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Omega-3 polyunsaturated fatty acid supplementation for improving peripheral nerve health: protocol for a systematic review

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ABSTRACT

Introduction: Damage to peripheral nerves occurs in a variety of health conditions. Preserving nerve integrity, to prevent progressive nerve damage, remains a clinical challenge. Omega-3 polyunsaturated fatty acids (PUFAs) are implicated in the development and maintenance of healthy nerves and may be beneficial for promoting peripheral nerve health. The aim of this systematic review is to assess the effects of oral omega-3 PUFA supplementation on peripheral nerve integrity, including both subjective and objective measures of peripheral nerve structure and/or function.

Methods and analysis: A systematic review of randomised controlled trials that have evaluated the effects of omega-3 PUFA supplementation on peripheral nerve assessments will be conducted. Comprehensive electronic database searches will be performed in OViD Medline, Embase, the Cochrane Central Register of controlled Trials (CENTRAL), US National Institutes of Health Clinical Trials Registry and the World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP). The title, abstract and keywords of identified articles will be assessed for eligibility by two reviewers. Full text articles will be obtained for all studies judged as eligible or potentially eligible; these studies will be independently assessed by two reviewers to determine eligibility. Disagreements will be resolved by consensus. Risk of bias assessment will be performed using the Cochrane Collaboration risk of bias tool to appraise the quality of included studies. If clinically meaningful, and there are a sufficient number of eligible studies, a meta-analysis will be conducted and a summary of findings table will be provided.

Ethics and dissemination: This is a systematic review that will involve the analysis of previously published data and therefore ethics approval is not required. A

manuscript reporting the results of this systematic review will be published in a peer-reviewed journal and may also be presented at relevant scientific conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This will be the first systematic review to consider the efficacy and safety of omega-3 PUFA supplementation on peripheral nerve structure and function.
- This systematic review will only consider data from randomised controlled trials, which provide the highest level of evidence for single intervention studies.
- This review will be conducted according to the Cochrane Handbook for Systematic Reviews of Interventions, and in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement.
- As we will include studies that have evaluated the use of omega-3 PUFA supplementation for treating any form of peripheral nerve damage, there may be limited scope to perform a meta-analysis due to clinical heterogeneity.
- There are currently no gold standard outcome measures for assessing peripheral neuropathy, which may affect the capacity to quantitatively synthesise data from individual studies to derive clear estimates of treatment effect(s).

BACKGROUND

Description of the condition

The peripheral nervous system encompasses the nerves mediating sensory, motor and autonomic functions that are located outside of the brain and spinal cord. Alterations to the anatomical integrity of the peripheral nerves can adversely affect their function, presenting clinically as abnormal or loss of sensation, weakness and/or as changes to autonomic function.[1] England describes peripheral neuropathy as "a general term that indicates any disorder of the peripheral nervous system;"[2] this is a broad definition that includes nerve damage due to a variety of aetiologies. The pathophysiological mechanisms underlying both the development and progression of peripheral neuropathy are complex and may depend on the cause. Mechanisms may involve altered metabolism and intracellular signaling,[3] vascular and inflammatory stress,[4] and reactive oxygen species formation.[5]

The most common systemic cause of peripheral neuropathy, which is evident in over 50% of individuals affected by the condition, is diabetes mellitus; the risk of peripheral neuropathy increases with longer disease duration,[6] and is correlated with the degree of glycaemic control.[7] Other causes include hereditary neuropathies (e.g., Charcot-Marie-Tooth Syndrome), post-infectious and inflammatory neuropathies (e.g., Guillain-Barré Syndrome) and drug-induced neuropathies (e.g. platinum analogues, thalidomide, alcohol).[8] Up to one-third of cases do not have an identified aetiology, and are thus defined as idiopathic peripheral neuropathies.[9]

Clinical evaluations of peripheral nerve integrity generally include a combination of symptoms, signs and electrodiagnostic studies, which aim to evaluate the extent of

nerve damage.[2] Symptoms and signs of nerve damage are often assessed using validated neuropathy scales or composite scores (which combine symptomatology with clinical measures of nerve function). Nerve biopsies are invasive and, as a result, not easily repeatable, and are therefore not frequently used as an outcome parameter in longitudinal studies, but are rather reserved for diagnostic purposes.[10]

Electrodiagnostic testing examines the characteristics of the conduction of an electrical signal that travels through a single nerve. These tests are useful in providing diagnostic information and for longitudinally monitoring disease progression.[11] Nerve conduction studies are reproducible and correlate well with underlying structural abnormalities,[12] but the precision of these tests is limited to detecting changes in large myelinated nerve fibres, as they are not sufficiently sensitive to detect small nerve fibre damage.[13, 14] Quantitative sensory tests, which quantify thermal and pain thresholds, can be used to evaluate small nerve fibre function.[14] Skin biopsies offer an alternative method to accurately diagnose and classify the extent of small fibre neuropathy, even in the absence of large fibre nerve damage.[14, 15] Cutaneous silent period (CSP) testing is a reproducible measurement of the nonciceptive spinal reflex where thinly myelinated A-delta fibres are the afferent arm. Quantitative sudomotor axonal reflex testing (QSART) assesses the function of unmyelinated post-ganglionic sudomotor C-fibres.[16, 17] These are amongst several other methods to assess various small fibre types, and as each individual test may have a relatively low sensitivity a combination of modalities is usually preferable to better assess small fibre function.[18]

Recently, corneal confocal microscopy has been applied to visualise small nerve fibres *in vivo*.[19] This technique has been shown to correlate well with intraepidermal nerve fibre biopsy results[20] and is useful for detecting and documenting various types of small fibre neuropathies.[21-24] Corneal confocal microscopy has also been suggested to be useful for monitoring disease progression, and as a marker for improvements in nerve function, in the investigation of therapeutic targets for diabetic peripheral neuropathy.[25-27]

Peripheral neuropathies are typically treated based on their subtype and/or underlying cause(s). Treatments primarily aim to manage the underlying condition to prevent progressive nerve damage and to treat any associated symptoms.[2, 13, 28] The consequences associated with symptoms of neuropathic impairment do not only affect an individual's quality of life, but remain an economic burden in the cost of healthcare and medical resources.[29-31] This is especially true in chronic conditions such as diabetes, where lifetime care is required.[3]

Description of the intervention

Omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids (EFAs) with multiple double bonds, the first of which is located at the third carbon from the methyl end of the molecule. Short-chain omega-3 PUFAs, alpha-linolenic acid (ALA), found in plant sources, is a metabolic precursor to the long-chain omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are present in high abundance in oily fish. As humans do not have the enzymes to synthesise these fatty acids *de novo*, omega-3 PUFAs must be obtained from the diet or through supplementation.[32] The other major class of EFAs are the omega-6 fatty

acids, which derive from the diet in the form of linolenic acid (LA) and are elongated *in vivo* to gamma-linoleic acid (GLC) and arachidonic acid (AA). Most eicosanoids derived from the omega-6 dependent AA-pathway are pro-inflammatory; in contrast, long-chain omega-3 fatty acids bias prostaglandin metabolism towards the production of anti-inflammatory eicosanoids.

As omega-3 PUFAs competitively inhibit the metabolic conversion of omega-6 PUFAs,[33] the balance of omega-3 to omega-6 fatty acid consumption can affect systemic inflammatory processes and immune activity. The ratio of consumed omega-6 to omega-3 in typical Western diets is approximately 15 to 1, whereas a ratio of 4 to 1 is considered optimal.[34] Increased consumption of omega-3 PUFAs is considered to provide a range of potential general health benefits, including a reduced risk of cardiovascular disease[35, 36] and lowered systemic triglycerides[37]. DHA, as an integral component in cellular membrane structures of the brain and retina, has been implicated in perinatal visual and neural development.[38-40] In ocular conditions, omega-3 fatty acids supplements can reduce the symptoms and clinical signs associated with ocular surface inflammation in dry eye disease.[41] The American Heart Foundation recommends a daily intake, for adults, of 500mg of long-chain omega-3 PUFAs,[42] and up to 4g/day in hypertriglyceridemia.[43]

How the intervention might work

Once consumed, omega-3 PUFAs alter membrane protein activity and cellular signaling response, to reduce immune activity and the concentration of systemic lipid inflammatory mediators.[44] The incorporation of omega-3 PUFAs into cellular membranes, and their subsequent effect on membrane activity, has been shown to

alter vascular function, improve sciatic nerve blood flow and enhance nerve conduction velocity in a rat model of experimental diabetic neuropathy.[45]

