

Review Article

Do long-chain *n*-3 fatty acids reduce arterial stiffness? A meta-analysis of randomised controlled trials

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Abstract

Fish oils, rich in long-chain *n*-3 PUFA, are known to reduce various risk factors for CVD. However, conclusive evidence regarding the benefits of *n*-3 on arterial stiffness, a risk factor for CVD, has not yet been established. Consequently, we conducted the first study aimed to quantify the effects of *n*-3 supplementation on arterial stiffness through meta-analysis. Multiple databases and clinical trial registries were systematically searched up until September 2010 for randomised and controlled adult human clinical trials to investigate the effects of long-chain *n*-3 fatty acids on arterial stiffness. No limits were set on dosage sizes or sample characteristics. A total of ten *n*-3 trials met the final inclusion criteria; four using pulse wave velocity (PWV) and six using arterial compliance, measured as capacitive compliance or systemic arterial compliance, as respective outcome measures. Meta-analysis revealed that *n*-3 was statistically significant in effectively improving both PWV ($g = 0.33$; 95% CI 0.12, 0.56; $P < 0.01$) and arterial compliance ($g = 0.48$; 95% CI 0.24, 0.72; $P < 0.001$). There was no evidence of heterogeneity or publication bias. Results were not influenced by changes in blood pressure, heart rate or BMI. The findings of the present study reveal that supplementation with *n*-3 offers a scientifically supported means of reducing arterial stiffness. Reduction in arterial stiffness by *n*-3 may account for some of its purported cardioprotective effects.

Key words: Arterial stiffness; Meta-analysis; *n*-3 PUFA; Pulse wave velocity; Arterial compliance; Hypertension; Fish oil

Arterial stiffness has recently emerged as a significant predictor of CVD. A recent meta-analysis of seventeen longitudinal studies confirmed aortic stiffness as a significant predictor of future cardiovascular events and all-cause mortality⁽¹⁾. Putative relationships also exist between arterial stiffness and brain as well as kidney end-organ damage⁽²⁾. Consequently, reducing arterial stiffness may reduce the risk of future cardiovascular events, all-cause mortality and end-organ damage, thus alleviating the socio-economic burden and personal costs associated with CVD. Thereby, evidence-based approaches to reducing arterial stiffness are of significant clinical importance.

n-3 PUFA, found abundantly in fatty fish, are emerging as a clinically useful treatment in cardiovascular medicine⁽³⁾.

Fish oils have long been known to be mildly hypotensive⁽⁴⁾, with more recent quantitative analysis suggesting benefits in cardiac electrophysiology in terms of lowering heart rate⁽⁵⁾ and preventing arrhythmia⁽⁶⁾ as well as benefits to those with CHD by reducing mortality⁽⁷⁾. Although current evidence from randomised and controlled human clinical trials suggests that fish oils may be beneficial in reducing arterial stiffness, no quantitative synthesis of this data has been conducted. Consequently, we aimed to assess the scientific evidence for *n*-3 PUFA in the treatment of arterial stiffness by conducting the first systematic review and meta-analysis of randomised and controlled clinical trials exploring the chronic effects of long-chain *n*-3 fish oils on pulse wave velocity (PWV) or arterial compliance. No limits were imposed on the nature

Abbreviations: BP, blood pressure; C1, capacitive arterial compliance; PWV, pulse wave velocity; SAC, systemic arterial compliance; SMD, standard mean difference.

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of the sample populations with the exception that trials must be conducted in adult human subjects.

Methods

Outcomes

To limit heterogeneity between study outcomes, we limited the analysis to only a few valid indices of arterial stiffness, including PWV, central haemodynamic indices and arterial compliance, measured as either capacitive arterial compliance (C1) or systemic arterial compliance (SAC). These specific indices of arterial stiffness were selected given their validity and frequency of use in the area of nutritional interventions and arterial stiffness^(8–13).

