Marine oil supplements for arthritis pain: *protocol for a systematic review and meta-analysis of randomised trials*

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ABSTRACT

Background

Arthritis is a group of several different musculoskeletal disorders defined by joint inflammation including synovitis, which has been shown to be associated with the patient's degree of pain. Since docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), found in marine oils, exert an anti-inflammatory and likely an analgesic effect, marine oil supplementation is a possible treatment for pain in several types of arthritis.

Objective

The aim is to assess the effect of oral marine oil supplementation compared to no marine oil supplementation on arthritis pain, but also on physical function and inflammation, by performing a systematic review and meta-analysis of available randomised controlled trials (RCTs).

Methods

The systematic review and meta-analysis will be conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Eligible studies will be acquired through a systematic search of MEDLINE via PubMed from 1950, Web of Science from 1900 via ISI Web of Knowledge, The Cochrane Central Register of Controlled Trials (CENTRAL) from 1898, EMBASE via OVID from 1980, ClinicalTrials.gov, and the World Health Organization International Clinical Trial Registry Platform portal (ICTRP). Eligibility criteria will be randomised or quasi-randomised controlled trials assessing the effect of marine oil supplementation compared with no marine oil supplementation (i.e., trials applying an add-on design) in patients with any type of arthritis, at any age and gender. Duration of the intervention must be at least two weeks. The study selection, data extraction and bias assessment will be conducted independently by two reviewers (NKS, SMN). Risk of bias (RoB) will be assessed using the *Cochrane Collaboration's tool for assessing risk of bias in randomised trials* and the tool for assessing risk of outcome reporting bias (ORB) developed by Dwan, et al. Additional analyses will be conducted stratifying for arthritis type, EPA/DHA ratio, total DHA and EPA dosage, duration, control treatment, funding source, and RoB. A dose-response and a duration-response plot will be created.

Perspectives

We anticipate that our findings can assist in clinical recommendations of whether or not to use marine oil supplements in managing arthritis pain. The results will be disseminated as article(s) in at least on peer-reviewed scientific journal.

INTRODUCTION

Arthritis is a musculoskeletal disorder [1], and includes the two common types, Osteoarthritis (OA) and Rheumatoid Arthritis (RA), which occur in synovial joints, and result in joint pain, swelling, stiffness, and restricted movement [2 3]. Joint inflammation is a hallmark of arthritis [1], and the extent of synovitis have been shown to be associated with pain severity [4 5].

A report from 2009 showed that the most common cause of disability in the United States was arthritis and rheumatism [6] and a more recent report from 2010-2012 showed that 22.7 % of American adults reported having a doctor-diagnosed arthritis [7]. Medical care costs and lost earnings from arthritis and other rheumatic conditions amounted to $128 billion in 2003 in the United States [8].

A study exploring The Oslo RA register showed that almost 70 % of RA-patients reported pain as a preferred area for improvement [9] and further studies suggests that pain may contribute more to RA-patients' disability than does the structural joint damage [10-12]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed drugs in managing arthritis and other rheumatic conditions [13] because of its analgesic and anti-inflammatory effects [14]. However, NSAIDs are known to cause serious gastro-intestinal [15] and cardiovascular adverse events [16 17], and the Oslo RA register study found that one-third of the RA-patients with preferences for pain improvement did not use symptom-modifying medications [9]. A US survey from 2004 found that 69.2 % used complementary and alternative medicine for managing arthritis [18], which has prompted the search for alternative treatments such as fish oil.

NSAIDs exerts their analgesic effect through inhibition of the cyclooxygenase isozymes, which is stimulated by inflammation, and converts arachidonic acid (AA; 20:4 n-6) into prostaglandin H_2, which is further converted to different isoforms of biologically active eicosanoids including prostaglandin E_2 (PGE_2) [19] (Figure 1a). PGE_2 is partly responsible for the pain mediation and acts on both peripheral sensory neurons and at the central nervous system [20]. Fish oil is thought to also have an analgesic effect, though the mechanism is not as clearly explained as for NSAIDs, and many theories exist. However, it is well established that the effect is mediated by the long chain n-3 polyunsaturated fatty acids (PUFAs) docosahexonoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3), which marine oil supplements, including fish oil, is a rich source of. One theory is that DHA and EPA inhibit AA metabolism by being homologues, resulting in the production of less potent eicosanoids, including PGE_3 [21-23], which may explain the anti-inflammatory effects and possible analgesic effect [21](Figure 1b). It is therefore anticipated that supplementation with EPA- and DHA-rich preparations exerts an anti-inflammatory effect [22], making it a possible treatment for synovitis and thereby arthritis pain.

