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META-ANALYSIS

Meta-analysis of the effects of *n*-3 polyunsaturated fatty acids on haematological and thrombogenic factors in type 2 diabetes

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Abstract
Aim/hypothesis To determine whether marine-derived *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) (also known as omega-3 fatty acids) have beneficial effects on haematological and thrombogenic risk markers in addition to dyslipidaemia, in patients with type 2 diabetes.
Methods A systematic review and meta-analysis of randomised controlled trials comparing dietary or non-dietary intake of *n*-3 PUFA with placebo in type 2 diabetes was conducted by systematically searching databases from 1966 to February 2006. Changes in C-reactive protein, IL-6, TNF-α, platelet function, fibrinogen, factor VII, von Willebrand factor, endothelial function, heart rate and blood pressure were recorded. Inclusion of studies, data extraction and quality were assessed independently in duplicate.
Results Twelve trials involving 847 subjects with a mean treatment duration of 8.5 weeks included sufficient data to permit pooling. Compared with placebo, *n*-3 PUFA supplementation had a significant effect on two outcomes: reducing the level of diastolic blood pressure (five trials, 248 subjects) by a mean of 1.8 mm Hg (95% CI 0.0–3.6, $p=0.05$) and increasing factor VII (two trials, 116 subjects) by 24.9% (95% CI 7.2–42.6, $p=0.006$). There were no significant effects on systolic blood pressure, fibrinogen or heart rate.
Conclusions/interpretation These results suggest that, in addition to the recognised effects on dyslipidaemia, *n*-3 PUFA decreases diastolic blood pressure, and appears to increase factor VII. Larger and more rigorously conducted clinical trials are required to establish conclusively the role of *n*-3 PUFA in cardiovascular risk markers and clinical outcomes in type 2 diabetes.

Keywords Cardiovascular disease · Cardiovascular risk factors · Haemostatic factors · Lipids · *n*-3 PUFA · Omega-3 fatty acids

Abbreviations
CRP C-reactive protein
PUFA polyunsaturated fatty acids

Introduction

Type 2 diabetes mellitus is characterised by multiple metabolic abnormalities, is associated with hypertension and results in a two- to four-fold increased risk of cardiovascular disease [1]. In patients without diabetes *n*-3 polyunsaturated fatty acid (*n*-3 PUFA) (also known as omega-3 fatty acids) supplementation has been reported to have a range of potential cardioprotective effects including anti-inflammatory effects, stabilisation of atherosclerotic plaques, anti-thrombotic effects due to the inhibition of platelet aggregation and enhancing fibrinolysis, as well as anti-hypertensive effects [2], and might therefore be

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expected to confer specific therapeutic benefits in the treatment of type 2 diabetes. There are, however, few available clinical outcome data. Two prospective cohort studies among women with type 2 diabetes showed the risk of CHD to be much lower among those with high intakes of *n*-3 PUFA [3, 4], but no randomised controlled clinical outcome trials have been reported and the evidence from secondary prevention trials in non-diabetic populations is conflicting [2, 3] with a recent meta-analysis casting doubt on the strength of the evidence [5].

The aim of this systematic review was to determine the effects of marine-derived *n*-3 PUFA on established and emerging cardiovascular risk markers, other than lipids, in patients with type 2 diabetes in randomised placebo-controlled clinical trials, and, where possible, derive pooled estimates of effect size.

Subjects and methods

We searched the Cochrane Register of Controlled Trials from 1986, MEDLINE from 1966, Embase from 1966, and the *meta*Register of Controlled Trials to 20 February 2006 for the terms 'fish oil,' 'omega-3 fatty acid,' 'polyunsaturated fatty acid,' 'eicosapentaenoic acid,' 'docosahexaenoic acid,' 'nutrition' and 'diet.' A standard search filter was used to identify randomised controlled trials among people with diabetes [6]. Additional trials were identified by searching references cited in identified primary trials. We restricted our search to trials in humans and imposed no language restrictions.