Study eligibility

Located studies were only considered for inclusion if they were randomised and controlled, used long-chain *n*-3 PUFA (i.e. DHA or EPA) supplementation as a monotherapy and at a specific dosage (the administration of an array of foods enriched with *n*-3 was not considered appropriate), involved chronic or sub-chronic administration periods (defined as >1 month), were conducted on adult human subjects and were written in English. No limits were set on the trial dosage sizes or the nature of the sample populations.

Literature search

MEDLINE (PubMed), Web of Science, The Cochrane Library and PsycINFO were searched up to September 2010 with the search terms 'fish oil' or 'omega-3' or 'polyunsaturated' or 'essential fatty acids' or 'EPA' or 'DHA' or 'Docosahexaenoic' or 'Eicosapentaenoic' combined with the terms; 'arterial stiffness' or 'pulse pressure' or 'wave reflections' or 'augmentation index' or 'pulse wave velocity' or 'aortic stiffness' or 'central pressure' or 'arterial compliance' or 'elasticity'. To locate unpublished literature, we searched clinical trial registries including the Australian and New Zealand Clinical Trials Registry and ClinicalTrials.gov for relevant trials. Corresponding investigators were contacted to locate appropriate data.

One out of two contacted investigators provided unpublished results that were included in the meta-analysis. Backward searches were performed manually on studies meeting the inclusion criteria while forward searches were performed using the Web of Science.

Data extraction

Article searching, assessment of inclusion criteria and article quality as well as data extraction were completed independently by two researchers (M. P. P. and N. A. G.) before results were combined via mutual group consensus. For consistency, PWV was converted into cm/s and C1 into ml/mmHg × 10. In trials with multiple treatment arms comprising parallel groups of participants, each group's data were extracted separately for analysis. In trials with multiple treatment arms and a single control group, the sample size of the control group was divided by the number of treatment groups, avoiding duplication of the control group sample size, thus reducing the probability of a type 1 error⁽¹⁴⁾. Where SD were not reported, they were back calculated using the sample size and either the SE or the CI. One trial did not report the number of dropouts or the mean age of participants⁽¹⁵⁾. Here the reported sample size was assumed to reflect the number of subjects who completed the trial and the mean age was reported as the mean of the sample age range.

Assessment of trial quality

The scientific quality of each study was assessed and given a score from 0 to 10 using a purpose-designed scale, with higher scores reflecting superior quality. Based on the original Jadad scale⁽¹⁶⁾, the augmented scale provides a more rigorous assessment of methodological quality and the assessment has been published in previous systematic reviews⁽¹⁷⁾.

Statistical analysis

Meta-analysis was conducted using Comprehensive Meta-Analysis version 2 (Biostat, Englewood, NJ, USA). Data were entered as pre- and post-intervention means and standard deviations as well as sample size for both the placebo and

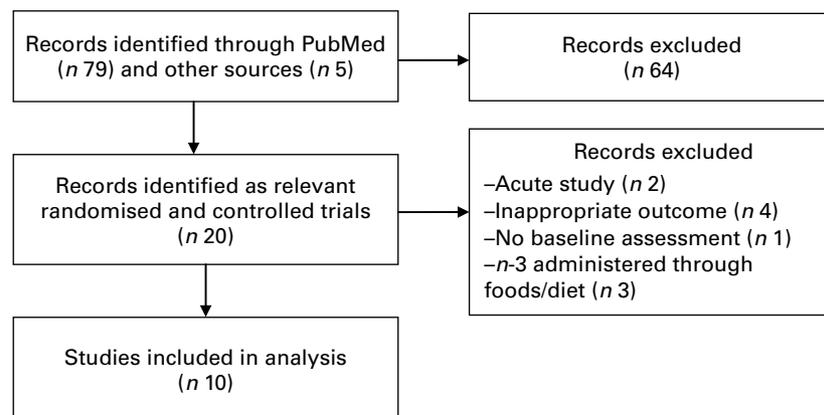


Fig. 1. Systematic review flowchart.