Omega-3 PUFAs also affect intracellular signalling pathways and the expression of genes, some of which may be associated with the regulation of neuron growth and neuroprotection.[46] In animal models of diabetes, omega-3 PUFA supplementation has been shown to attenuate adverse changes in nerve structure and function.[47, 48] Mice enriched with genes that increase endogenous profiles of omega-3 PUFAs have been shown to have reduced neuronal cell death and increased recovery to mechanical stress and peripheral nerve injury.[49] Omega-3 PUFAs have also been demonstrated to promote neurite growth in rat sensory neurons.[50]

Derivatives of omega-3 PUFA metabolism, resolvins and protectins, which are oxygenated metabolites from EPA and DHA respectively, may further promote neuronal function. Neuroprotectin D-1 has been shown to facilitate the regeneration of corneal nerves following refractive surgery and neurite growth from the trigeminal ganglion of mice [51] and to prevent neuropathic pain after peripheral nerve injury.[52]

Why it is important to do this review

This will be the first systematic review to consider the potential effects of omega-3 PUFA supplementation on peripheral nerve integrity. Omega-3 PUFA supplementation has been shown to reduce neuronal damage and enhance recovery following nerve injury in experimental animal models of peripheral neuropathy. Confirmation of these effects in clinical populations would contribute significantly

towards enhancing the clinical management of peripheral neuropathy. A therapeutic agent to prevent the pathogenesis of, or slow the progression of, peripheral nerve damage has the potential to greatly improve clinical outcomes. Furthermore, the potential for omega-3 PUFA supplements to alleviate neuropathy-associated symptoms would be predicted to reduce impairment on an individual's quality of life and lessen the economic burden of peripheral neuropathy in the community.

OBJECTIVES

The primary objective of this systematic review is to evaluate the efficacy and safety of oral omega-3 PUFA supplements for improving peripheral nerve health. Efficacy outcomes will consider both subjective endpoints (i.e., symptoms) and objective clinical measures, including changes to peripheral nerve structure and function.

METHODS AND ANALYSIS

We will conduct the proposed systematic review and meta-analyses according to the recommendations stated in the *Cochrane Handbook for Systematic Reviews of Interventions*,[53] and following the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement.[54] The protocol for this review will be prospectively published on the PROSPERO online registry.

Eligibility criteria

All studies published from the date of database inception until 21st November 2017 will be included. Studies will be selected according to the following eligibility criteria:

Types of studies

We will include randomised controlled trials (RCTs) where participants were allocated to consume oral omega-3 PUFA supplements. We will exclude quasi-randomised trials. We will not exclude studies based upon language, publication status, year, or the number of participants. In cases where more than one publication reporting data from the same cohort of participants exist (i.e., from the same trial), the study reporting on the largest number of participants will be included. Published conference abstracts will be eligible for inclusion.

Types of participants

We will include studies involving adults (i.e., aged 18 years or older), recruited from within any study setting, where the structure and/or function of peripheral nerves was assessed. To be eligible for inclusion in the review, studies need to include at least one subjective measure of peripheral neuropathy (e.g., symptom score), one composite measure of peripheral neuropathy (i.e., combining subjective and objective measures), or one objective measure of peripheral nerve structure (e.g., nerve biopsy) or function (e.g., nerve conduction studies).

Types of interventions

We will consider interventions where participants were randomised to oral supplementation with short-chain and/or long-chain omega-3 PUFAs. We will accept studies that administered omega-3 supplements in any form or dosage. We will exclude studies where the intervention was administered in the form of dietary manipulation (i.e., a food-based intervention), and where omega-3 PUFA supplements were administered in combination with another intervention (including other

nutritional interventions), unless the intervention was administered in the same dose and frequency in the comparator group. We will consider studies where omega-3 PUFA supplements were compared to placebo or no treatment.

Types of outcome measures

There are no gold standard or universally accepted outcome measures for peripheral nerve assessment. In selecting the outcome measures for this review, we considered the recommendations provided by the European Neuromuscular Centre (ENMC) International workshop: Selection of Outcome Measures for Peripheral Neuropathy Clinical Trials (10-12 December 2014), taking into account both subjective and clinical measures.[55]

We will assess all outcome measures at six months of follow-up, with an acceptable follow-up range of between three and nine months from baseline. If studies do not report the change from baseline, we will utilise data reported at the end of the follow-up period.

Primary outcomes

The primary outcome will be the change, from baseline, in peripheral neuropathy impairments, as quantified by validated, composite (i.e., combining symptoms plus objective measures) neuropathy measures. We have not been prescriptive in our selection of particular scales as there are no universally agreed scoring systems; examples of validated, composite neuropathy assessment scales include the Michigan Diabetic Neuropathy Score (MDNS),[56] Neuropathy Impairment Score (NIS)[57]

and Total Neuropathy Score (TNS).[58] For the purpose of this review, we define a 'validated' measure being a survey instrument that has been psychometrically tested.

Secondary outcomes

We will consider the following secondary outcomes:

1. Symptoms: change, from baseline, in symptoms of peripheral neuropathy, measured by a validated, patient-assessed symptom score;
2. Pain: change, from baseline, in mean scores of pain, measured by a validated, patient-assessed pain scale. Examples of validated scales include the visual analogue scale (VAS),[59] Likert scales[60, 61] and the McGill Pain Questionnaire (MPQ).[62]
3. Disability: change, from baseline, in the mean score of a patient-reported disability measure. Examples of validated disability measures include the Overall Neuropathy Limitation Scale[63] and the Overall Disability Sum Score (ODSS).[64]
4. Anatomical markers:
    a. Change, from baseline in central corneal nerve fibre length (CNFL), defined as the total length of nerves in a given area, measured in mm/mm^2, using a laser-scanning in vivo confocal microscope (IVCM);
    b. Change, from baseline in intraepidermal nerve fibre density (IENFD).
5. Nerve Conduction Studies (NCS): Change, from baseline, in nerve conduction study parameters, as recommended by England (2005):[65]
    a. Sensory nerve action potential (SNAP) amplitudes of the sural, median and ulnar nerves;
    b. SNAP latencies of the sural, median and ulnar nerves;

c. Sensory nerve conduction velocity (NCV) of the sural, median and ulnar nerves;

d. Distal compound motor action potential (CMAP) amplitude of the peroneal, tibial, median and ulnar nerves;

e. CMAP latency of the peroneal, tibial, median and ulnar nerves;

f. Motor NCV of the peroneal and ulnar Motor NCV of the peroneal, tibial, medium and ulnar nerves;

g. Minimum F-wave latency of the peroneal, tibial, median and ulnar nerves.

6. Sensory function in the cornea: change, from baseline, in corneal sensation, as quantified using:

   a. Contact aesthesiometry, to quantify mechanical detection thresholds using the Cochet-Bonnet aesthesiometer (measured in millimetres);

   b. Non-contact aesthesiometry, to quantify corneal sensation quantified using an air-based aesthesiometer (measured in millibars).

7. Sensory function in the skin: Change, from baseline, in sensory function test scores:

   a. Mechanical detection thresholds, measured using pressure aesthesiometry (e.g., von Frey hair aesthesiometer);

   b. Cold detection thresholds measured using quantitative sensory testing (QST) methods;

   c. Warm detection thresholds measured using QST methods;

   d. Thermal pain thresholds, for cold and hot stimuli, measured using QST methods.

8. Adverse events: we will consider all adverse events, and will analyse them in the following categories: (i) any adverse events, (ii) adverse events leading to discontinuation of the interventions, and (iii) serious adverse events, being those leading to hospitalization or prolonged admission, a life-threatening event or death.

Search methods for identification of studies

We will conduct comprehensive electronic database searches in: the Cochrane Central Register of Controlled Trials (CENTRAL) (Issue 10, October 2017), Ovid MEDLINE, Ovid MEDLINE Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE Daily (January 1946 to November 2017), EMBASE (January 1947 to November 2017). We will also search the US National Institutes of Health Clinical Trials Registry (www.clinicaltrials.gov) and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/en/). We will not impose any restrictions on date or language in our search strategies. Search strategies for all electronic searches are included in Appendices 1 – 5. We will additionally search the bibliographies of included RCTs to identify any other potentially relevant studies.

Data collection and analysis

After the search strategies are performed within each electronic database, the reference lists will be imported into EndNote. Duplicate entries will be identified and removed. The final reference library will be imported into Covidence,[66] the standard production platform for Cochrane reviews, for the study selection process.