Studies were included if they were randomised trials of marine-derived *n*-3 PUFA and had a control or comparison arm. Trials were excluded if they included multiple risk factor interventions on lifestyle factors other than diet and dietary supplements, unless their effect could be separated from other interventions. No restrictions were placed on trial duration. Trials were excluded from the pooled analysis if outcome or change data were not obtainable or data were only available from a single trial. Criteria for assessment of trial quality included the reporting of the method of randomisation, blinding or objective measurements, loss to follow-up, and systematic difference in care between intervention groups. Potential scores ranged from 0 to a maximum of 5 [7].

The outcomes evaluated were changes in fibrinogen, blood pressure, heart rate, factor VII, C-reactive protein (CRP), IL-6, TNF-α, platelet function, von Willebrand factor and endothelial function, as well as adhesion molecules and selectins.

Statistical analysis In trials with a cross-over design, where outcomes were reported for each intervention period, only data from the first intervention period were pooled. Where serial measurement of an outcome was given during the intervention phase, change in the outcome was measured from beginning to final measurement. Where a trial compared two intervention groups with a control group, or compared an intervention with more than one control group, an analysis was carried out to determine the comparison with the smallest effect size [8], which was then included in the pooled analysis. A fixed-effect model with weighted mean difference was used except where heterogeneity was observed, when a random-effects model was applied. Where trials reported different units of measurement for the same outcome, the effect sizes were calculated from the standardised mean difference using a random-effects model and converted to a standardised unit for the outcome according to International System notation. Cohen's *d* was calculated as the difference between the means of the two groups, divided by a sample-size-weighted average of the SDs of the scores in the two groups [9]. We evaluated potential publication bias using funnel plots.

For each trial, we recorded or calculated mean change for each outcome from beginning to end of the intervention. If the SD of change was not provided, it was derived from the 95% CI or SE, assuming a degree of correlation of 0.5 between the beginning and end of the intervention [9]. A p value of 0.05 was considered to be statistically significant. All analyses used Review Manager (Version 4.2.7; Update Software, Oxford, UK).

Results

Description of studies A total of 876 abstracts were identified from the electronic searches, of which 189 papers were considered appropriate for further consideration (Fig. 1). One hundred and fifty-seven were excluded because 49 were not randomised, 12 were not placebo controlled, 45 had multifactorial interventions from which *n*-3 PUFA effects could not be separated or did not use *n*-3 PUFA derivates, 47 included non-type-2 diabetic patients, two did not include human participants, and two lacked data or did not report on outcomes included in this review. A further 11 papers meeting the inclusion criteria were excluded as they did not report on outcomes included in the meta-analyses or this review. From 21 published papers we identified 12 trials of *n*-3 PUFA supplementation (Table 1), reporting results on 56 conventional and emerging cardiac risk markers in 847 subjects with type 2 diabetes [10–29]. Nine of the 12 trials involving 297 participants measured the same outcomes, including only sufficient data to allow