Table 1. Study characteristics of *n*-3 trials included in meta-analyses

First author	Dose	Design	Duration (weeks)	<i>n</i>	Concomitant medications	Outcome	Sample	Mean age (years)	Male (%)	Dropout (%)	Quality rating*
Hill <i>et al.</i> (2007) ^{(19)†}	1560 mg DHA + 360 mg EPA/d v. placebo (6 g sunflower oil/d)	Ran PG DB PC	6 and 12	38	No BP or lipid-lowering medications	C1	Overweight and high BP, cholesterol or TAG	FO = 52 PL = 51	FO = 35 PL = 39	7	8/10
McVeigh <i>et al.</i> (1994) ⁽¹⁵⁾	1800 mg EPA + 1200 mg DHA/d v. placebo (olive oil)	Ran CR DB PC	6	20	No cardiovascular medications	C1	Diabetic	53	80	0	5/10
Sjoberg <i>et al.</i> (2010) ⁽²¹⁾	520 mg DHA + 120 mg EPA/d v. 1040 mg DHA + 240 mg EPA/d v. 1560 DHA + 360 mg EPA/d v. placebo (sunola oil)	Ran PG DB PC	12	67	No blood-thinning, BP- or lipid-lowering medications	C1	Overweight	FO = 52-54 PL = 51	FO = 45-50 PL = 57	11	8/10
Wang <i>et al.</i> (2008) ⁽²²⁾	540 mg EPA + 360 mg DHA v. placebo capsules	Ran PG DB PC	8	52	Ceased 2 weeks before study	C1	Overweight, hypertensive	FO = 43 PL = 43	FO = 90 PL = 78	17	7/10
Meyer <i>et al.</i> (2007) ⁽²⁰⁾	1080 mg DHA/d v. 2160 mg/d v. placebo (olive oil)	Ran PG PC	12 and 24	35	Statins	C1	Hyperlipidaemic	FO = 53-58 PL = 59	67	5	6
Anderson‡	1800 mg EPA and 1100 mg DHA/d v. placebo (olive oil) in context of low or high <i>n</i> -6 diet (2 × 2 factorial design)	Ran PG DB PC	8	64	No medications	rc-PWV	Healthy young males	25-6	100	NA	NA
Mita <i>et al.</i> (2007) ⁽²⁶⁾	1800 mg EPA/d v. control (no EPA)	Ran PG	105	60	Matched across groups	ba-PWV	Type 2 diabetes	FO = 59 PL = 61	FO = 53 PL = 67	26	5/10
Satoh <i>et al.</i> (2009) ⁽²⁴⁾	1800 mg EPA/d + diet v. control (diet only)	Ran PG SB	12	92	No statins, ACE inhibitors or ARB	ca-PWV	Metabolic syndrome	FO = 51 PL = 52	FO = 43 PL = 41	0	6/10
Tomiyama <i>et al.</i> (2005) ⁽²⁵⁾	1800 mg EPA/d v. control (diet therapy)	Ran PG	52	84	Not specified	ba-PWV	Dyslipidaemia	FO = 65 PL = 64	FO = 55 PL = 43	4	5/10
Nestel <i>et al.</i> (2002) ⁽²³⁾	3000 mg EPA/d v. 3000 mg DHA/d v. placebo (olive oil)	Ran PG DB PC	7	38	No BP- or lipid-lowering medications	SAC	Dyslipidaemic	FO = 55-57 PL = 61	FO = 42 PL = 64	7	7/10

Ran, randomised; PG, parallel groups; DB, double blind; PC, placebo controlled; BP, blood pressure; C1, capacitive arterial compliance; FO, fish oil; PL, placebo; CR, cross-over; rc, radial-carotid; PWV, pulse wave velocity; NA, not available to be calculated; ba, brachial-ankle; SB, single blind; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; ca, cardio-ankle; SAC, systemic arterial compliance.

* Quality rating derived from augmented Jadad scale.