Selection of studies

The titles, keywords and abstracts of all unique articles, identified by the search strategies, will be independently reviewed by two review authors to identify potentially eligible studies. The full text papers of articles identified as relevant or potentially relevant, by at least one reviewer, will be retrieved for full text screening. Full text articles will be independently screened by two review authors and assessed for eligibility according to the pre-specified inclusion and exclusion criteria. Reasons for exclusion will be identified and recorded for ineligible studies that progress to the full text screening stage. Any disagreements in eligibility assessment will be adjudicated between the two reviewers; if consensus cannot be achieved, a third independent author will be consulted to reach consensus. We will include a PRISMA flow diagram (summarising the article selection process) and a 'Characteristics of excluded studies' table (with reasons for study exclusion).

Data extraction and management

Two review authors will independently extract outcome data and key study characteristics for all included studies. Any discrepancies will be resolved by discussion and consensus. We will contact the study authors of relevant trials, by email, if further information or clarification is required. If we fail to receive a response from the corresponding author within four weeks, or if the authors are unable to provide us with the requested information, we will use the information that is available.

For each study, we will extract the following information:

1. Article details: year of publication, journal of publication, language, publication status;
2. Study details: dates study conducted, trial registration number, country, study setting, corresponding author details (name, institution, email, address) and whether the study investigators were contacted for further information;
3. Methods: exclusions after randomisation, losses to follow-up, how missing data were handled, whether a sample size calculation was reported;
4. Participants: number of participants in each intervention group, participant baseline characteristics (i.e., age, gender and/or sex (as specified by the study authors), underlying conditions, peripheral neuropathy diagnostic criteria), participant inclusion criteria and exclusion criteria, comparison of groups at baseline;
5. Interventions: intervention(s) and comparator (type (long- or short-chain), dose (milligrams/day), duration of treatment), concomitant medications or treatments;
6. Outcomes: pre-specified primary and secondary outcome measures, time points of assessments;
7. Other: sources of funding statement (i.e., present or absent), actual source of funding (e.g., industry funding), conflicts of interest statement (i.e., present of absent), nature of conflict of interest (e.g., industry employee).

Assessment of risk of bias in included studies

Two review authors will independently assess the risk of bias for each included study using *the Cochrane Handbook for Systematic Reviews* guidelines. Assessment of bias will be considered using the following domains:

1. Selection bias (random sequence generation and allocation concealment);
2. Performance bias (blinding of participants and all study personnel);
3. Detection bias (blinding of outcome assessors);
4. Attrition bias (incomplete outcome data);
5. Reporting bias (selective reporting of outcomes);
6. Other sources of bias (funding source, conflicts of interest).

Each review author will judge the risk of bias in each domain as: (i) low risk, (ii) unclear risk or (iii) high risk. Disagreements in bias assessment will be resolved by consensus between the two reviewers.

Measures of treatment effect

We will analyse data according to the methods described in Chapter 9 of the *Cochrane Handbook for Systematic Reviews of Interventions*.[53]

As all of the pre-defined outcomes are continuous measures, we will extract information on the change in mean from baseline, and standard deviations of change, for the intervention and comparison groups. If change from baseline is not reported, we will extract information on the mean and standard deviation of the outcome, for the intervention and comparator groups, at the specified follow-up period. The effects of the interventions will be expressed as the mean difference (MD), with 95% confidence intervals (CIs), between the intervention and comparator groups.

Unit of analysis issues

The unit of analysis for this review will be the individual participant. In studies where outcomes were measured in ocular tissues, the unit of analysis will be the enrolled study eye of the participant. In studies where participants were randomly allocated to treatment, there will be no unit of analysis issues if only one eye per person is included in the trial, or if both eyes per person are included and the average value of both eyes are reported. In studies where participants were randomly allocated to treatment and both eyes were included, but reported separately, we will analyse this as clustered data (i.e. adjusted for within-person correlation). We may have to contact the trial investigators for further information to do this.

Dealing with missing data

For any studies where missing outcome data (e.g., omitted standard deviations, standard errors) are identified, we will attempt to contact the study authors. If we fail to receive a response from the corresponding authors in four weeks, or if the authors are unable to provide us with the requested information, we will use the information that is available. We will use imputed data, if this has been derived by the trial investigators using an appropriate method, but will not impute missing data ourselves.

Assessment of heterogeneity

We will assess clinical and methodological heterogeneity in the included studies by examining differences in the intervention (e.g., type, dose, form), participant characteristics at baseline (e.g., age, gender/sex, cause of neuropathy, eligibility criteria, etc.), and risk of bias. We will quantify statistical heterogeneity using the I^2 statistic, as outlined in Chapter 9 of the *Cochrane Handbook for Systematic Reviews*

of Interventions;[53] we will consider an I^2 statistic of 60% or more as consistent with a moderate level of heterogeneity. In measuring heterogeneity, we will also consider the: (i) magnitude and direction of the effects of individual studies, and (ii) strength of evidence for heterogeneity (using a p-value < 0.10 from the Chi-squared test as the criterion for significant heterogeneity).

Assessment of reporting biases

If at least ten studies are included in a meta-analysis, we will use a funnel plot to assess for any potential publication bias. We will interpret any asymmetries in the funnel plot in association with the trial characteristics, considering relevant factors such as sample size.

Data synthesis

We will undertake meta-analyses, for the primary and/or secondary outcomes, when this would be clinically meaningful (i.e., for studies where the treatment, participants and the underlying clinical questions are similar). If fewer than three RCTs are to be included in a meta-analysis, we will use a fixed-effect model, otherwise we will use a random-effects model.

If there is inconsistency between individual study results, such that the pooled results may not provide a fair representation of the trial findings (e.g., the effects are in opposite directions, or I^2 > 60% or the Chi-squared test p-value is <0.10), we will not pool the study data but will instead describe the pattern of the individual study results. If there is statistical heterogeneity but all of the effect estimates are in the same

direction, such that a pooled estimate would seem to provide a good summary of the individual trial results, we may pool the data.

If a meta-analysis is not deemed appropriate, we will provide a descriptive or tabulated results summary.

Subgroup analysis and investigation of heterogeneity

If sufficient data are available, we will perform subgroup analyses by prognostic factors (e.g., severity of peripheral neuropathy at baseline, and age) and by potential intervention effect modifiers (e.g., dose, duration and type of omega-3 PUFA supplement), as these factors are potentially important to any observed treatment effects.

Sensitivity analysis

Provided there are sufficient data available, we will perform a sensitivity analysis for the primary outcome (i.e., change in peripheral neuropathy impairment score), to assess the impact of excluding studies that: i) were appraised as having a high risk of bias due to lack of allocation concealment or lack of blinding of participants and study personnel, ii) had more than 20 percent of participants that were lost to follow-up, iii) were unpublished, and iv) were funded by industry.

Summary of findings table

Provided that sufficient data are available, we will provide a "Summary of findings" table for the primary outcome (change in peripheral neuropathy as measured using a validated composite neuropathy assessment) and the following secondary outcomes

(as previously defined): (i) Symptoms, (ii) Pain, (iii) Corneal nerve fibre length (CNFL), (iv) Intraepidermal nerve fibre density (IENFD), (v) SNAP amplitudes of the sural nerve, and (vi) Motor NCV of the peroneal nerve. We will follow the recommendations specified in Chapter 11 of the *Cochrane Handbook for Systematic Reviews of Interventions.[53]* The strength and quality of the evidence for each outcome will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[67]

CONCLUSIONS

The term "peripheral neuropathy" describes a heterogeneous group of disorders that cause damage to the peripheral nervous system. Currently, management approaches for peripheral neuropathy are aimed primarily at addressing the underlying cause and/or managing symptoms. For many causes of peripheral neuropathy, including diabetes, reversing or even limiting the progression of nerve damage remains a challenge with currently available therapeutics.

Omega-3 PUFAs are reported to be associated with a range of general health benefits that include reducing the risk of cardiovascular disease,[35, 36] lowering systemic triglycerides[37] and improving clinical symptoms of dry eye disease.[41] In animal models of experimental peripheral nerve injury, increasing endogenous levels of omega-3 PUFAs have been shown to improve sciatic blood flow and accelerate the recovery of neuronal function.[47, 49, 50]

The aim of this systematic review is to assess the safety and efficacy of oral omega-3 PUFA supplementation for improving peripheral nerve health. If it is demonstrated

that omega-3 supplements can improve measures of peripheral nerve function and/or quality of life, it is anticipated that this therapy would make a valuable contribution to the current clinical management of peripheral neuropathy.

