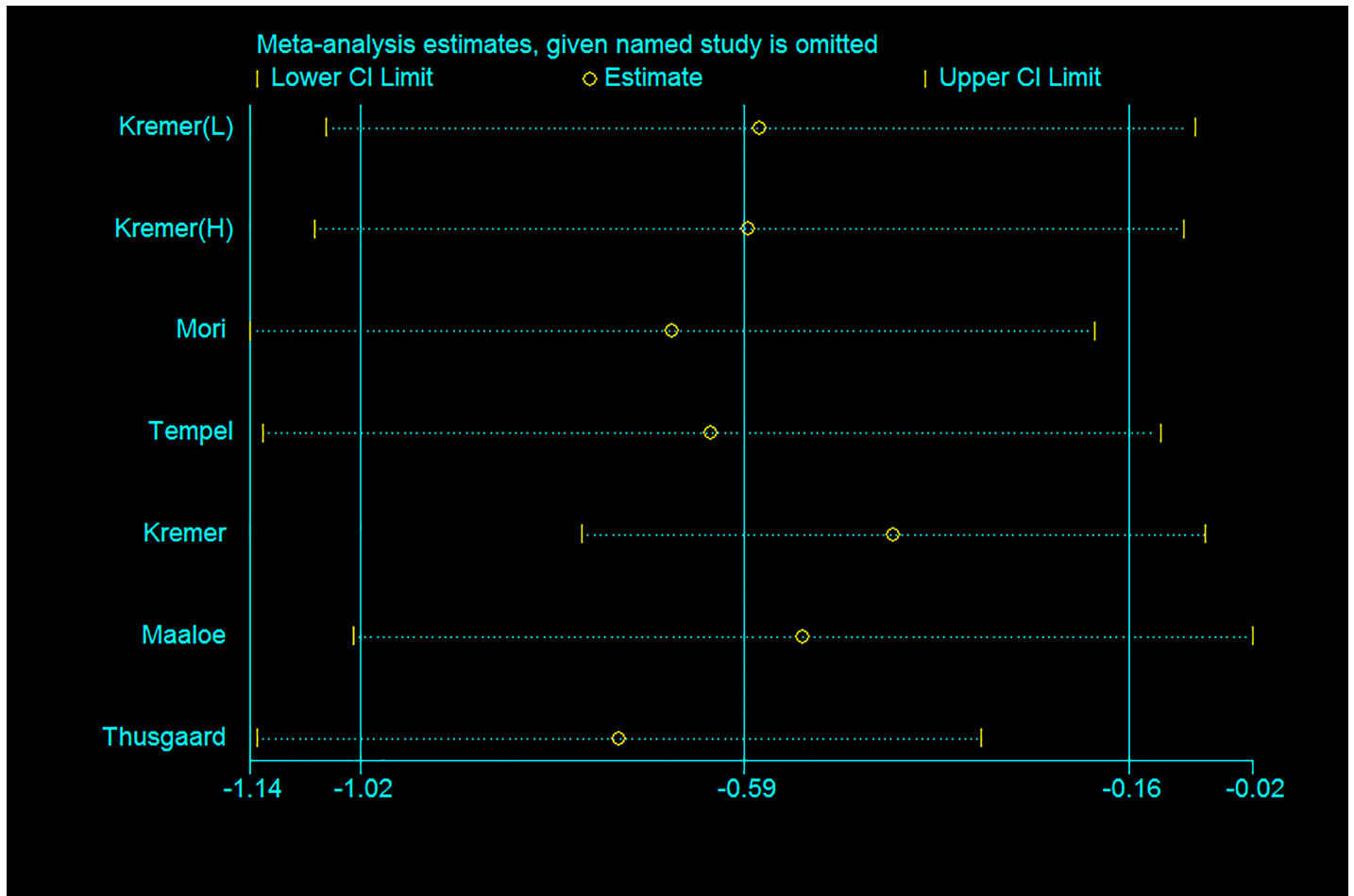


Fig 6. Sensitive analysis with pseudo 95% confidence limits for studies with LTB₄.

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PUFA [16], and a slightly but not significant increase was also found in RA patients [27]. In addition, marine-derived n-3 PUFA did not result in significantly decreased formation of plasma PGE₂ in mildly hypertriglycerolemic subjects [39]. A hypothesis was put forward to explain the different results between PGE₂ and LTB₄: a decrease in proteinuria and an improvement in glomerular filtration rate was reported [40], which may be relevant to the function of n-3 PUFA on immunoglobulin A nephropathy. Increased DHA and EPA could result in the production of inactive LTB₅ along with decreased synthesis of the inflammatory LTB₄. These eicosanoids were produced through different pathways, LTB₄ was generated through lipoxygenase pathway with 5-lipoxygenases enzymes, while PGE₂ and TXA₂ /TXB₂ were from cyclo-oxygenase (COX) pathway. So LTB₅ blocked the lipoxygenase pathway and arachidonic acid was metabolized to produce PGE₂ through the cyclooxygenase pathway, which led to a decrease in LTB₄ and corresponding increase in PGE₂.