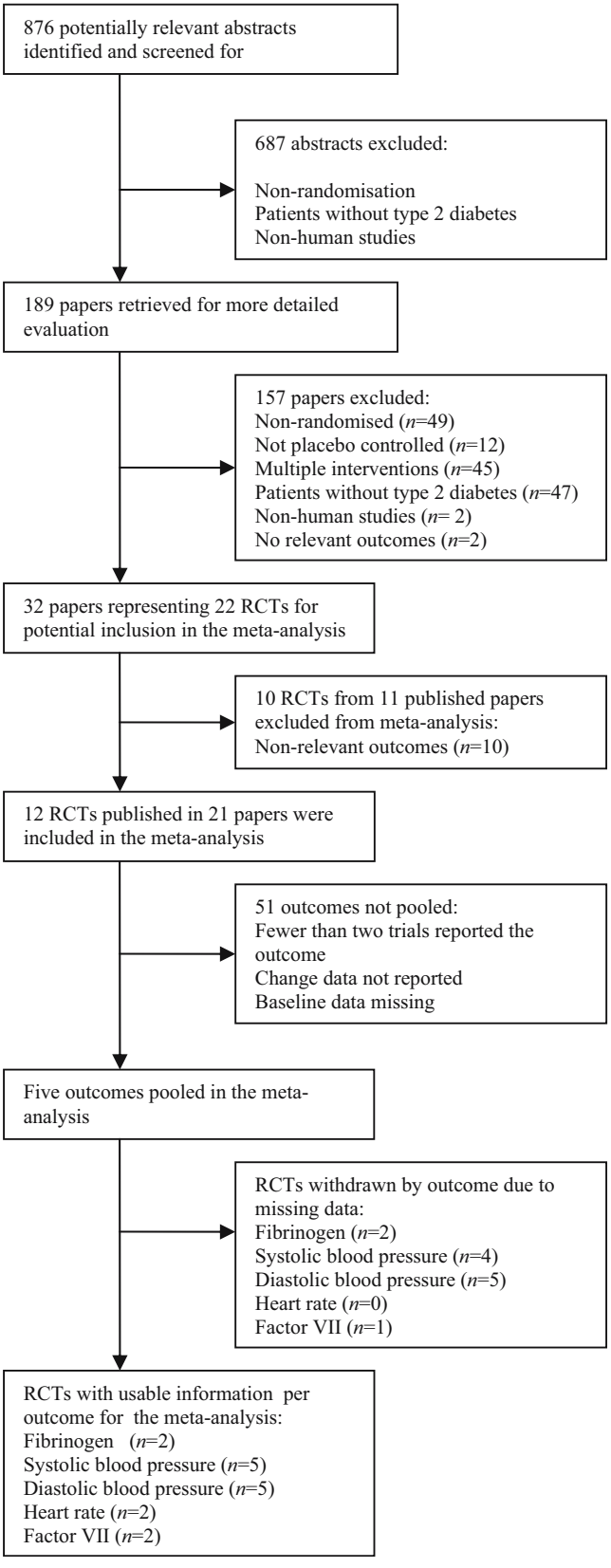

Fig. 1 Progress through the stages of the meta-analysis of randomised controlled trials (*RCT*s) of the effects of *n*-3 fatty acids on haemostatic and thrombogenic markers in type 2 diabetes. *RCT*, randomised controlled trial

pooling of five risk markers. Table 1 shows the quality score, trial size and dose of *n*-3 PUFA used in each trial. The mean dose was 4.3 g/day (median 3 g/day; range 0.9–10 g/day) with a mean trial duration of 8.5 weeks. The size of the trials included in the pooled analysis was small, with a median of 40 patients. The results of six trials were reported in more than one publication, accounting for 17 of the published papers [10, 13, 16–23, 25, 28–33]. Eight of the trials, contained in 14 publications, obtained a quality score ranging from 1 to 3 [10, 12–15, 17–21, 26, 28, 29, 34], while four trials, reporting results in seven publications, scored above 3 [11, 16, 22–25, 27]. Table 2 lists the 56 haemostatic, vascular and inflammatory markers studied, but most of these were measured only in one trial and in small numbers of patients.

One trial compared different preparations of *n*-3 PUFA with the same control group [28] and another two trials compared two different control groups with the same *n*-3 PUFA supplement [24, 35]. Three of the trials used a cross-over design, all of which presented data on the first experimental period [12, 17, 26], while the remaining nine trials used a parallel design. Eleven trials described measures undertaken to control for changes in diet during the trial [11, 15, 24, 28, 33, 35–40], and six trials advised the participants to keep their diet constant throughout the intervention [12, 14, 17, 25, 27, 32]. All of the trials included both male and female participants but two trials excluded pre-menopausal women [25, 28]. Having carried out funnel plots, the results for systolic blood pressure, but not diastolic, were found to be scattered asymmetrically.