AUTHORS' CONTRIBUTIONS

All authors (ACZ, LED, LB, JK, RJM, LR and JPC) made contributions to the conception and/or design of the work; and drafted (ACZ and LED) or revised (LB, JK, RJM, LR and JPC) the protocol; and approved the final version of the manuscript; and agree to be accountable for all aspects of the submitted work.

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COMPETING INTERESTS STATEMENT

All authors declare no conflicts of interest relevant to this review.

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APPENDIX 1

MEDLINE (OViD) search strategy

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to November 2017

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. (randomised OR randomized).ab,ti.
4. placebo.ab.
5. drug therapy.fs.
6. randomly.ab.
7. trial.ab.
8. groups.ab.
9. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. exp Diabetic Neuropathies/
13. exp Diabetic Foot/
14. exp Peripheral Nervous System Diseases/
15. neuropath*.tw.
16. exp Neuralgia/

17. neuralgia.tw.

18. exp Charcot-Marie-Tooth Disease/

19. exp Facial Nerve Diseases/

20. exp Nerve Regeneration/

21. exp Nerve Degeneration/

22. nerve*.tw.

23. exp Hyperalgesia/

24. allodynia.tw.

25. exp Sensation/

26. sensation.tw.

27. exp Sensation Disorders/

28. an?esthesiomet*.tw.

29. esthesiomet*.tw.

30. aesthesiomet*.tw.

31. (guillain and barre).tw.

32. (polyradiculoneuritis OR polyradiculoneuropath* OR polyradiculopath*).tw.

33. (inflammatory adj3 (polyneuropath* OR mononeuropath* OR polyradiculoneuropath*)).tw.

34. (amyloid adj3 neuropath*).tw.

35. (motor and sensory and neuropath* and hereditary).mp.

36. (hereditary and sensory and autonomic and neuropath*).mp.

37. (heredit* adj6 neuropath*).mp.

38. or/12-37

39. (omega-3 OR omega 3).tw.

40. (PUFA* OR LCPUFA* OR polyunsaturated OR poly-unsaturated).tw.

41. eicosapentaenoic.tw.

42. EPA.tw.

43. docosahexaenoic.tw.

44. DHA.tw.

45. exp Alpha-Linolenic Acid/

46. alpha-linolenic.tw.

47. Fatty acids/

48. Fatty Acids, Unsaturated/

49. Fatty Acids, Essential/

50. exp Fatty Acids, Omega-3/

51. (Fatty adj3 acid*).tw.

52. or/39-51

53. 11 and 38 and 52

APPENDIX 2

EMBASE (OViD) search strategy

Embase Classic + Embase 1947 to November 2017

1. exp Randomized Controlled Trial/
2. Exp controlled clinical trial
3. (randomised OR randomized).ab,ti.
4. placebo.ab.
5. drug therapy.fs.
6. randomly.ab.
7. trial.ab.
8. groups.ab.
9. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. exp neuropathy/
13. exp neuralgia/
14. exp nerve regeneration/
15. exp peripheral nervous system/
16. neuropath*.tw.
17. neuralgia.tw.
18. nerve*

19. an?esthesiomet*.tw.

20. esthesiomet*.tw.

21. aesthesiomet*.tw.

22. sensation*.tw.

23. exp sensation/

24. exp hyperalgesia/

25. exp allodynia/

26. hyperalgesia.tw.

27. allodynia.tw.

28. (guillain and barre).tw.

29. (polyradiculoneuritis OR polyradiculoneuropath* OR polyradiculopath*).tw.

30. (inflammatory adj3 (polyneuropath* OR mononeuropath* OR polyradiculoneuropath*)).tw.

31. (amyloid adj3 neuropath*).tw.

32. (motor and sensory and neuropath* and hereditary).mp.

33. (hereditary and sensory and autonomic and neuropath*).mp.

34. (heredit* adj6 neuropath*).mp.

35. or/12-34

36. exp unsaturated fatty acid/

37. exp fish oil/

38. (PUFA* OR LCPUFA* OR polyunsaturated OR poly-unsaturated).tw.

39. (omega-3 OR omega 3).tw.

40. eicosapentaenoic.tw.

41. docosahexaenoic.tw.

42. alpha-linolenic.tw.

43. (Fatty adj3 acid*).tw.

44. EPA.tw.

45. DHA.tw.

46. or/36-45

47. 11 and 35 and 46

APPENDIX 3

Cochrane Central Register of controlled Trials (CENTRAL) search strategy

1. MeSH descriptor: [Diabetic Neuropathies] explode all trees

2. MeSH descriptor: [Diabetic Foot] explode all trees

3. MeSH descriptor: [Peripheral Nervous System Diseases] explode all trees

4. MeSH descriptor: [Neuralgia] explode all trees

5. MeSH descriptor: [Charcot-Marie-Tooth Disease] explode all trees

6. MeSH descriptor: [Facial Nerve Diseases] explode all trees

7. MeSH descriptor: [Nerve Regeneration] explode all trees

8. MeSH descriptor: [Nerve Degeneration] explode all trees

9. (neuropath* OR Neuralgia* OR nerve*):ti,ab,kw

10. MeSH descriptor: [Hyperalgesia] explode all trees

11. MeSH descriptor: [Sensation] explode all trees

12. MeSH descriptor: [Sensation Disorders] explode all trees

13. (sensation* OR hyperalgesia OR allodynia):ti,ab,kw

14. (an?esthesiomet* OR esthesiomet* OR aesthesiomet*):ti,ab,kw

15. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14

16. (omega-3 OR omega 3 OR ω-3 OR ω3 OR "ω 3"):ti,ab,kw

17. (PUFA* OR LCPUFA* OR polyunsaturated OR poly-unsaturated):ti,ab,kw

18. MeSH descriptor: [Eicosapentaenoic Acid] explode all trees

19. (Eicosapentaenoic OR EPA):ti,ab,kw

20. MeSH descriptor: [Docosahexaenoic Acids] explode all trees

21. (Docosahexaenoic OR DHA):ti,ab,kw

22. MeSH descriptor: [Alpha-Linolenic Acid] explode all trees

23. (Alpha-Linolenic):ti,ab,kw

24. MeSH descriptor: [Fatty acids] this term only

25. MeSH descriptor: [Fatty Acids, Unsaturated] this term only

26. MeSH descriptor: [Fatty Acids, Essential] this term only

27. MeSH descriptor: [Fatty Acids, Omega-3] explode all trees

28. (Fatty near/3 acid*):ti,ab,kw

29. #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28

30. #15 and #29

APPENDIX 4

US National Institutes of Health Clinical Trials Registry (ClinicalTrials.gov) search strategy

Condition = neuropathy OR nerve OR sensation

Intervention = omega-3 OR "omega 3" OR polyunsaturated OR PUFA OR "docosahexaenoic" OR DHA OR "eicosapentaenoic" OR EPA OR "fish oil" OR "fish-oil"

APPENDIX 5

World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP) search strategy (www.who.int/ictrp/)

Condition = nerve disorders OR nerve disease OR neuropathy OR nerve OR sensation

Intervention = omega-3 OR omega 3 OR polyunsaturated OR PUFA OR docosahexaenoic OR eicosapentaenoic OR fish oil

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported in manuscript (page number + section details)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	Not applicable
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	10 (methods and analysis)
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1 (title page)
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	23 (contribution of authors)
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	Not applicable
Support:			
Sources	5a	Indicate sources of financial or other support for the review	23 (funding statement)
Sponsor	5b	Provide name for the review funder and/or sponsor	23 (funding statement)
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	23 (funding statement)
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	5 (background)
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	10 (objectives)
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	10 (eligibility criteria)
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	15 (search methods for identification of studies)

Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	15 (search methods for identification of studies), appendix
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	16 (data collection and analysis)
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	16 (selection of studies)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	17 (data extraction and management)
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	17 (data extraction and management)
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	12 (types of outcome measures)
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	18 (assessment of risk of bias in included studies)
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	21 (data synthesis)
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	21 (data synthesis)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	20/21 (subgroup analysis and investigation of heterogeneity, sensitivity analysis)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	21 (data synthesis)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	20 (assessment of reporting biases)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	22 (summary of findings table)

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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Omega-3 polyunsaturated fatty acid supplementation for improving peripheral nerve health: protocol for a systematic review

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Omega-3 polyunsaturated fatty acid supplementation for improving peripheral nerve health: protocol for a systematic review

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ABSTRACT

Introduction: Damage to peripheral nerves occurs in a variety of health conditions. Preserving nerve integrity, to prevent progressive nerve damage, remains a clinical challenge. Omega-3 polyunsaturated fatty acids (PUFAs) are implicated in the development and maintenance of healthy nerves and may be beneficial for promoting peripheral nerve health. The aim of this systematic review is to assess the effects of oral omega-3 PUFA supplementation on peripheral nerve integrity, including both subjective and objective measures of peripheral nerve structure and/or function.