However, for another product of the cyclooxygenase pathway, TXB₂ showed a reduction with supplementation of marine-derived n-3 PUFA in populations with high risk of CVD. Mehta et al. [41] found that EPA supplementation had no effect on concentrations of PGE₂ in Barrett’s mucosa. This study also suggested no significant alteration in AA and increase in phospholipase A₂ activity. Hishinuma et al. [42] demonstrated that an increased availability of AA resulted in an increase in 2-series eicosanoid production in murine mast cells, and the

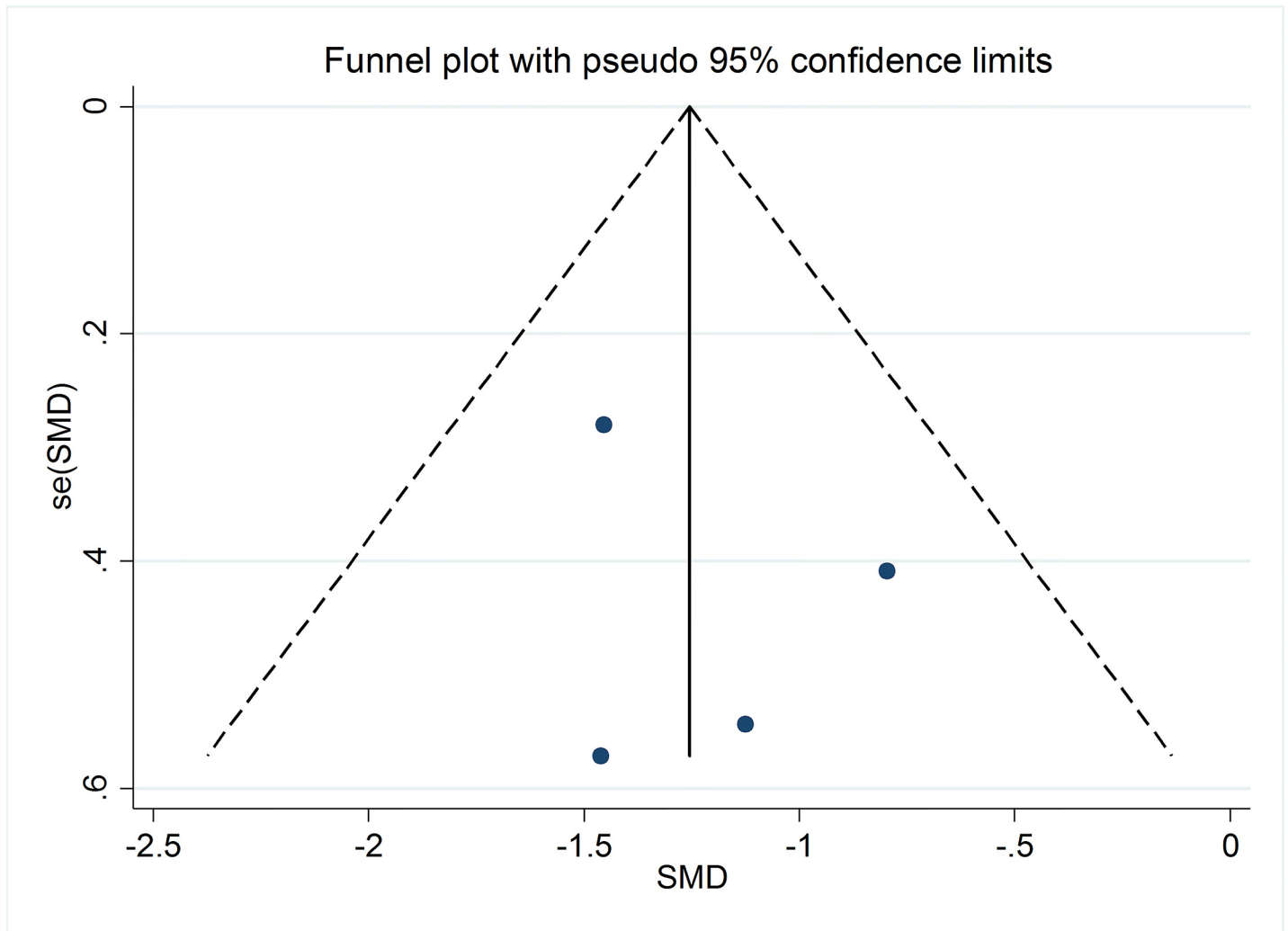


Fig 7. Funnel plot with pseudo 95% confidence limits for studies with TXB₂.