† Only those arms relevant to meta-analysis are presented.

‡ Unpublished results (AD Andersen, M Seest, N Wiinberg, C Haedersdal, CT Damsgaard and L Lauritzen (unpublished results)). See Damsgaard *et al.*⁽²⁷⁾ for associated publication.

treatment groups (standardised by the post-score standard deviations). The mean effect size (standard mean difference, SMD) across trials was computed using a random effect model and was expressed as a Hedges's *g*. The existence of bias was tested statistically using Begg and Egger regression tests, and by a funnel plot to assess diagrammatic symmetry. Heterogeneity in effect sizes between studies was tested using Higgins I^2 test⁽¹⁸⁾. All results were considered statistically significant at $P < 0.05$. Meta-analyses were conducted separately for PWV and arterial compliance (combining both C1 and SAC). Meta-regressions were also conducted to examine whether SMD were correlated with trial sample sizes, trial quality or changes in blood pressure (BP), heart rate or BMI.

Results

Qualitative summary

As detailed in Fig. 1, the initial search revealed seventy-nine hits in PubMed. An addition four relevant, randomised and controlled studies were searched through other databases and one through a clinical trial registry. Of the twenty relevant studies, ten did not meet the inclusion criteria and were eliminated from analysis.

In total, ten trials met the inclusion criteria, creating a pooled sample of 550 participants (Table 1). Of the ten trials, five implemented C1^(15,19–22) and one SAC⁽²³⁾. A further three published trials^(24–26) and one unpublished trial (AD Andersen, M Seest, N Wiinberg, C Haedersdal, CT Damsgaard and L Lauritzen, unpublished results from Damsgaard *et al.*⁽²⁷⁾) implemented PWV (two at the brachial-ankle and two at the cardio-ankle and radial-carotid sites, respectively). No published trials used central haemodynamic indices or carotid-femoral PWV as outcomes of arterial stiffness. Samples ranged from healthy men to participants who were overweight, diabetic, hypertensive, dyslipidaemic or hyperlipidaemic. Trial durations ranged from 6 to 105 weeks while dosages ranged from 640 to 3000 mg of combined EPA and DHA/d, administered through capsules. In all the published trials, respective changes in arterial stiffness occurred in the absence of significant changes in BP.

The average trial quality rating was 6.3/10. Although all trials were randomised and controlled, the three published trials that used PWV as the primary outcome of arterial stiffness did not implement matching placebos and were thereby not double blind^(24–26).

Quantitative summary

As displayed in Fig. 2, when examining the four trials to implement PWV (five data sets, n 300), meta-analysis revealed a significant pooled SMD of 0.33 (95% CI 0.12, 0.56; $P < 0.01$) indicating a beneficial effect of *n*-3 supplementation on PWV compared with control. There was no evidence of heterogeneity between studies ($I^2 = 0\%$, $P = 0.91$). When examining only those studies to measure PWV across the aorta (data not shown)^(24–26), the pooled SMD remained significant (SMD = 0.29, 95% CI 0.03, 0.54; $P < 0.05$) without evidence of heterogeneity ($I^2 = 0\%$, $P = 0.84$). As seen in Fig. 3, across the six trials to investigate the effects of *n*-3 on either C1 or SAC (thirteen data sets, n 250), meta-analysis revealed a significant pooled SMD of 0.48 (95% CI 0.24, 0.72; $P < 0.001$), suggesting a beneficial effect of *n*-3 on arterial compliance relative to placebo. There was no evidence of heterogeneity ($I^2 = 0\%$, $P = 0.99$). Analysis of only those studies using C1 as an outcome of arterial stiffness (data not shown) revealed that the effect of *n*-3 was independently significant on C1 (SMD of 0.45; 95% CI 0.20, 0.70; $P < 0.001$) without evidence of heterogeneity ($I^2 = 0\%$, $P = 0.98$) or bias (Begg test, $P = 0.16$; Egger test, $P = 0.10$).