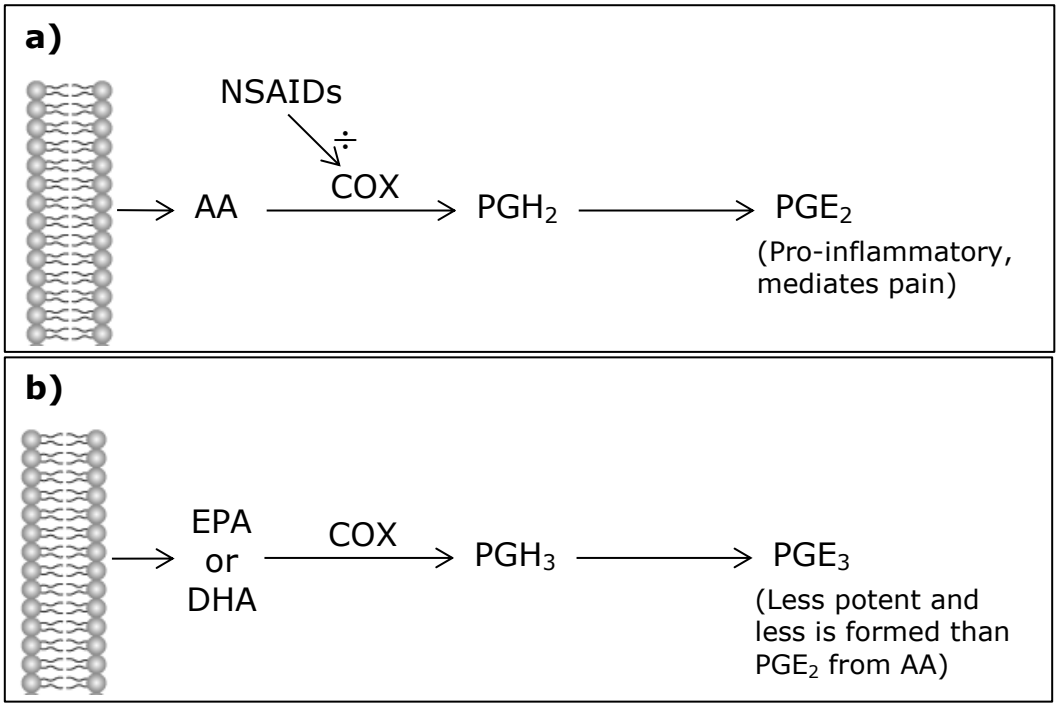

Figure 1: The action of a) NSAIDs and b) EPA on inflammation and pain
a) AA is released from cell membranes and converted into prostaglandin H_2 (PGH_2) by cyclooxygenase isozymes (COX) and further into prostaglandin E_2 (PGE_2), which induces inflammation and pain. Non-steroidal anti-inflammatory drugs (NSAIDs) exerts their effect by inhibiting COX, and thereby less PGE_2 is formed. b) Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is a homologue for AA, but results in formation of PGE_3, which is less potent and formed in smaller amounts, than PGE_2, and thereby inducing an anti-inflammatory and analgesic effect. [14 19-23]

Evidence-based research

In order to examine if a new meta-analysis was needed to provide evidence for clinical purpose, a survey of existing meta-analyses, on the effect of n-3 PUFAs on arthritis, was conducted by applying the following search strategy to Web of Science via ISI Web of Knowledge:
TOPIC: ((fish oil* OR marine oil* OR DHA OR Docosahexaenoic OR EPA OR Eicosapentaenoic OR omega-3 OR omega 3 OR n-3) AND (arthrit* OR spondylitis OR spondylarth*) AND meta-analysis)

The search yielded 27 results. Twenty three articles were excluded; exclusion was done primarily based on title, but reading abstract or article for 10 of the studies was necessary [24-33]. Four systematic reviews and meta-analyses have been conducted, as described in table 1, all of them only dealing with RA.