Methods and analysis: A systematic review of randomised controlled trials that have evaluated the effects of omega-3 PUFA supplementation on peripheral nerve assessments will be conducted. Comprehensive electronic database searches will be performed in OViD Medline, Embase, the Cochrane Central Register of controlled Trials (CENTRAL), US National Institutes of Health Clinical Trials Registry and the World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP). The title, abstract and keywords of identified articles will be assessed for eligibility by two reviewers. Full text articles will be obtained for all studies judged as eligible or potentially eligible; these studies will be independently assessed by two reviewers to determine eligibility. Disagreements will be resolved by consensus. Risk of bias assessment will be performed using the Cochrane Collaboration risk of bias tool to appraise the quality of included studies. If clinically meaningful, and there are a sufficient number of eligible studies, a meta-analysis will be conducted and a summary of findings table will be provided.

Ethics and dissemination: This is a systematic review that will involve the analysis of previously published data and therefore ethics approval is not required. A

manuscript reporting the results of this systematic review will be published in a peer-reviewed journal and may also be presented at relevant scientific conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This will be the first systematic review to consider the efficacy and safety of omega-3 PUFA supplementation on peripheral nerve structure and function.
- This systematic review will only consider data from randomised controlled trials, which provide the highest level of evidence for single intervention studies.
- This review will be conducted according to the Cochrane Handbook for Systematic Reviews of Interventions, and in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement.
- As we will include studies that have evaluated the use of omega-3 PUFA supplementation for treating any form of peripheral nerve damage, there may be limited scope to perform a meta-analysis due to clinical heterogeneity.
- There are currently no gold standard outcome measures for assessing peripheral neuropathy, which may affect the capacity to quantitatively synthesise data from individual studies to derive clear estimates of treatment effect(s).

BACKGROUND

Description of the condition

The peripheral nervous system encompasses the nerves mediating sensory, motor and autonomic functions that are located outside of the brain and spinal cord. Alterations to the anatomical integrity of the peripheral nerves can adversely affect their function, presenting clinically as abnormal or loss of sensation, weakness and/or as changes to autonomic function.[1] England describes peripheral neuropathy as "a general term that indicates any disorder of the peripheral nervous system;"[2] this is a broad definition that includes nerve damage due to a variety of aetiologies. The pathophysiological mechanisms underlying both the development and progression of peripheral neuropathy are complex and may depend on the cause. Some of these mechanisms include altered metabolism and intracellular signaling,[3] vascular and inflammatory stress,[4] and reactive oxygen species formation[5].

The most common systemic cause of peripheral neuropathy, which is evident in over 50% of individuals affected by the condition, is diabetes mellitus; the risk of peripheral neuropathy increases with longer disease duration,[6] and may be correlated with the degree of glycaemic control, particularly in type-1 diabetes. [7-10] Other causes include hereditary neuropathies (e.g., Charcot-Marie-Tooth Syndrome), post-infectious and inflammatory neuropathies (e.g., Guillain-Barré Syndrome) and drug-induced neuropathies (e.g., platinum analogues, thalidomide, alcohol).[11] Up to one-third of cases do not have an identified aetiology, and are thus defined as idiopathic peripheral neuropathies.[12]

Clinical evaluations of peripheral nerve integrity generally include a combination of symptoms, signs and electrodiagnostic studies, which aim to evaluate the extent of nerve damage.[2] Symptoms and signs of nerve damage are often assessed using validated neuropathy scales or composite scores (which combine symptomatology with clinical measures of nerve function). Nerve biopsies are invasive and, as a result, not easily repeatable, and are therefore not frequently used as an outcome parameter in longitudinal studies, but are rather reserved for diagnostic purposes.[13]

Electrodiagnostic testing examines the characteristics of the conduction of an electrical signal that travels through a single nerve. These tests are useful in providing diagnostic information and for longitudinally monitoring disease progression.[14] Nerve conduction studies are reproducible and correlate well with underlying structural abnormalities,[15] but the precision of these tests is limited to detecting changes in large myelinated nerve fibres, as they are not sufficiently sensitive to detect small nerve fibre damage.[16, 17] Quantitative sensory tests, which quantify thermal and pain thresholds, can be used to evaluate small nerve fibre function.[17] Skin biopsies offer an alternative method to accurately diagnose and classify the extent of small fibre neuropathy, even in the absence of large fibre nerve damage.[17, 18] Cutaneous silent period (CSP) testing is a reproducible measurement of the nonciceptive spinal reflex where thinly myelinated A-delta fibres are the afferent arm. Quantitative sudomotor axonal reflex testing (QSART) assesses the function of unmyelinated post-ganglionic sudomotor C-fibres.[19, 20] These are amongst several other methods to assess various small fibre types, and as each individual test may have a relatively low sensitivity a combination of modalities is usually preferable to better assess small fibre function.[21]

Recently, corneal confocal microscopy has been applied to visualise small nerve fibres *in vivo*.[22] This technique has been shown to correlate well with intraepidermal nerve fibre biopsy results[23] and is useful for detecting and documenting various types of small fibre neuropathies.[24-27] Corneal confocal microscopy has also been suggested to be useful for monitoring disease progression, and as a marker for improvements in nerve function, in the investigation of therapeutic targets for diabetic peripheral neuropathy.[28-30]

Peripheral neuropathies are typically treated based on their subtype and/or underlying cause(s). Treatments primarily aim to manage the underlying condition to prevent progressive nerve damage and to treat any associated symptoms.[2, 16, 31] The consequences associated with symptoms of neuropathic impairment do not only affect an individual's quality of life, but remain an economic burden in the cost of healthcare and medical resources.[32-34] This is especially true in chronic conditions such as diabetes, where lifetime care is required.[3]

Description of the intervention

Omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids (EFAs) with multiple double bonds, the first of which is located at the third carbon from the methyl end of the molecule. Short-chain omega-3 PUFAs, alpha-linolenic acid (ALA), found in plant sources, is a metabolic precursor to the long-chain omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are present in high abundance in oily fish. As humans do not have the enzymes to synthesise these fatty acids *de novo*, omega-3 PUFAs must be obtained from the diet

or through supplementation.[35] The other major class of EFAs are the omega-6 fatty acids, which derive from the diet in the form of linolenic acid (LA) and are elongated *in vivo* to gamma-linoleic acid (GLC) and arachidonic acid (AA). Most eicosanoids derived from the omega-6 dependent AA-pathway are pro-inflammatory; in contrast, long-chain omega-3 fatty acids bias prostaglandin metabolism towards the production of anti-inflammatory eicosanoids.

As omega-3 PUFAs competitively inhibit the metabolic conversion of omega-6 PUFAs,[36] the balance of omega-3 to omega-6 fatty acid consumption can affect systemic inflammatory processes and immune activity. The ratio of consumed omega-6 to omega-3 in typical Western diets is approximately 15 to 1, whereas a ratio of 4 to 1 is considered optimal.[37] Increased consumption of omega-3 PUFAs is considered to provide a range of potential general health benefits, including a reduced risk of cardiovascular disease[38, 39] and lowered systemic triglycerides[40]. DHA, as an integral component in cellular membrane structures of the brain and retina, has been implicated in perinatal visual and neural development.[41-43] In ocular conditions, omega-3 fatty acids supplements can reduce the symptoms and clinical signs associated with ocular surface inflammation in dry eye disease.[44] The American Heart Foundation recommends a daily intake, for adults, of 500mg of long-chain omega-3 PUFAs,[45] and up to 4g/day in hypertriglyceridemia.[46]

How the intervention might work

Once consumed, omega-3 PUFAs alter membrane protein activity and cellular signaling response, to reduce immune activity and the concentration of systemic lipid inflammatory mediators.[47] The incorporation of omega-3 PUFAs into cellular

membranes, and their subsequent effect on membrane activity, has been shown to alter vascular function, improve sciatic nerve blood flow and enhance nerve conduction velocity in a rat model of experimental diabetic neuropathy.[48]