Inspection of funnel plots (Fig. 4) revealed no significant asymmetry while the Begg and Egger tests confirmed no evidence of bias across those trials using PWV (Begg test, $P = 0.22$; Egger test, $P = 0.13$) and arterial compliance (Begg test, $P = 0.75$; Egger test, $P = 0.64$).

Meta-regression between each study's SMD and their sample size (figures not shown) revealed that effect sizes were not correlated with trial sample size (regression coefficient = 0.77, $P = 0.38$). Moreover, meta-regression indicated that trial results were not influenced by their objectively rated quality (regression coefficient: -1.16 , $P = 0.28$).

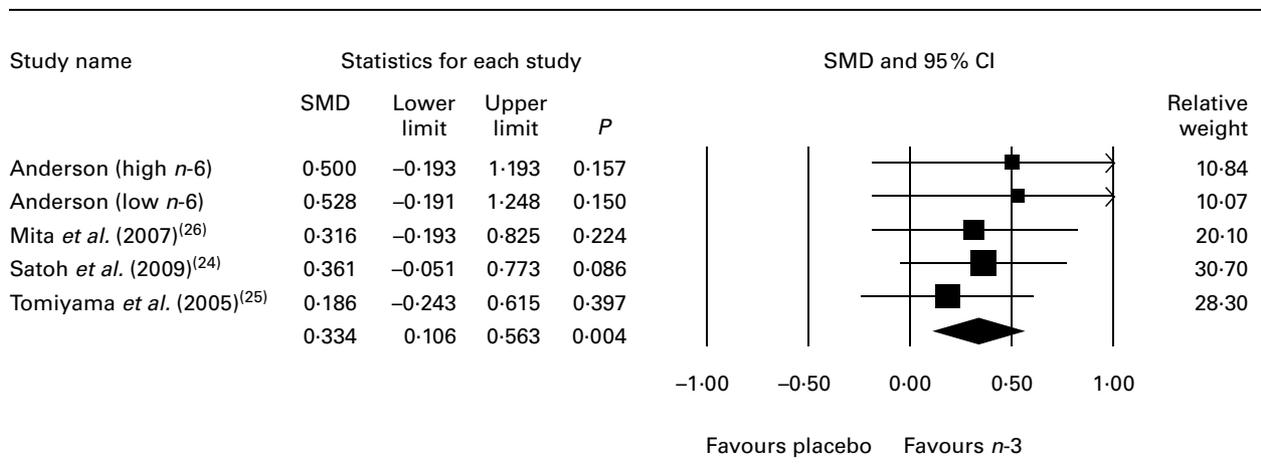


Fig. 2. Forest plot showing the effects of *n*-3 on pulse wave velocity. SMD, standard mean difference.