Fibrinogen Four trials measured changes in plasma fibrinogen [12, 14, 24, 27]. The pre-specified pooled mean difference using a random-effects model based on data reported by two trials [14, 24] was −0.91 μmol/l (95% CI −3.11 to 1.28, $p=0.42$, Fig. 2) when compared with a fibre control group, but in an exploratory analysis was significant at −1.96 μmol/l (95% CI −3.13 to −0.79, $p=0.001$) when compared with an olive oil control group. The two trials [12, 27] in which the mean change and SD could not be calculated showed no change in fibrinogen (Table 2) in the *n*-3 PUFA group compared with controls.

Systolic and diastolic blood pressure Ten trials measured changes in blood pressure between groups [10–12, 14, 15, 19, 25, 26, 28, 34], but only five of these, involving 253 subjects [10, 14, 15, 19, 28] reported data that could be pooled. The pooled analysis showed *n*-3 PUFA reduced both diastolic and systolic blood pressure compared with control groups by 1.79 mmHg (95% CI −3.56 to −0.02, $p=0.05$) and 1.69 mmHg (95% CI −5.04 to 1.65, $p=0.32$), respectively (Fig. 2). There was no heterogeneity ($p>0.10$) between the results of trials measuring systolic or diastolic blood pressure

Table 1 Randomised controlled trials using *n*-3 fatty acids for management of cardiovascular risks in type 2 diabetes

References	Quality of study design (0=min, 5=max score)	Treatment (EPA/DHA content vs placebo)	No. of subjects (intervention vs placebo)	Age (years)[a]	Hypertriacylglycerolaemic patients[b]	Exclusion criteria	Subjects withdrawn (*n*)	Length of treatment (weeks)
Parallel design								
[24]	4	1.4 g EPA/0.3 g DHA vs 12 g olive oil	42 vs 21	30–73	No	Duodenal ulcer, non-compliance or unreliable, alcoholism, epilepsy, obesity, malabsorption, use of insulin, unstable angina or recent heart attack, severe hypertension, severe dyslipidaemia	5	8 weeks per 8-week washout
[14]	3	1.8 g EPA/2.4 g DHA vs 10 g olive oil	40 vs 40	56.0± 1.3	No	Pregnancy, oral contraceptives, hypercholesterolaemia, recent heart attack or stroke	NA	6 weeks
[34]	2	Low dose: 2.6 g EPA/2.4 g DHA High dose: 5.2 g EPA/4.8 g DHA vs 9 or 18 g corn oil	20 vs 20	53.9± 6.2	Yes	NA	NA	12 weeks with 4-week run-in
[21, 28, 29]	2	4 g EPA or 4 g DHA vs 4 g olive oil	35 vs 16	40–75	No	Smokers, non-hypertensive pre-menopausal, diagnosis for less than 3 months, insulin use, more than two fish meals/week or supplements, symptomatic heart disease, myocardial infarction, stroke, liver or renal disease, symptomatic autonomic neuropathy, regular use of non-steroidal anti-inflammatory drugs, recent major surgery	8	6 weeks
[10, 20]	1	8 g/day Eiconol in cod-liver oil vs standard diet with 15 g/day sunflower oil	30 vs 30	55.5± 6.6	No	Diabetes confirmed <1 year	NA	4 weeks
[15]	2	1.8 g EPA/1.2 g DHA vs placebo and diet	25 vs 15	52.3± 8.8	No	No antioxidant medication, non-obese	NA	6-week phases 4-week washout
[27]	4	Low dose: 0.9 g EPA; High dose: 1.8 g EPA vs 1.6 g olive oil	16 vs 8	37–71	No	Renal or hepatic disease, cardiovascular disease in the last 3 months, insulin use, haematological disease	0	8 weeks
[16, 22, 23, 25]	4	1.5 g EPA/1 g DHA for 2 months then 1 g EPA/0.7 g DHA for 4 months vs 3 g olive oil for 6 months	211 vs 207	58.5± 9	Yes	Duodenal ulcer, non-compliance or unreliable, alcoholism, epilepsy, obesity, malabsorption, use of insulin, unstable angina or recent heart attack, severe hypertension, severe dyslipidaemia	4	24 weeks
[11]	5	1.1 g EPA/1.5 g DHA vs 5 g of safflower oil	10 vs 10	21–65	No	Bleeding, anaemia, steroids, poorly controlled diabetes, retinopathy, use of aspirin, non-steroidal anti-inflammatory drugs	2	6 weeks
Cross-over design								
[13, 17–19]	3	1.8 g EPA/2 g DHA vs 10 g olive oil	23	45–61	No	Cerebrovascular disease, ischaemic heart disease, peripheral vascular disease, hypertension, renal impairment, cardiovascular drugs, vitamin use, lipid-lowering drugs	NA	6 weeks per phase 6-week washout
[12]	3	1.8 g EPA/1.2 g DHA vs 10 g olive oil	14	55–75	No	Renal and liver failure, hypothyroidism	0	8 weeks per phase
[26]	3	1.8 g EPA/1.2 g DHA vs 10 g olive oil	14	39–72	No	Use of lipid-lowering agents	NA	8 weeks per phase