Omega-3 PUFAs also affect intracellular signalling pathways and the expression of genes, some of which may be associated with the regulation of neuron growth and neuroprotection.[49] In animal models of diabetes, omega-3 PUFA supplementation has been shown to attenuate adverse changes in nerve structure and function.[50, 51] Mice enriched with genes that increase endogenous profiles of omega-3 PUFAs have been shown to have reduced neuronal cell death and increased recovery to mechanical stress and peripheral nerve injury.[52] Omega-3 PUFAs have also been demonstrated to promote neurite growth in rat sensory neurons.[53]

Derivatives of omega-3 PUFA metabolism, resolvins and protectins, which are oxygenated metabolites from EPA and DHA respectively, may further promote neuronal function. Neuroprotectin D-1 has been shown to facilitate the regeneration of corneal nerves following refractive surgery and neurite growth from the trigeminal ganglion of mice [54] and to prevent neuropathic pain after peripheral nerve injury.[55]

Why it is important to do this review

This will be the first systematic review to consider the potential effects of omega-3 PUFA supplementation on peripheral nerve integrity. Omega-3 PUFA supplementation has been shown to reduce neuronal damage and enhance recovery following nerve injury in experimental animal models of peripheral neuropathy.

Confirmation of these effects in clinical populations would contribute significantly towards enhancing the clinical management of peripheral neuropathy. A therapeutic agent to prevent the pathogenesis of, or slow the progression of, peripheral nerve damage has the potential to greatly improve clinical outcomes. Furthermore, the potential for omega-3 PUFA supplements to alleviate neuropathy-associated symptoms would be predicted to reduce impairment on an individual's quality of life and lessen the economic burden of peripheral neuropathy in the community.

OBJECTIVES

The primary objective of this systematic review is to evaluate the efficacy and safety of oral omega-3 PUFA supplements for improving peripheral nerve health. Efficacy outcomes will consider both subjective endpoints (i.e., symptoms) and objective clinical measures, including changes to peripheral nerve structure and function.

METHODS AND ANALYSIS

We will conduct the proposed systematic review and meta-analyses according to the recommendations stated in the *Cochrane Handbook for Systematic Reviews of Interventions*,[56] and following the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement.[57] The protocol for this review has been registered in the PROSPERO International prospective register of systematic reviews, (ID 86297).

Eligibility criteria

All studies published from the date of database inception until 21st November 2017 will be included. Studies will be selected according to the following eligibility criteria:

Types of studies

We will include randomised controlled trials (RCTs) where participants were allocated to consume oral omega-3 PUFA supplements. We will exclude quasi-randomised trials. We will not exclude studies based upon language, publication status, year, or the number of participants. In cases where more than one publication reporting data from the same cohort of participants exist (i.e., from the same trial), the study reporting on the largest number of participants will be included. Published conference abstracts will be eligible for inclusion.

Types of participants

We will include studies involving adults (i.e., aged 18 years or older), recruited from within any study setting, where the structure and/or function of peripheral nerves was assessed. To be eligible for inclusion in the review, studies need to include at least one subjective measure of peripheral neuropathy (e.g., symptom score), one composite measure of peripheral neuropathy (i.e., combining subjective and objective measures), or one objective measure of peripheral nerve structure (e.g., nerve biopsy) or function (e.g., nerve conduction studies).

Types of interventions

We will consider interventions where participants were randomised to oral supplementation with short-chain and/or long-chain omega-3 PUFAs. We will accept

studies that administered omega-3 supplements in any form or dosage. We will exclude studies where the intervention was administered in the form of dietary manipulation (i.e., a food-based intervention), and where omega-3 PUFA supplements were administered in combination with another intervention (including other nutritional interventions), unless the intervention was administered in the same dose and frequency in the comparator group. We will consider studies where omega-3 PUFA supplements were compared to placebo or no treatment.

Types of outcome measures

There are no gold standard or universally accepted outcome measures for peripheral nerve assessment. In selecting the outcome measures for this review, we considered the recommendations provided by the European Neuromuscular Centre (ENMC) International workshop: Selection of Outcome Measures for Peripheral Neuropathy Clinical Trials (10-12 December 2014), taking into account both subjective and clinical measures.[58]

We will assess all outcome measures at six months of follow-up, with an acceptable follow-up range of between three and nine months from baseline. If studies do not report the change from baseline, we will utilise data reported at the end of the follow-up period.

Primary outcomes

The primary outcome will be the change, from baseline, in peripheral neuropathy impairments, as quantified by validated, composite (i.e., combining symptoms plus objective measures) neuropathy measures. We have not been prescriptive in our

selection of particular scales as there are no universally agreed scoring systems; examples of validated, composite neuropathy assessment scales include the Michigan Diabetic Neuropathy Score (MDNS),[59] Neuropathy Impairment Score (NIS)[60] and Total Neuropathy Score (TNS).[61] For the purpose of this review, we define a 'validated' measure being a survey instrument that has been psychometrically tested.

Secondary outcomes

We will consider the following secondary outcomes:

1. Symptoms: change, from baseline, in symptoms of peripheral neuropathy, measured by a validated, patient-assessed symptom score.
2. Pain: change, from baseline, in mean scores of pain, measured by a validated, patient-assessed pain scale. Examples of validated scales include the visual analogue scale (VAS),[62] Likert scales[63, 64] and the McGill Pain Questionnaire (MPQ).[65]
3. Disability: change, from baseline, in the mean score of a patient-reported disability measure. Examples of validated disability measures include the Overall Neuropathy Limitation Scale[66] and the Overall Disability Sum Score (ODSS).[67]
4. Anatomical markers:
    a. Change, from baseline in central corneal nerve fibre length (CNFL), defined as the total length of nerves in a given area, measured in mm/mm^2, using a laser-scanning in vivo confocal microscope (IVCM);
    b. Change, from baseline in intraepidermal nerve fibre density (IENFD).
5. Nerve Conduction Studies (NCS): Change, from baseline, in nerve conduction study parameters, as recommended by England (2005):[68]

a. Sensory nerve action potential (SNAP) amplitudes of the sural, median and ulnar nerves;

b. SNAP latencies of the sural, median and ulnar nerves;

c. Sensory nerve conduction velocity (NCV) of the sural, median and ulnar nerves;

d. Distal compound motor action potential (CMAP) amplitude of the peroneal, tibial, median and ulnar nerves;

e. CMAP latency of the peroneal, tibial, median and ulnar nerves;

f. Motor NCV of the peroneal and ulnar Motor NCV of the peroneal, tibial, medium and ulnar nerves;

g. Minimum F-wave latency of the peroneal, tibial, median and ulnar nerves.

6. Sensory function in the cornea: change, from baseline, in corneal sensation, as quantified using:

   a. Contact aesthesiometry, to quantify mechanical detection thresholds using the Cochet-Bonnet aesthesiometer (measured in millimetres);

   b. Non-contact aesthesiometry, to quantify corneal sensation quantified using an air-based aesthesiometer (measured in millibars).

7. Sensory function in the skin: Change, from baseline, in sensory function test scores:

   a. Mechanical detection thresholds, measured using pressure aesthesiometry (e.g., von Frey hair aesthesiometer);

   b. Cold detection thresholds measured using quantitative sensory testing (QST) methods;

   c. Warm detection thresholds measured using QST methods;

d. Thermal pain thresholds, for cold and hot stimuli, measured using QST methods.

8. Adverse events: we will consider all adverse events, and will analyse them in the following categories: (i) any adverse events, (ii) adverse events leading to discontinuation of the interventions, and (iii) serious adverse events, being those leading to hospitalization or prolonged admission, a life-threatening event or death.

Search methods for identification of studies

We will conduct comprehensive electronic database searches in: the Cochrane Central Register of Controlled Trials (CENTRAL) (Issue 10, October 2017), Ovid MEDLINE, Ovid MEDLINE Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE Daily (January 1946 to November 2017), EMBASE (January 1947 to November 2017). We will also search the US National Institutes of Health Clinical Trials Registry (www.clinicaltrials.gov) and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/en/). We will not impose any restrictions on date or language in our search strategies. Search strategies for all electronic searches are included in Appendices 1 – 5. We will additionally search the bibliographies of included RCTs to identify any other potentially relevant studies.

Data collection and analysis

After the search strategies are performed within each electronic database, the reference lists will be imported into EndNote. Duplicate entries will be identified and

removed. The final reference library will be imported into Covidence,[69] the standard production platform for Cochrane reviews, for the study selection process.