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predominant COX2 pathway products were PGE₂ > PGF_{2α} > TXB₂ > 6-keto-PGF_{1α} [43, 44] when AA is in abundance. Therefore, it may lead to no significant change in PGE₂ and a reduction in TXB₂ concentration.

An animal experiment by Kang et al [45] examined this issue from another aspect. They measured the inflammatory indicators in wild type (WT) mice and fat-1 mice (a kind of transgenic mice rich in endogenous n-3 PUFA), both of which were induced to have colitis. Results showed that in colon tissue, no significant differences in the content of AA, LTB₄ and PGE₂ were found, but there was a remarkable increase in the amounts of EPA and DHA as well as their potent bioactive products including resolvins and protectins (RvE1, RvD3, and PD1/NPD1), and the less potent products including LTB₅ and PGE₃. The author assumed that marine-derived n-3 PUFA exerts anti-inflammatory functions by the potent bioactive rather than AA-derived eicosanoids. This hypothesis could explain why there was no difference in PGE₂ levels after marine-derived n-3 PUFA supplementation, but this conflicted with our results for TXB₂ and LTB₄. The inconsistency may be most likely due to the difference in the ratio of n-3/n-6 PUFA. In the study by Kang et al [45], the n-3/n-6 ratio was so high in fat-1 mice that it could supply limited AA as substrate to rise PGE₂, TXB₂ and LTB₄. More animal

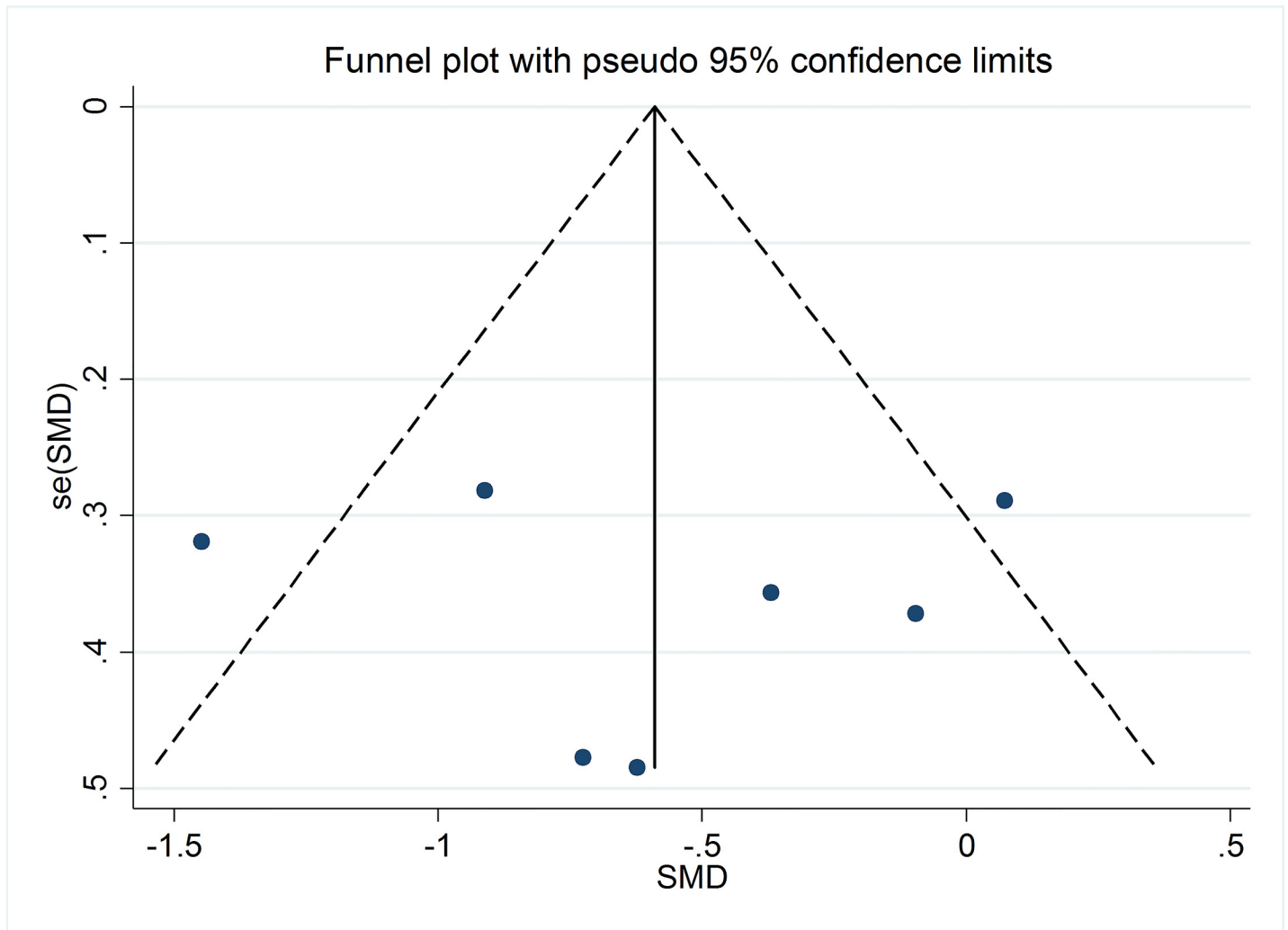


Fig 8. Funnel plot with pseudo 95% confidence limits for studies with LTB₄.