EPA Eicosapentaenoic acid; *DHA* docosahexaenoic acid

[a] Range or mean±SD

[b] Baseline triacylglycerol >4 mmol/l

Table 2 Haematological and thrombogenic risk markers measured in randomised controlled trials of *n*-3 fatty acids on cardiovascular risk markers in type 2 diabetes

Risk factor	References	Risk factor	References
Vascular regulation		Blood viscosity	
Diastolic blood pressure	[10, 11, 14, 15, 19, 25, 26, 29, 34]	Plasma viscosity	[27]
Systolic blood pressure	[10, 11, 14, 15, 19, 25, 29, 34]	Whole blood viscosity	[27]
Mean blood pressure	[26]	Erythrocyte deformability	[27]
Heart rate	[19, 29]	Bleeding time	[24, 27]
Mean arterial pressure	[19]	Clotting time	[24]
Cardiac output	[19]	Thrombogenic factors: clotting	
Stroke volume	[19]	Fibrinogen	[12, 14, 24, 27]
Blood flow and regulation		Factor IV	[14]
Basal blood flow	[29]	Factor VII	[14, 24]
Reactive hyperaemia	[29]	Factor VII	[14, 24]
Forearm mediated dilatation	[29]	Factor X	[14]
Glyceryl trinitrate-mediated flow	[29]	Thrombogenic factors: aggregation	
Forearm blood flow	[13, 18]	Plasma β-thromboglobulin	[14]
Forearm blood flow response to L-NNMA	[18]	vWf	[29]
Forearm blood flow response to glycerol trinitrate	[13, 18]	tPA:PAI-1 ratio	[29]
Forearm blood flow after four doses of acetylcholine	[13, 18]	Collagen-induced thromboxane A2	[11]
Forearm blood flow after low dose of acetylcholine	[13]	Collagen-stimulated TXB2	[11, 14]
Forearm vascular response	[18]	ADP-stimulated TXB2	[14]
Pulse contour A2	[19]	Epinephrine-stimulated TXB2	[14]
Pulse contour A4	[19]	Serum TXB2	[11]
Pulse contour A5	[19]	TXB2 generation	[11, 29]
Vascular impedance C1	[19]	Platelet adhesion	[27]
Vascular impedance C2	[19]	Platelet aggregation to collagen	[11, 27, 29]
Vascular impedance (total peripheral resistance) R Dyne	[19]	Agonist-induced platelet aggregation	[14]
Vascular impedance Lml	[19]	Epinephrine-induced platelet aggregation	[14]
Cytokines, inflammatory markers and adhesion molecules		ADP-induced platelet aggregation	[11, 27]
IL-6	[29]	Spontaneous platelet aggregation	[14]
TNF-α	[29]	Platelet aggregation to platelet-activating factor	[27, 29]
CRP	[29]	PAI-1	[12, 29]
P-selectin	[29]	tPA	[29]