Selection of studies

The titles, keywords and abstracts of all unique articles, identified by the search strategies, will be independently reviewed by two review authors to identify potentially eligible studies. The full text papers of articles identified as relevant or potentially relevant, by at least one reviewer, will be retrieved for full text screening. Full text articles will be independently screened by two review authors and assessed for eligibility according to the pre-specified inclusion and exclusion criteria. Reasons for exclusion will be identified and recorded for ineligible studies that progress to the full text screening stage. Any disagreements in eligibility assessment will be adjudicated between the two reviewers; if consensus cannot be achieved, a third independent author will be consulted to reach consensus. We will include a PRISMA flow diagram (summarising the article selection process) and a 'Characteristics of excluded studies' table (with reasons for study exclusion).

Data extraction and management

Two review authors will independently extract outcome data and key study characteristics for all included studies. Any discrepancies will be resolved by discussion and consensus. We will contact the study authors of relevant trials, by email, if further information or clarification is required. If we fail to receive a response from the corresponding author within four weeks, or if the authors are unable to provide us with the requested information, we will use the information that is available.

For each study, we will extract the following information:

1. Article details: year of publication, journal of publication, language, publication status;
2. Study details: dates study conducted, trial registration number, country, study setting, corresponding author details (name, institution, email, address) and whether the study investigators were contacted for further information;
3. Methods: exclusions after randomisation, losses to follow-up, how missing data were handled, whether a sample size calculation was reported;
4. Participants: number of participants in each intervention group, participant baseline characteristics (i.e., age, gender and/or sex (as specified by the study authors), underlying conditions, peripheral neuropathy diagnostic criteria), participant inclusion criteria and exclusion criteria, comparison of groups at baseline;
5. Interventions: intervention(s) and comparator (type (long- or short-chain), dose (milligrams/day), duration of treatment), concomitant medications or treatments, compliance measures (i.e., whether compliance was assessed, and the method used, e.g., returned capsule counts, red blood cell fatty acid profiles, etc.);
6. Outcomes: pre-specified primary and secondary outcome measures, time points of assessments;
7. Other: sources of funding statement (i.e., present or absent), actual source of funding (e.g., industry funding), conflicts of interest statement (i.e., present of absent), nature of conflict of interest (e.g., industry employee).

Assessment of risk of bias in included studies

Two review authors will independently assess the risk of bias for each included study using *the Cochrane Handbook for Systematic Reviews* guidelines. Assessment of bias will be considered using the following domains:

1. Selection bias (random sequence generation and allocation concealment);
2. Performance bias (blinding of participants and all study personnel);
3. Detection bias (blinding of outcome assessors);
4. Attrition bias (incomplete outcome data);
5. Reporting bias (selective reporting of outcomes);
6. Other sources of bias (funding source, conflicts of interest).

Each review author will judge the risk of bias in each domain as: (i) low risk, (ii) unclear risk or (iii) high risk. Disagreements in bias assessment will be resolved by consensus between the two reviewers.

Measures of treatment effect

We will analyse data according to the methods described in Chapter 9 of the *Cochrane Handbook for Systematic Reviews of Interventions*.[56]

As all of the pre-defined outcomes are continuous measures, we will extract information on the change in mean from baseline, and standard deviations of change, for the intervention and comparison groups. If change from baseline is not reported, we will extract information on the mean and standard deviation of the outcome, for the intervention and comparator groups, at the specified follow-up period. The effects of the interventions will be expressed as the mean difference (MD), with 95%

confidence intervals (CIs), between the intervention and comparator groups for significant outcomes ($p<0.05$); exact p-values will be reported.

Unit of analysis issues

The unit of analysis for this review will be the individual participant. In studies where outcomes were measured in ocular tissues, the unit of analysis will be the enrolled study eye of the participant. In studies where participants were randomly allocated to treatment, there will be no unit of analysis issues if only one eye per person is included in the trial, or if both eyes per person are included and the average value of both eyes are reported. In studies where participants were randomly allocated to treatment and both eyes were included, but reported separately, we will analyse this as clustered data (i.e. adjusted for within-person correlation). We may have to contact the trial investigators for further information to do this.

Dealing with missing data

For any studies where missing outcome data (e.g., omitted standard deviations, standard errors) are identified, we will attempt to contact the study authors. If we fail to receive a response from the corresponding authors in four weeks, or if the authors are unable to provide us with the requested information, we will use the information that is available. We will use imputed data, if this has been derived by the trial investigators using an appropriate method, but will not impute missing data ourselves.

Assessment of heterogeneity

We will assess clinical and methodological heterogeneity in the included studies by examining differences in the intervention (e.g., type, dose, form), participant

characteristics at baseline (e.g., age, gender/sex, cause of neuropathy, eligibility criteria, etc.), and risk of bias. We will quantify statistical heterogeneity using the I^2 statistic, as outlined in Chapter 9 of the *Cochrane Handbook for Systematic Reviews of Interventions*;[56] we will consider an I^2 statistic of 60% or more as consistent with a moderate level of heterogeneity. In measuring heterogeneity, we will also consider the: (i) magnitude and direction of the effects of individual studies, and (ii) strength of evidence for heterogeneity (using a p-value < 0.10 from the Chi-squared test as the criterion for significant heterogeneity).

Assessment of reporting biases

If at least ten studies are included in a meta-analysis, we will use a funnel plot to assess for any potential publication bias. We will interpret any asymmetries in the funnel plot in association with the trial characteristics, considering relevant factors such as sample size.

Data synthesis

We will undertake meta-analyses, for the primary and/or secondary outcomes, when this would be clinically meaningful (i.e., for studies where the treatment, participants and the underlying clinical questions are similar). If fewer than three RCTs are to be included in a meta-analysis, we will use a fixed-effect model, otherwise we will use a random-effects model.

If there is inconsistency between individual study results, such that the pooled results may not provide a fair representation of the trial findings (e.g., the effects are in opposite directions, or $I^2 > 60\%$ or the Chi-squared test p-value is <0.10), we will not

pool the study data but will instead describe the pattern of the individual study results. If there is statistical heterogeneity but all of the effect estimates are in the same direction, such that a pooled estimate would seem to provide a good summary of the individual trial results, we may pool the data.

If a meta-analysis is not deemed appropriate, we will provide a descriptive or tabulated results summary.

Subgroup analysis and investigation of heterogeneity

If sufficient data are available, we will perform subgroup analyses by prognostic factors (e.g., type of disease (including sub-type of diabetes), severity of peripheral neuropathy at baseline, and age) and by potential intervention effect modifiers (e.g., dose, duration and type of omega-3 PUFA supplement), as these factors are potentially important to any observed treatment effects.

Sensitivity analysis

Provided there are sufficient data available, we will perform a sensitivity analysis for the primary outcome (i.e., change in peripheral neuropathy impairment score), to assess the impact of excluding studies that: i) were appraised as having a high risk of bias due to lack of allocation concealment or lack of blinding of participants and study personnel, ii) had more than 20 percent of participants that were lost to follow-up, iii) were unpublished, and iv) were funded by industry.

Summary of findings table

Provided that sufficient data are available, we will provide a "Summary of findings" table for the primary outcome (change in peripheral neuropathy as measured using a validated composite neuropathy assessment) and the following secondary outcomes (as previously defined): (i) Symptoms, (ii) Pain, (iii) Corneal nerve fibre length (CNFL), (iv) Intraepidermal nerve fibre density (IENFD), (v) SNAP amplitudes of the sural nerve, and (vi) Motor NCV of the peroneal nerve. We will follow the recommendations specified in Chapter 11 of the *Cochrane Handbook for Systematic Reviews of Interventions.[56]* The strength and quality of the evidence for each outcome will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[70]

CONCLUSIONS

The term "peripheral neuropathy" describes a heterogeneous group of disorders that cause damage to the peripheral nervous system. Currently, management approaches for peripheral neuropathy are aimed primarily at addressing the underlying cause and/or managing symptoms. For many causes of peripheral neuropathy, including diabetes, reversing or even limiting the progression of nerve damage remains a challenge with currently available therapeutics.