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experiments are needed to explore the mechanism of how marine-derived n-3 PUFA exerts its effect on inflammation.

In healthy subjects, marine-derived n-3 PUFA supplementation had no significant effect on TXB₂ [21, 24], which is consistent with the result from Murphy et al. [46], who found that levels of TXB₂ tended to decrease with n-3 PUFA supplementation in both groups, but this did not reach statistical significance. It was most likely due to the low production of eicosanoids at baseline in healthy subjects. However, Andrade et al. found swimming athletes who had supplementation of marine-derived n-3 PUFA showed decreased PGE₂ in plasma [28], which was also confirmed in common healthy subjects reported by Tartibian et al [26], and EPA also led to a significant reduction in the levels of PGE₂ in mononuclear cell (MNC) [25] and skin unexposed to ultraviolet radiation [47]. The different results between PGE₂ and TXB₂ may result from the different dose of n-3 PUFA. Individuals with a higher concentrations of EPA and DHA into erythrocyte membranes also showed the greatest reduction in erythrocyte AA content [48]. A substantial increase in the EPA content of MNC phospholipids (> 4-fold) and a corresponding decrease in AA (25%) was required to affect PGE₂ production [25].

We only included studies for meta-analysis where PGE₂ were measured in plasma of healthy subjects, and TXB₂ were tested in in serum/plasma in subjects with high risk of CVD (Fig 4) and LTB₄ were measured in neutrophils of unhealthy subjects (Fig 5). Others were only included for systematic review and reported in discussion. Additionally, LTB₄ was determined by HPLC, while PGE₂ and TXB₂ were measured by radioimmunoassay or ELISA. We conducted SMD as the estimated effect size and random effects model was used for meta-analysis.

We first utilized pooled data to quantitatively assess the effect of marine-derived n-3 PUFA on AA-derived major eicosanoids (PGE₂, TXA₂/TXB₂ and LTB₄), and subgroup analyses were conducted to evaluate the effects of confounding factors on overall effect size of LTB₄ in unhealthy subjects. In addition, we discovered the effects of marine-derived n-3 PUFA on PGE₂ and TXB₂ were different, and future studies can recruit volunteers with different health status to explore the effect of n-3 PUFA on eicosanoids. Lastly, no significant publication bias was shown in the meta-analysis according to the results of funnel plots and Egger's test.

There are several limitations in the present study. First, to explore the potential influence on effect size by several confounding factors (such as age of subjects and dose of n-3 PUFA), 'shared' control group was split into two or more control groups to couple the intervention group according to the method by Cochrane handbook [18]. Studies included in one meta-analysis were so limited that all trials in a subgroup were likely to be from one study in subsequent subgroup analysis. Secondly, the placebo in control group showed variety including olive oil, safflower, mineral oil, corn oil and others, and subgroup analysis by the type of placebo couldn't be performed for too few trials in each subgroup. Lastly, dose-response of the effect of marine-derived n-3 PUFA on major eicosanoids was also not conducted due to limited trials. Therefore, more well-controlled RCTs to investigate the effect of marine-derived n-3 PUFA on major eicosanoids are needed to solve the problems above.

Conclusions

In conclusion, this systematic review and meta-analysis provided evidence that marine-derived n-3 PUFA had a significant benefit on the reduction of TXB₂ content in subjects with high risk of CVD and LTB₄ concentrations in unhealthy subjects. Subgroup analyses showed that marine-derived n-3 PUFA significantly decreased LTB₄ in RA patients, but not in non-autoimmune chronic disease patients, and duration of intervention with marine-derived n-3 PUFA also affected the overall effect size. High quality RCTs are needed to explore the effects of marine-derived n-3 PUFA on different eicosanoids in subjects with different health status.

Supporting Information

S1 PRISMA Checklist. PRISMA 2009 checklist.
(DOC)

S1 Fig. PRISMA flow diagram of the search and study selection process.
(DOC)

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Author Contributions

Conceived and designed the experiments: JJ DL. Analyzed the data: JJ BY YF. Wrote the paper: JJ JZ. Searched and selected relevant studies: JJ KL FW. Extracted data: JJ KL.

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