The first published systematic review and meta-analysis [34] is the only one dealing exclusively with fish oil and not vegetable oils. In addition to the meta-analysis, they conducted an extended analysis by using primary data (a 'mega-analysis'). From both analyses they found an improvement in number of tender joints and duration of morning stiffness. The next published systematic review and meta-analysis [35] examined the effect of n-3 PUFAs on several diseases, including RA. They found no effect in any of their outcomes. The systematic review and meta-analysis by Goldberg and Katz [36] examined joint

Table 1: Results from survey of existing meta-analyses on the effect of n-3 PUFAs on arthritis

Study	Title (journal)	PICOs and abstract conclusion
Fortin, et al. [34] (1995)	Validation of a meta-analysis: the effects of fish oil in rheumatoid arthritis. (Journal of clinical epidemiology)	P: Patients with RA. I: Fish oil. C: Placebo (dietary control oils). O: Number of tender/painful joints, tender joint index, number of swollen joints, swollen joint index, morning stiffness, grip strength, patient's global assessments, physician's and patient's global assessments, and ESR. **Abstract conclusion:** "Use of fish oil improved the number of tender joints and duration of morning stiffness at 3 months as analyzed by both meta- and mega-analysis. The fuller mega-analysis confirmed the results of the meta-analysis."
MacLean, et al. [35] (2004)	Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis. (Evidence Report/Technology Assessment)	P: Patients with either diabetes mellitus, metabolic syndrome, IBD, RA, renal disease, systemic lupus erythematosus or osteoporosis. I: n-3 PUFAs. C: Placebo. O: For the RA part: Patient assessment of pain, swollen joint count, ESR, patient's global assessment and joint damage. **Abstract conclusion:** "There appears to be no effect on most clinical outcomes in rheumatoid arthritis, although tender joint count may be reduced." **Clarification*:** A MA was only conducted for diabetes, RA and IBD resp. For RA, a MA was not conducted for the variables joint damage or tender joint count. They draw their conclusion regarding tender joint count by referring to a previously published MA by Fortin, et al. [34].
Goldberg and Katz [36] (2007)	A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain (International Association for the Study of Pain)	P: Patients with RA, or joint pain secondary to IBD or dysmenorrhea. I: n-3 PUFAs. C: Inert substance. O: Patient assessed pain, physician assessed pain, duration of morning stiffness, number of painful and/or tender joints, Ritchie articular index (assessment of joint tenderness), NSAID consumption. **Abstract conclusion:** "The results suggest that omega-3 PUFAs are an attractive adjunctive treatment for joint pain associated with rheumatoid arthritis, inflammatory bowel disease, and dysmenorrhea." **Clarification***: They only found improvements in the subgroup with duration of ≥3 months. No improvement was found in Ritchie articular index in any of the subgroups. Furthermore they found a greater improvement in the subgroup using >2.7 g/d and in the subgroup using non-olive oil placebo.
Lee, et al. [37] (2012)	Omega-3 Polyunsaturated Fatty Acids and the Treatment of Rheumatoid Arthritis: A Meta-analysis (Archives of medical research)	P: Patients with RA. I: >2.7 g/d n-3 PUFA for ≥ 3 months. C: Placebo. O: Tender joint count, swollen joint count, patient global assessment of disease-associated pain, physician global assessment of disease activity, pain, morning stiffness, physical function, ESR, CRP, NSAID consumption. Abstract conclusion: "This meta-analysis suggests that the use of omega-3 PUFAs at dosages >2.7 g/day for >3 months reduces NSAID consumption by RA patients. Further studies are needed to explore the clinical and NSAID-sparing effects of omega-3 PUFAs in RA."

*Clarification obtained from reading the full-text, and added when substantial details are missing from the abstract conclusion. PICOS, Population, Intervention, Control and Outcome; RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; NSAID, non-steroidal anti-inflammatory drug; IBD, inflammatory bowel disease; MA, meta-analysis; VAS, visual analogue scale.

pain in both RA, inflammatory bowel disease and dysmenorrhea and found an improvement in various pain measures at 3-4 months supplementation. A further improvement was found when using at least 2.7 g/day n-3 PUFAs or when using a non-olive oil placebo. The most resent systematic review and meta-analysis [37] only included studies with at least 2.7 g/d n-3 PUFAs supplementation for ≥3 months. They only found a reduction in NSAIDs consumption and concluded that further studies are needed. The study by Lee, et al. [37] was published only three years ago. However, as already mentioned, they included vegetable oils, which do not contain DHA or EPA, and the conversion from α-linolenic acid (ALA; 18:3 n-3), found in vegetable oils, to DHA and EPA is limited [38]. They only searched two databases, MEDLINE and the Cochrane Controlled Trial Register, in addition to the references of the studies, and applied a simple search strategy, which altogether may have failed to capture all relevant studies. Furthermore they only assessed publication bias, while other types of bias, especially outcome reporting bias (ORB), are of great importance [39 40].