L-NNMA N^G-methyl-L-arginine; *PAI-1* plasminogen activator inhibitor-1; *tPA* tissue plasminogen activator; *TXB2* thromboxane B2; *vWf* von Willebrand factor

(Fig. 2). The outcomes for systolic blood pressure were found to be scattered asymmetrically on a funnel plot.

Of the three trials measuring systolic blood pressure that were not pooled, two showed no change [25, 34] and one showed a statistically significant decrease after *n*-3 PUFA [11]. Three of the four trials measuring diastolic blood pressure that were not pooled showed no change [11, 25, 34], and one showed a statistically significant reduction in diastolic blood pressure [26].

Heart rate Heart rate was assessed in two trials [19, 28], which included a total of 54 subjects. Heart rate in the *n*-3 PUFA group compared with the control group was 2.0 beats/min lower (95% CI −8.1 to 4.1, $p=0.52$) with no significant heterogeneity between the trials (Fig. 2).

Other risk markers and factor VII Seven studies reported outcomes on 51 measures of thrombotic factors, endothelial and vascular function and inflammation (Table 2) [11, 12, 14, 17, 24, 27, 28]. Of these, 37 individual thrombotic factors were measured by six trials [11, 12, 14, 24, 27, 28]. However, the only risk maker for which data were available from more than one trial was factor VII measured by two trials [14, 24], which had a pooled effect size of 24.9% (95% CI 7.2 to 42.6, $p=0.006$) after *n*-3 PUFA when compared with a fibre and olive oil supplemented control group