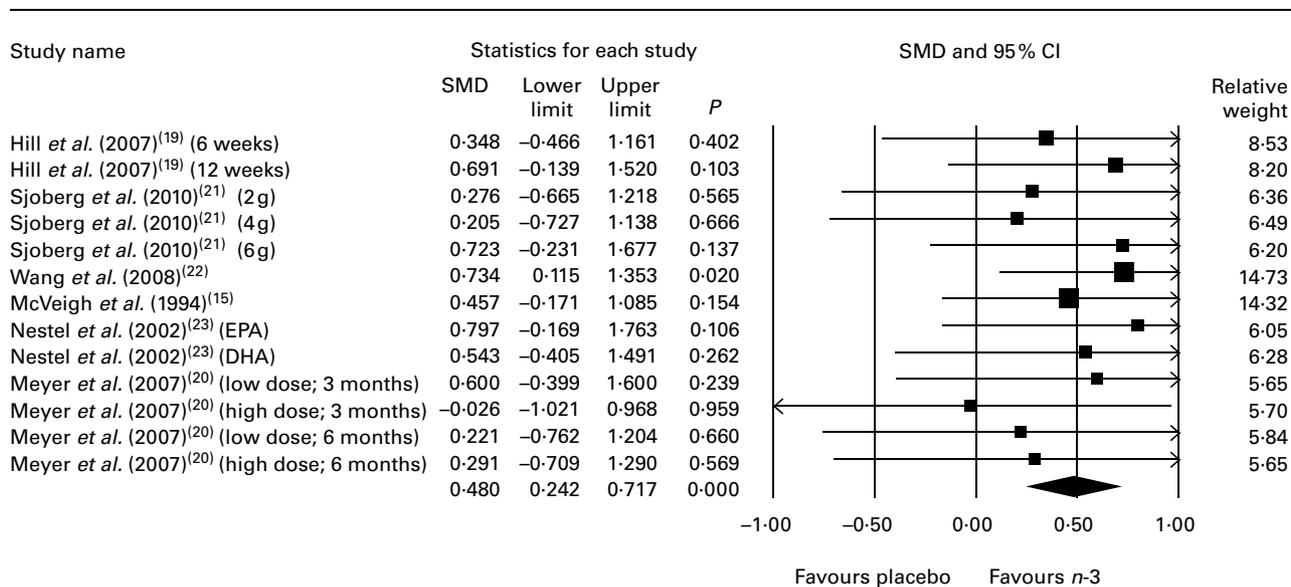


Fig. 3. Forest plot showing the effects of *n*-3 on capacitive and systemic arterial compliance. SMD, standard mean difference.

Meta-regression was also conducted to investigate whether results were correlated with concomitant changes in other physiological variables that could potentially influence arterial stiffness. Results indicated that SMD were independent of changes in systolic BP (regression coefficient = -0.82, $P=0.41$) and diastolic BP (regression coefficient = -0.72, $P=0.47$). Although the appropriate data were reported in only five studies, results appeared neither dependent on changes in heart rate (regression coefficient = 0.03, $P=0.98$) nor on BMI (regression coefficient = 0.07, $P=0.50$).

Discussion

Meta-analysis of randomised and controlled human clinical trials provides compelling evidence that supplementation with long-chain *n*-3 PUFA offers a scientifically supported means of reducing arterial stiffness. It was found that *n*-3 fatty acids were effective in independently improving both PWV and arterial compliance with small-to-moderate clinical

effects. There was no statistical evidence of heterogeneity or publication bias, thus confidence can be extended to these results.

All published trials that reported a benefit of *n*-3 supplementation on arterial stiffness did so in the absence of significant BP changes. While suggesting that *n*-3 fatty acids improve arterial stiffness through a BP-independent mechanism, these results are surprising given the documented hypotensive actions of *n*-3⁽²⁸⁾. Providing additional insight, a meta-regression revealed no significant association between trial effect sizes and changes in BP in response to *n*-3; this further suggests a BP-independent mechanism. Available data also suggested that reductions in arterial stiffness were not related to changes in heart rate or BMI. However, given that most studies included in meta-analysis were of relatively short duration, it is likely that observed reductions in stiffness were the result of *n*-3 modulating the functional rather than structural mechanisms underpinning arterial stiffness.