Omega-3 PUFAs are reported to be associated with a range of general health benefits that include reducing the risk of cardiovascular disease,[38, 39] lowering systemic triglycerides[40] and improving clinical symptoms of dry eye disease.[44] In animal models of experimental peripheral nerve injury, increasing endogenous levels of omega-3 PUFAs have been shown to improve sciatic blood flow and accelerate the recovery of neuronal function.[50, 52, 53]

The aim of this systematic review is to assess the safety and efficacy of oral omega-3 PUFA supplementation for improving peripheral nerve health. If it is demonstrated that omega-3 supplements can improve measures of peripheral nerve function and/or quality of life, it is anticipated that this therapy would make a valuable contribution to the current clinical management of peripheral neuropathy.

AUTHORS' CONTRIBUTIONS

All authors (ACZ, LED, LB, JK, RJM, LR and JPC) made contributions to the conception and/or design of the work; and drafted (ACZ & LED) or revised (LB, JK, RJM, LR and JPC) the protocol; and approved the final version of the manuscript; and agree to be accountable for all aspects of the submitted work.

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COMPETING INTERESTS STATEMENT

All authors declare no conflicts of interest relevant to this review.

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APPENDIX 1

MEDLINE (OViD) search strategy

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to November 2017

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. (randomised or randomized).ab,ti.
4. placebo.ab.
5. drug therapy.fs.
6. randomly.ab.
7. trial.ab.
8. groups.ab.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. exp Diabetic Neuropathies/
13. exp Diabetic Foot/
14. exp Peripheral Nervous System Diseases/
15. neuropath*.tw.
16. exp Neuralgia/

17. neuralgia.tw.

18. exp Charcot-Marie-Tooth Disease/

19. exp Facial Nerve Diseases/

20. exp Nerve Regeneration/

21. exp Nerve Degeneration/

22. nerve*.tw.

23. exp Hyperalgesia/

24. allodynia.tw.

25. exp Sensation/

26. sensation.tw.

27. exp Sensation Disorders/

28. an?esthesiomet*.tw.

29. esthesiomet*.tw.

30. aesthesiomet*.tw.

31. (guillain and barre).tw.

32. (polyradiculoneuritis or polyradiculoneuropath* or polyradiculopath*).tw.

33. (inflammatory adj3 (polyneuropath* or mononeuropath* or polyradiculoneuropath*)).tw.

34. (amyloid adj3 neuropath*).tw.

35. (motor and sensory and neuropath* and hereditary).mp.

36. (hereditary and sensory and autonomic and neuropath*).mp.

37. (heredit* adj6 neuropath*).mp.

38. or/12-37

39. (omega-3 or omega 3).tw.

40. (PUFA* or LCPUFA* or polyunsaturated or poly-unsaturated).tw.

41. eicosapentaenoic.tw.

42. EPA.tw.

43. docosahexaenoic.tw.

44. DHA.tw.

45. exp Alpha-Linolenic Acid/

46. alpha-linolenic.tw.

47. Fatty acids/

48. Fatty Acids, Unsaturated/

49. Fatty Acids, Essential/

50. exp Fatty Acids, Omega-3/

51. (Fatty adj3 acid*).tw.

52. or/39-51

53. 11 and 38 and 53

APPENDIX 2

EMBASE (OViD) search strategy

Embase Classic + Embase 1947 to November 2017

1. exp Randomized Controlled Trial/
2. Exp controlled clinical trial
3. (randomised or randomized).ab,ti.
4. placebo.ab.
5. drug therapy.fs.
6. randomly.ab.
7. trial.ab.
8. groups.ab.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. exp neuropathy/
13. exp neuralgia/
14. exp nerve regeneration/
15. exp peripheral nervous system/
16. neuropath*.tw.
17. neuralgia.tw.
18. nerve*

19. an?esthesiomet*.tw.

20. esthesiomet*.tw.

21. aesthesiomet*.tw.

22. Sensation*.tw.

23. exp sensation*/

24. exp hyperalgesia/

25. exp allodynia/

26. hyperalgesia.tw.

27. allodynia.tw.

28. (guillain and barre).tw.

29. (polyradiculoneuritis or polyradiculoneuropath* or polyradiculopath*).tw.

30. (inflammatory adj3 (polyneuropath* or mononeuropath* or polyradiculoneuropath*)).tw.

31. (amyloid adj3 neuropath*).tw.

32. (motor and sensory and neuropath* and hereditary).mp.

33. (hereditary and sensory and autonomic and neuropath*).mp.

34. (heredit* adj6 neuropath*).mp.

35. or/12-34

36. exp unsaturated fatty acid/

37. exp fish oil/

38. (PUFA* or LCPUFA* or polyunsaturated or poly-unsaturated).tw.

39. (omega-3 or omega 3).tw.

40. eicosapentaenoic.tw.

41. docosahexaenoic.tw.

42. alpha-linolenic.tw.

43. (Fatty adj3 acid*).tw.

44. EPA.tw.

45. DHA.tw.

46. or/36-45

47. 11 and 35 and 46

APPENDIX 3

Cochrane Central Register of controlled Trials (CENTRAL) search strategy

1. MeSH descriptor: [Diabetic Neuropathies] explode all trees

2. MeSH descriptor: [Diabetic Foot] explode all trees

3. MeSH descriptor: [Peripheral Nervous System Diseases] explode all trees

4. MeSH descriptor: [Neuralgia] explode all trees

5. MeSH descriptor: [Charcot-Marie-Tooth Disease] explode all trees

6. MeSH descriptor: [Facial Nerve Diseases] explode all trees

7. MeSH descriptor: [Nerve Regeneration] explode all trees

8. MeSH descriptor: [Nerve Degeneration] explode all trees

9. (neuropath* or Neuralgia* or nerve*):ti,ab,kw

10. MeSH descriptor: [Hyperalgesia] explode all trees

11. MeSH descriptor: [Sensation] explode all trees

12. MeSH descriptor: [Sensation Disorders] explode all trees

13. (sensation* or hyperalgesia or allodynia):ti,ab,kw

14. (an?esthesiomet* OR esthesiomet* OR aesthesiomet*):ti,ab,kw

15. #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14

16. (omega-3 or omega 3 or ω-3 or ω3 or "ω 3"):ti,ab,kw

17. (PUFA* or LCPUFA* or polyunsaturated or poly-unsaturated):ti,ab,kw

18. MeSH descriptor: [Eicosapentaenoic Acid] explode all trees

19. (Eicosapentaenoic OR EPA):ti,ab,kw

20. MeSH descriptor: [Docosahexaenoic Acids] explode all trees

21. (Docosahexaenoic OR DHA):ti,ab,kw

22. MeSH descriptor: [Alpha-Linolenic Acid] explode all trees

23. (Alpha-Linolenic):ti,ab,kw

24. MeSH descriptor: [Fatty acids] this term only

25. MeSH descriptor: [Fatty Acids, Unsaturated] this term only

26. MeSH descriptor: [Fatty Acids, Essential] this term only

27. MeSH descriptor: [Fatty Acids, Omega-3] explode all trees

28. (Fatty near/3 acid*):ti,ab,kw

29. #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28

30. #15 and #29

APPENDIX 4

US National Institutes of Health Clinical Trials Registry (ClinicalTrials.gov) search strategy

Condition = neuropathy or nerve or sensation

Intervention = omega-3 or “omega 3” or polyunsaturated OR PUFA or “docosahexaenoic” or DHA OR “eicosapentaenoic” or EPA or “fish oil” or "fish-oil"

APPENDIX 5

World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP) search strategy

Condition = nerve disorders or nerve disease or neuropathy or nerve or sensation

Intervention = omega-3 or omega 3 or polyunsaturated or PUFA or docosahexaenoic or eicosapentaenoic or fish oil

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported in manuscript (page number + section details)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	Not applicable
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	10 (methods and analysis)
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1 (title page)
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	23 (contribution of authors)
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	Not applicable
Support:			
Sources	5a	Indicate sources of financial or other support for the review	23 (funding statement)
Sponsor	5b	Provide name for the review funder and/or sponsor	23 (funding statement)
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	23 (funding statement)
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	5 (background)
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	10 (objectives)
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	10 (eligibility criteria)
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	15 (search methods for identification of studies)

Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	15 (search methods for identification of studies), appendix
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	16 (data collection and analysis)
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	16 (selection of studies)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	17 (data extraction and management)
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	17 (data extraction and management)
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	12 (types of outcome measures)
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	18 (assessment of risk of bias in included studies)
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	21 (data synthesis)
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	21 (data synthesis)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	20/21 (subgroup analysis and investigation of heterogeneity, sensitivity analysis)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	21 (data synthesis)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	20 (assessment of reporting biases)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	22 (summary of findings table)

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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