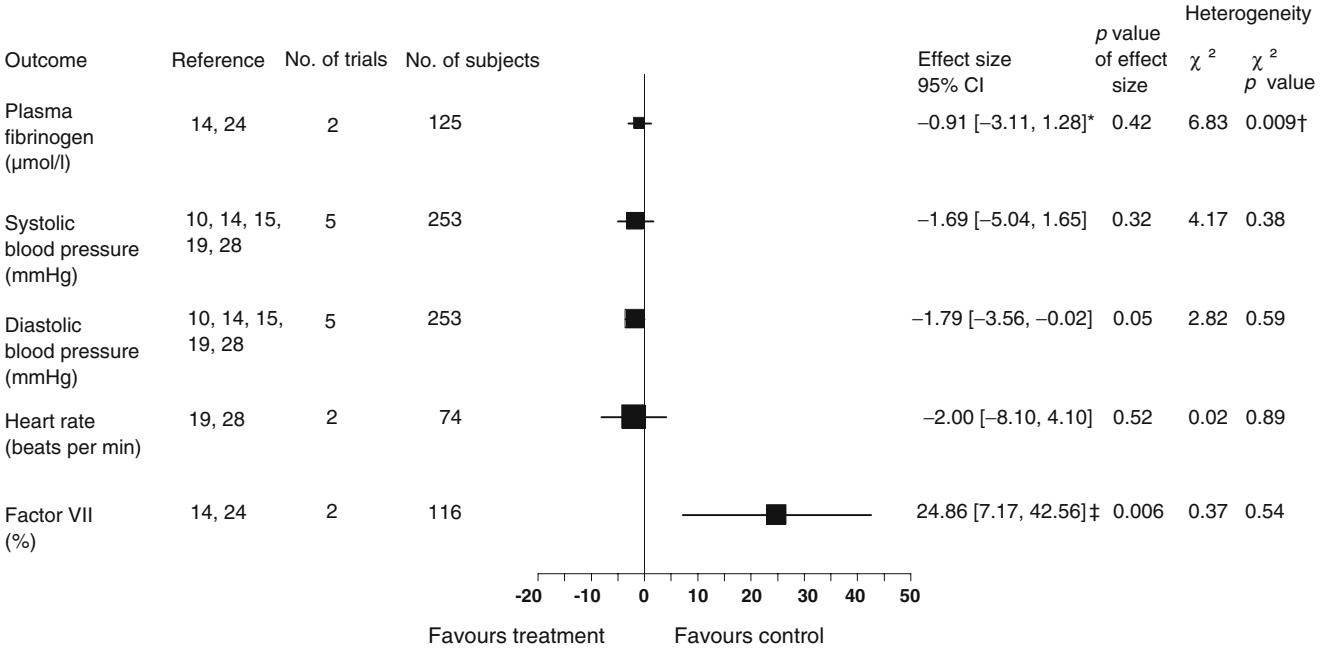

Fig. 2 Summary of the pooled effect of *n*-3 fatty acids on cardiovascular risk markers in type 2 diabetes where two or more trials reported on the marker. *Weighted mean difference, random-effects model; †heterogeneity, $p<0.10$; ‡standardised mean difference, random-effects model

(Fig. 2). There was no heterogeneity between the trials ($p>0.10$). Factor VII was unchanged in a third trial [12] but data were not reported. One trial, reported in two papers, measured the inflammatory markers IL-1, IL-6, TNF-α, CRP, P-selectin, tissue plasminogen activator and von Willebrand factor [21, 29], as well as plasminogen activator inhibitor, but reported no significant changes, although the latter risk marker was increased in another trial [12]. Another trial measured plasma viscosity, platelet adhesion and bleeding time but reported no significant change after *n*-3 PUFA [27]. However, a second study measured bleeding time after *n*-3 PUFA and noted a significant increase [24].

Discussion

Our systematic review provides the most comprehensive assessment to date of the possible effects of *n*-3 PUFA on established and emerging cardiovascular risk markers in type 2 diabetes. The results suggest that *n*-3 PUFA supplementation significantly reduces diastolic blood pressure by about 2 mm Hg. The impact on systolic blood pressure, fibrinogen and heart rate may be clinically important, although these changes did not reach conventional levels of statistical significance.

The limitations of our systematic review include the small number of trials available, with a median trial size of only 40 participants. Some of the trials failed to describe the methods of randomisation or blinding used. It was not possible to pool all the outcomes due to variability between the trials in the outcomes measured, non-standardised measurement units, failure to report change, and, importantly, a lack of two or more trials to pool for a specific outcome. The number of included trials was too few to allow us to draw firm conclusions or to undertake any subgroup analyses, and we did not obtain individual patient data. Although we included trials reported in any language to reduce selection bias, and assessed their quality, funnel plots showed that outcomes for some trials were scattered asymmetrically, which may indicate bias regarding reporting, selection or methodology of the trials. However, the funnel–plot analysis needs to be interpreted cautiously because of the small number of trials.

We are not aware of other systematic reviews including only randomised control trials that have evaluated the effect of *n*-3 PUFA on established and emerging cardiovascular risk factors in type 2 diabetes. A recent review included patients with diabetes as part of a high-risk-group analysis, but also included non-randomised control trials [41]. There are three previous systematic reviews evaluating the effect of *n*-3 PUFA on cardiovascular events, lipid and glycaemic markers in type 2 diabetes [42–44], which found *n*-3 PUFA reduced triacylglycerol, modestly increased LDL-cholesterol, and had no significant effect on fasting glucose, HbA_{1c}, or total cholesterol and HDL-cholesterol. However, unlike previous systematic reviews, we also assessed the effects on other established and emerging cardiovascular risk factors.