Rationale for this systematic review and meta-analysis

Based on the survey of existing meta-analyses, we conclude that there is not enough evidence currently for recommending marine oil supplements as a treatment for RA or any other type of arthritis.

This systematic review and meta-analysis will be unique by including all types of arthritis and all kinds of marine oil supplements (e.g. fish oil, fish liver oil, krill oil and algae supplements), but no vegetable oils, and by applying a broad search strategy. No limit on dose will be applied and the minimum duration is set low (2 weeks), but stratified analyses will be carried out to assess whether these factors could have an effect. In addition, the data will be stratified according to the ratio of EPA/DHA in order to assess if one of the fatty acids is more effective. Emphasis will be placed on the outcome relevant to patients with arthritis, i.e. pain [9]. Different measures of pain will be pooled in order include more studies. The GRADE approach will be applied for grading the quality of the evidence.

Objectives

The objective is to assess the effect of oral marine oil supplementation on pain, but also on the secondary outcomes physical function and inflammation, in patients with arthritis, by performing a systematic review and a meta-analysis of the available randomised controlled trials (RCTs).

METHODS

Protocol and registration

This protocol is drafted following the *Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015* [41] and registered on PROSPERO (CRD42015016817). Study selection, assessment of eligibility criteria, data extraction, and statistical analyses will be performed based on this predefined protocol according to the

Cochrane Collaboration guidelines and the Updated Method Guidelines for Cochrane Musculoskeletal Group (CMSG) Systematic Reviews and Meta-analyses [42]. The manuscript will be reported following the guidelines from EQUATOR[43] on systematic reviews (the PRISMA statement [44]).

Eligibility criteria

Randomised or quasi-randomised controlled trials assessing the effect of any kind of marine oil supplementation therapy compared with no marine oil supplementation therapy in patients with any kind of diagnosed arthritis will be considered eligible (i.e. trials applying an add-on design). Participants at any age and gender will be included.

In order to exclude studies assessing postprandial effects, a minimum duration of two weeks will be applied. This limit is reasoned by a study, which have shown, that the rate of incorporation of DHA and EPA into plasma phosphatidylcholine, the main phospholipid in the outer membrane of erythrocytes [45], reaches maximum in 1-2 weeks and subsequently slows down [46]. No minimum dosage will be applied as an eligibility criterion. Whether the outcomes of interest have been reported, is not a criterion for entering the systematic review, in order to assess ORB. However, reports to be included in the meta-analysis must present suitable quantitative data on a change in at least one of the outcomes of interest, or present figures that are sufficiently comprehensive, allowing data extraction. No language restrictions will be applied for the systematic review, however the full-text must be written in English, Danish, Swedish or Norwegian to be included in the subsequent meta-analysis. No restrictions on publication date will be applied.

Information sources

Studies will be found by searching electronic databases and by screening reference lists from relevant articles. The search will be applied to MEDLINE via PubMed from 1950, the Cochrane Library including The Cochrane Central Register of Controlled Trials (CENTRAL) from 1898, EMBASE via OVID from 1980, ClinicalTrials.gov, and the World Health Organization International Clinical Trial Registry Platform portal (ICTRP), as recommended by the CMSG[42]. The search will also be applied to Web of Science via ISI Web of Knowledge from 1900.

Search

The search strategy will be developed by NKS and SMN with assistance from ST. It will consist of the three search concepts (i) marine oil supplements, (ii) arthritis, and (iii) RCT. Within each search concept various keywords and, when available, Medical Subject Heading (MeSH) terms will be used, aiming to make the search sensitive rather than specific. Different terms for common marine oil supplements were found searching the Dietary Supplement Label Database (http://www.dsld.nlm.nih.gov/dsld/). The RCT search concept consists of the *Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximizing version (2008 revision); PubMed*

format [47] with exception of ‘randomized [tiab]´, which has been replaced with ´random* [tiab]´ in order to make it more sensitive. Other terms is used in addition in order to make it even more sensitive.