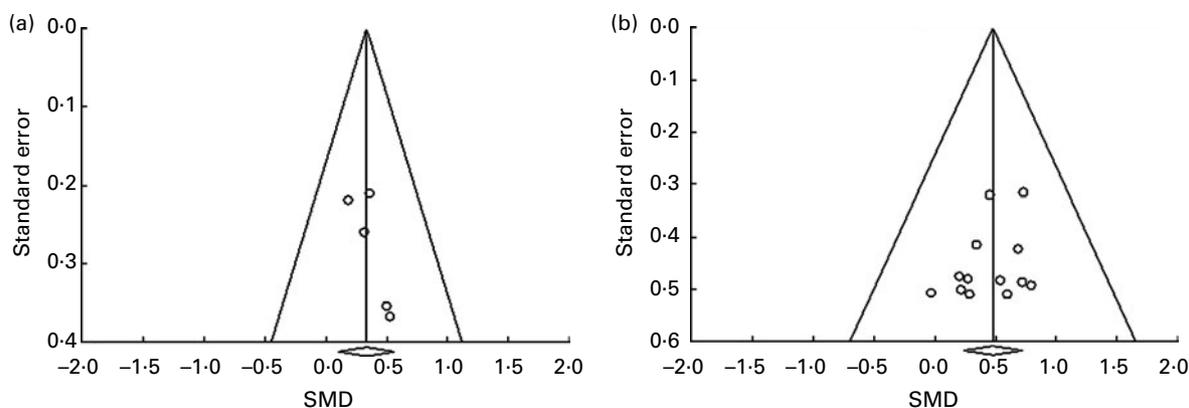


Fig. 4. Funnel plots of SE by standard difference in means (SMD). Plots are presented separately for both (a) pulse wave velocity and (b) arterial compliance (combining capacitive and systemic arterial compliance) meta-analyses.

Such functional mechanisms may include changes in aortic BP and wave reflections, which are distinct from brachial BP.

Others have posited that n-3 PUFA may reduce the risk of CVD through anti-arrhythmic, anti-thrombogenic, hypotensive, anti-inflammatory, hypertriacylglycerolaemic, endothelial NO-stimulating and atherosclerotic plaque growth-inhibiting mechanisms^(29,30). The findings of this study reveal that the cardioprotective effects of n-3 may also be explained by the ability of n-3 to reduce arterial stiffness. As inflammatory processes⁽³¹⁾, BP⁽³²⁾ and NO production⁽³³⁾ are all implicated in the functional regulation of arterial stiffness, the aforementioned mechanisms may work synergistically to reduce both arterial stiffness and CVD risk in the context of n-3 supplementation. Given the predictive value of arterial stiffness in CVD, its measurement has widely increased in clinical settings⁽³⁴⁾. The present findings, therefore, further support the use of n-3 fatty acids in clinical settings to reduce CVD risk.

The following potential limitations of the meta-analyses warrant discussion. First, as only English language articles were searched we cannot exclude the possibility of omitting articles published in foreign languages. Second, a number of trials implementing PWV were found to have low methodological quality. However, supporting the validity of our findings, meta-regression indicated that results were independent of trial quality. Carotid-femoral PWV is regarded as the 'gold standard' in the measurement of arterial stiffness and has the highest predictive validity⁽³⁴⁾. As no located trial has assessed the effects of n-3 on carotid-femoral PWV, the pooled estimate of PWV in this study reflects the recording of this measure at other sites. Central haemodynamic indices, such as aortic pulse pressure and augmentation index, are also established predictors of CVD⁽¹⁰⁾. To the best of our knowledge, no trial has been reported on the chronic effects of n-3 on these measures. Although the measures used in the present meta-analysis, such as brachial-ankle PWV and SAC, are also valid and predictive of CVD and or mortality^(13,35–37), given the superior predictive validity of carotid-femoral PWV and central haemodynamic indices, exploring the effects of n-3 on these 'gold standard' measures remains an important area for future research. Extending our findings, future large-scale clinical trials could investigate whether reducing arterial stiffness, by way of n-3 supplementation, leads to a decreased risk of CVD and end-organ damage. Furthermore, although we report that n-3 fatty acids are effective in reducing arterial stiffness across diverse samples and with varying dosages, further research is still required to determine the optimal dosages of EPA and DHA for reducing arterial stiffness.

The present meta-analytic study suggests that long-chain n-3 PUFA offer a scientifically supported means of reducing arterial stiffness and this may account for some of the purported cardioprotective effects of n-3. As increased arterial stiffness is a risk factor for CVD, n-3 supplementation may provide a means of reducing the risk of CVD and end-organ damage. Given that carotid-femoral PWV is a strong predictor of future cardiovascular events⁽³⁴⁾, exploring the effects of n-3 on this 'gold standard' measure constitutes an important area for future research.

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