An earlier systematic review reported the effect of *n*-3 PUFA on blood pressure [45] in a subgroup analysis of

three trials of patients with diabetes, but that analysis included a trial that was not placebo-controlled and two of the trials had subjects with type 1 diabetes. Our pooled blood pressure results are consistent with this and an additional meta-analysis on controlled trials of the effect of *n*-3 PUFA on blood pressure in normotensive and hypertensive subjects [46], both showing a similar reduction in blood pressure. Although the reduction in blood pressure of about 2 mmHg would be expected to produce small clinical effects, it would substantially reduce population risk of cardiovascular disease [47], and even more so in patients with type 2 diabetes [48]. Tight blood pressure control has also been shown to reduce the risk of diabetes-related micro- and macrovascular complications [49].

There was a non-significant reduction in heart rate of 2 beats/min with *n*-3 PUFA, which is consistent with the results in a cross-sectional analysis of healthy men [50], a randomised control trial of overweight hypertensive subjects [51], and a prospective population-based study [52]. A recent systematic review measured changes in heart rate after *n*-3 PUFA combining all patients groups, and including two trials with patients with type 2 diabetes, showing pooled reductions of about 2 beats/min [53]. In the population-based study, 4 beats/min was shown to be the difference between patients who died a sudden death and the controls. Even small reductions in heart rate may lead to a significant public health impact because of the linear relationship between heart rate and the risk of sudden cardiac death [54], especially in type 2 diabetes [55]. However, larger trials are required to confirm whether *n*-3 supplementation in type 2 diabetes reduces heart rate.

Although our prospective analysis plan did not indicate that *n*-3 PUFA reduced plasma fibrinogen levels in type 2 diabetes compared with a fibre control, there was significant heterogeneity between the trials. An exploratory pooled comparison with an olive oil group showed a statistically significant change without heterogeneity. The heterogeneity in the pooled analysis using the fibre controls could be due to methodological differences, small study populations and high intra- and inter-individual variation. Non-significant changes in fibrinogen levels were reported by trials of hyperlipidaemic [56], hypertensive [57] and healthy subjects [58], but fibrinogen was significantly reduced in a trial on healthy individuals with genetic variations leading to high baseline fibrinogen levels [59], and significantly increased in a trial of patients with type 1 diabetes [60]. Even minor reductions in fibrinogen levels are potentially clinically important in reducing the risk of atherosclerosis [61], especially as this risk is high in diabetes [62]. It was not possible to pool the results of other reported thrombotic factors to explore the reported hypocoagulating effect of *n*-3 PUFA [2].

Increased fasting coagulant factor VII after *n*-3 PUFA supplementation has been reported in two other trials in healthy subjects [63, 64]. However, one of these two trials found a simultaneous reduction in prothrombin factor X required in the process of clotting [64], and the authors suggested that clotting was therefore not necessarily activated. An earlier study [65] also found that postprandial elevation of activated factor VII did not cause a concomitant activation of factor X, thereby not confirming enhanced coagulation. Factors IV and X and thromboglobulin were measured in one of the trials identified in our pooled analysis [14], and were reported not to be significantly changed after *n*-3 PUFA supplementation. The increase of coagulant factor VII observed in this review may be due to chance but might indicate an adverse effect of *n*-3 PUFA. This requires further research as increased plasma activity of factor VIIc is associated with CHD risk factors such as hypertriacylglycerolaemia, reduced glucose tolerance, overweight and hyperinsulinaemia, but may also be an independent risk factor for CHD [66–68], and higher levels were observed in healthy subjects with coronary events [69]. However, the independent association with CHD was not shown in another cohort study [70].

More rigorously designed and conducted large randomised controlled trials of longer duration reporting details of randomisation are required that measure both established and emerging cardiovascular risk markers in type 2 diabetes using standardised assays to provide sufficient statistical power to assess outcomes convincingly. Larger population samples will also improve the precision of the effect size estimates and establish conclusively the role of *n*-3 PUFA in CHD risk reduction in type 2 diabetes. One trial subgroup analysis awaits reporting [71], and four additional trials are in progress [72–75].

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Duality of interest The authors declare no duality of interest.

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