PubMed search strategy:

(essential fatty acids OR polyunsaturated OR PUFA OR PUFAs OR omega 3 OR n-3 fatty acids OR Docosahexaenoic Acid OR Eicosapentaenoic Acid OR ((fish OR marine OR krill OR haddock OR cod OR salmon OR mackerel OR herring OR anchovy OR sardine OR tuna OR skipjack OR halibut OR coalfish OR shark OR whale OR seal OR calamari OR algae OR algal OR spirulina OR seaweed OR euphausia superba OR haematococcus pluvialis OR hematococcus pluvialis OR lithothamnion corallioides OR nova scotia dulce OR ascophyllum nodosum OR chlorella OR lithothamnion calcareum OR gigartina OR mussel OR perna canaliculus) AND (oil OR oils OR fatty acids OR lipid OR lipids OR triglyceride OR triglycerides)))

AND

(musculoskeletal diseases OR polyarthritis OR polyarthritides OR arthritides OR arthriti* OR joint pain OR rheumatoid OR rheuma* OR osteoarthritis OR Chondrocalcinosis OR calcium pyrophosphate deposition disease OR Gout OR Periarthritis OR Sacroiliitis OR Spondylarthritis OR Spondylarthropathies OR Spondylarthropathy OR Spondyloarthritis OR Spondyloarthropathies OR Spondyloarthropathy OR ((Still's OR caplan OR caplan’s OR Felty OR Felty’s OR Sjogren OR Sjogren’s OR gouty OR Wissler OR Wissler's OR Wissler-Fanconi) AND (syndrome OR disease OR arthritis)))

AND

(randomized controlled trial [pt] OR controlled clinical trial [pt] OR placebo [tiab] OR drug therapy [sh] OR trial [tiab] OR groups [tiab] OR clinical trials as topic [mesh: noexp] OR Clinical Trial OR random* [tiab] OR random allocation [mh] OR single-blind method [mh] OR double-blind method [mh] OR cross-over studies)

NOT

(animals [mh] NOT humans [mh])

When using words, which are mapped in the MeSH database, the search will automatically explode by including all narrower terms within the MeSH terms indexed for the articles. Searching e.g. `omega 3 fatty acids´ will include `docosahexaenoic acid´ and `eicosapentaenoic acid´ within the MeSH terms indexed for the articles. The following words are all automatically linked to MeSH terms (shown in parentheses):

Essential fatty acids (fatty acids, essential), PUFAs (fatty acids, unsaturated), omega 3 (fatty acids, omega-3), n-3 fatty acids (fatty acids, omega-3), docosahexaenoic acid (docosahexaenoic acids), eicosapentaenoic acid (eicosapentaenoic acid), fish (fishes), krill (euphausiacea), salmon (salmon), mackerel (perciformes), tuna (tuna), flounder (flounder), shark (sharks), whale (whales), seal (seals, earless), spirulina (spirulina), seaweed (seaweed), euphausiacea (euphausiacea), nova scotia (nova scotia), ascophyllum (ascophyllum), chlorella (chlorella), mussel (bivalvia), perna canaliculus (perna), oils (oils), fatty acids (fatty acids), lipids (lipids), triglycerides (triglycerides), musculoskeletal diseases (musculoskeletal diseases), polyarthritis (arthritis), joint pain (arthralgia), osteoarthritis

(osteoarthritis), disease (disease), syndrome (syndrome), chondrocalcinosis (chondrocalcinosis).

Date for the final search will be stated. Search strategies for the other databases can be found in appendix 1.

Study selection

The initial screening of the records will be based on title and abstract and the subsequent assessment on full-texts. The whole procedure will be done independently by two reviewers (NKS, SMN) and disagreements will be resolved by discussion until consensus is reached or otherwise solved by a third reviewer (RC). The reference software EndNote X7.2.1 will be used.

Data collection process

Data extraction from eligible studies will be done in a systematic, standardised way using a customised data-extraction form, and will be done independently by two reviewers (NKS, SMN). In case of disagreement, consensus will be reached by discussion or with help from a third reviewer (RC) when necessary. The preferred time point of measurements, in case of multiple time points reported, is the latest possible, i.e. where a control group is still used. Only data from the first period of intervention in cross-over trials will be included, because of risk of accumulating carry-over effect in pooled efficacy meta-analyses [48]. We anticipate that pain, physical function and inflammation can be measured in several ways within each study, and thus for each construct we will use the outcome stated to be the primary outcome in the study or study protocol, or the outcome proposed by the rheumatologist (HB). The corresponding author of the article will be contacted in order to obtain underlying data if it is not extractable.

Data items

Included studies will be assigned an ID number and information will be extracted on: main author, year of publication, publication status, and funding source. Details of the trial methodology will be extracted on: study design, duration, number of patients randomised (N_{total}), number of patients allocated to each group (n_I and n_C), type of statistical analysis (e.g. intention-to-treat analysis [ITT]), random sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, completeness of outcome data and handling of missing values, and a list of all reported outcomes in the study.

Information on patient characteristics will be extracted on: mean age, % female, mean body mass index (BMI), country, arthritis type, disease duration, joints affected, eligibility criteria, and baseline values of outcomes of interest.

Details of the intervention used will be extracted on: Supplementation formulation (e.g. capsule), supplementation origin (e.g. cod liver), daily dose of DHA and EPA, and control treatment, including dose of PUFAs other than DHA and EPA.

Outcome measures of interest will be extracted on: patient assessed pain (e.g. visual analogue scale [VAS]), objective outcome for physical function (e.g. hand grip strength), and markers of inflammation (e.g. c-reactive protein [CRP]).

On the adverse effects we will extract data on tolerance (i.e. number of completers), harm assessed as withdrawals due to adverse events (WD d/t AEs), and the number of serious adverse events (SAEs).

Risk of bias in individual studies

Trials with low internal validity, because of inadequate methodological quality, may distort the results from meta-analyses [49]. Therefore two reviewers (NKS, SMN) will independently assess RoB of each full text using *The Cochrane Collaboration's tool for assessing risk of bias in randomised trials* [40]. The RoB tool includes five bias domains (i) Selection bias (appropriate sequence generation and allocation concealment), (ii) Performance bias (blinding of participants and personnel), (iii) Detection bias (blinding of outcome assessor), (iv) Attrition bias (proper use of ITT), and (v) Reporting bias (i.e. ORB). Selective ORB is a novel RoB domain, and is important since there is strong evidence that statistically significant outcomes have higher odds of being fully reported [50]; ORB will be assessed using the tool developed by Dwan, et al. [39]. Results from the RoB assessment will be used in stratified analyses.

Summary measures

Due to different ways of measuring pain, physical function and inflammation, treatment effect sizes for each of the studies will be expressed as Standardised Mean Differences (SMDs), by dividing the difference in mean values by the pooled standard deviation for the given outcome. The variance (SE^2) will be calculated based on the SMD and number of patients in each group ($SE^2 = 1/n_I+1/n_C + SMD^2 / [2x\{n_I+n_C\}]$) [51]. Since this tends to overestimate SMDs for small samples, a correction will be applied by default by calculating Hedges' g ($g=J$ x SMD; $J=1–3/[4 \text{ x } df – 1]$; $df=n_I+n_C–2$) [52]. A negative SMD will indicate a beneficial effect of the intervention for pain and inflammation, and a harmful effect on physical function. As the CMSG recommends, the SMDs will be transformed into a measure that is easier to interpret [42]; SMDs will be transformed into average improvement in percentage compared to placebo alone, corresponding to the conversion suggested by Bliddal and Christensen [53].

Risk ratios (RR) will be calculated for binary outcomes, i.e. number of completers (tolerance), withdrawals due to adverse events, and serious adverse events, in order to make results easier to interpret, unlike odds ratios, as recommended by CMSG [42]. All results will be presented with 95 % confidence intervals (95 % CIs).

Synthesis of results

Random-effects meta-analysis will be used as default option, whereas the fixed-effect analysis will be applied for sensitivity analyses. Each study effect size will be weighted by

the inverse variance of the effect size. Homogeneity statistics will be computed in order to evaluate the consistency of the individual trial results by applying the chi-squared test. We will measure inconsistency by calculating the I^2-statistic [54], which describes the percentage of total variation across trials due to heterogeneity rather than to chance. I^2 values below 25 %, from 25 % to 50 %, and from 50 % to 75 %, correspond to low, moderate, and high between-trial heterogeneity, respectively [55].

As recommended by The Cochrane Collaboration a summary of findings (SoF) and/or evidence profile table will be constructed including assessing the quality of the evidence using the GRADE approach [56]. Analyses will be performed using Review Manager (Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), and R Software (version 3.1.2) [57].

Risk of bias across studies

Funnel plots will be used to support assessment of publication bias across studies for pain. The horizontal axis will show the SMDs and the vertical axis will show the standard errors of SMDs. Since the data are continuous, the test proposed by Egger will be applied [58]. The test will only be applied if at least 10 studies are included and if the studies have similar standard errors [59].

For the remaining RoBs and funding source, stratified analyses of the primary outcome (pain) will be performed. For this purpose univariable Restricted Maximum Likelihood (REML)-based (i.e. random-effect) meta-regression models will be applied. Trials will be stratified according to:

- Funding source:
    - Industry source
    - Not reported
    - Non-profit source
- Randomisation, i.e. random sequence generation and allocation concealment (selection bias):
    - Adequate
    - Unclear
    - Inadequate
- Blinding of participants and personnel (performance bias):
    - Adequate
    - Unclear
    - Inadequate
- Blinding of outcome assessment (detection bias) :
    - Adequate
    - Unclear
    - Inadequate
- Adequacy of statistical analysis (attrition bias) :
    - Adequate

    - Unclear
    - Inadequate
- Outcome reporting (ORB)
    - Adequate
    - Unclear
    - Inadequate

Additional analyses

A number of additional analyses of the primary outcome (pain) will be performed, stratifying the available trials according to pre-specified trial characteristics. Univariable Restricted Maximum Likelihood (REML)-based (i.e. random-effect) meta-regression models will be applied. Trials will be stratified according to:

- Type of arthritis; RA is characterised by inflammation, OA by a smaller degree of inflammation and manifestations different from RA, hence the following stratification will be applied:
    - RA
    - OA
    - Others
- Ratio of EPA/DHA; in order to assess if effectiveness varies with EPA/DHA ratio, and which ratio EPA/DHA is most effective, hence the following stratification will be applied:
    - Contains the ratio of EPA/DHA > 1.5
    - Contains the ratio of EPA/DHA ≤ 1.5
    - Unspecified
- Total dosage of DHA and EPA; a review and a meta-analysis has established that supplementation with ≥2.6 g DHA and EPA daily for 12 weeks will reduce symptoms of RA [36 60], hence the following stratification will be applied:
    - Dosage of EPA and DHA <2.6 g/d
    - Dosage of EPA and DHA ≥2.6 g/d and <3.6 g/d
    - Dosage of EPA and DHA ≥3.6 g/d
    - Unspecified
- Duration of intervention, cf. the latter point, data will be stratified according to:
    - Duration of <12 weeks
    - Duration of ≥12 weeks and <24 weeks
    - Duration of ≥24 weeks
    - Unspecified
- Control; the type of control treatment may affect the measured effect from the marine oil supplement. The cut-off for PUFA content in PUFA oils is chosen to be ≥30 %, based on the general content in vegetable oils. The following stratification will be applied:
    - PUFA oils, not containing DHA or EPA

- Non-PUFA oils
- Non-oil placebo
- No placebo treatment
- Unspecified

A plot of the dose-response and of the duration-response relationship will be made by plotting each study SMD against total daily DHA and EPA dosage and duration of intervention respectively. Studies containing an EPA/DHA-ratio of >1.5 and ≤1.5 respectively will be highlighted in distinct colours.

ETHICS AND DISSEMINATION

We believe that the findings of this systematic review and meta-analysis will have important implications for future research strategies. Hopefully it will also assist directly in clinical practice when deciding whether to recommend and apply marine oil supplements in patients with arthritis pain.

The results will be disseminated as article(s) in peer-reviewed scientific journal(s), and will be communicated via scientific meetings as well as presented for public outreach to patients and the public via suitable sources (incl. the Danish Rheumatism Association). Papers will be drafted by the co-primary investigators (NKS, SMN) and revised by the collaborators, who will, according to the standards of ICMJE, be authors when they provide substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; are part of drafting the work or revising it critically for important intellectual content; and will be part of the final approval of the version to be published. Finally, all authors need to be in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Contributors

NKS, SMN, JRA, and RC conceived and designed the study; NKS, SMN, JRA, ST, LL and RC contributed to the development of the protocol. All of the authors (NKS, SMN, JRA, HB, ST, LL, and RC) assisted in the final protocol and agreed to its final approval before submission.

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had no role in study design, data collection, data synthesis, data interpretation, writing the report, or the decision to submit the manuscript for publication.

Competing interests

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