The effect of omega-3 polyunsaturated fatty acids on arthritic pain: A systematic review

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Running title: Omega-3 fatty acids and arthritic pain

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Highlights

- Eighteen RCTs published between 1985 and 2013 involving 1143 patients were included in a systematic review of omega-3 fatty acids and pain in rheumatoid arthritis (RA)
- Ten studies support the hypothesis that there is a reduction in patient or physician assessment of pain associated with RA after intake of omega-3 fatty acids
- Omega-3 fatty acids may have a therapeutic role in decreasing pain associated with RA, with doses of 3-6 g/d appearing to have a greater effect.
- More research is needed to investigate omega-3 fatty acids and pain in larger populations and over extended periods of time

Abstract

Background & aims: Pain is a significant problem in rheumatoid arthritis (RA), and is associated with prostaglandins derived from the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA). The omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to reduce inflammation, with some studies observing clinical improvements in RA. The aim of this systematic review is to investigate the effect of omega-3 PUFAs on arthritic pain.

Method: A systematic literature review of omega-3 PUFAs and pain associated with RA was performed up to December 2015. Randomised controlled trials (RCTs) investigating the effect of omega-3 PUFAs (> 2 g/d) on patient and/or physician assessment of pain were included. The Cochrane Collaboration's tool for assessing risk of bias was employed. Data for outcomes of interest were extracted and collated for interpretation.

Results: Eighteen RCTs published between 1985 and 2013 involving 1143 patients were included. Dosage of omega-3 PUFAs used was 2.1-9.1 g/day, with study durations of 12-52 weeks. Ten studies supported the hypothesis that there is a reduction in patient or physician assessment of pain associated with RA after intake of omega-3 PUFAs. Eight studies found no statistically significant effect of omega-3 PUFAs on arthritic pain.

Conclusions: Omega-3 PUFAs may have a therapeutic role in decreasing pain associated with RA, with doses of 3-6 g/d appearing to have a greater effect. Due to the limitations identified in studies included in this review, more research is needed to investigate omega-3 PUFAs in larger populations and over extended periods of time.

Keywords: EPA, DHA, fish oil, rheumatoid arthritis, pain

Abbreviations used: AA, arachidonic acid; ALA, α-Linolenic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; DMARD, disease-modifying anti-rheumatic drug; EPA, eicosapentaenoic acid; LTB_5, leukotriene B5; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PGE, Prostaglandin E; PUFA, polyunsaturated fatty acid; RA; rheumatoid arthritis; VAS, visual analogue scale

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease which causes chronic inflammation and pain in the joints, leading to destruction of the cartilage over time [1]. Pain is one of the most significant problems for patients with RA, and is caused by infiltration of inflammatory cells into the synovium of the joints [2]. Activation of these cells results in secretion of pro-inflammatory cytokines, eicosanoids and other mediators resulting in inflammation, pain and swelling of the joint [1].

The infiltrating cells are a source of the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA) which resides in the cell membrane phospholipids. Under inflammatory conditions, AA is released from the phospholipids and the expression of cyclooxygenase (COX) enzymes is increased, resulting in oxidation of free AA to form pro-inflammatory lipid mediators such as the prostaglandins (PGs) and thromboxanes, particularly PGE_2 [3]. PGE_2 increases vascular permeability and vasodilation leading to enhancement of pain and oedema [4]. PGE_2 further increases pro-inflammatory cytokine release and production of destructive matrix metalloproteinase (MMPs) [1].

Treatment of RA including its pain component, involves anti-inflammatory medications such as non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and disease-modifying anti-rheumatic drugs (DMARDs) [5]. NSAIDs inhibit the COX enzymes involved in AA metabolism, thereby reducing the formation of pro-inflammatory, pain causing AA-derived prostaglandins. However, long-term use of NSAIDs can cause side effects such as stomach ulceration due to their unselective inhibition of both COX enzyme isoforms [6]. Therefore, there is interest in exploring therapeutic alternatives that could help alleviate the painful symptoms of RA and slow disease progression without side effects.

Oily fish and fish oil supplements contain the long chain omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Many studies have shown that EPA and DHA partly inhibit some inflammatory processes and that, as a result, they may be useful in inflammatory diseases such as RA [7]. There have been several studies using animal models and isolated inflammatory cells that have provided information about the likely mechanisms involved [8]. It has been shown that increased intake of EPA and DHA results in increased EPA and DHA content in inflammatory cell membranes, partly displacing AA and thus decreasing availability of AA for prostaglandin synthesis [8,9]. EPA is itself a substrate for prostaglandin synthesis, but gives rise to less potent and less inflammatory mediators such as PGE_3 and LTB_5 [10]. Furthermore, both EPA and DHA are substrates for synthesis of inflammation-resolving resolvins, protectins and maresins [7,11]. EPA and DHA also act through lowering COX-2 gene expression thereby reducing the availability of the enzyme that converts AA to the more potent inflammatory mediators [8].

A number of studies have investigated the effect of omega-3 PUFAs on various clinical outcomes in RA. These studies provide some evidence that, through their anti-inflammatory and inflammation-resolving properties, omega-3 PUFAs can lower the duration of morning stiffness, reduce the number of tender and swollen joints, and improve physician and patient assessed pain in patients with RA [7]. This may in turn reduce the consumption of anti-inflammatory drugs such as NSAIDs in RA. However, not all studies support the use of omega-3 PUFAs in RA, and this is reflected in three systematic reviews conducted over the past decade in this area [7,12,13]. Lee et al. conducted a meta-analysis of 10 RCTs that found no improvement in arthritic pain, but this was limited by the exclusion of trials using < 2.7 g/d omega-3 PUFAs or having a duration of less than 3 months [13]. Conversely, Goldberg & Katz's meta-analysis of 17 RCTs found that intake of omega-3 PUFAs for 3-4 months reduced patient-reported joint pain intensity and NSAID consumption, with a greater effect observed with higher doses of omega-3 PUFAs [12]. This is supported further by Miles

& Calder who conducted a systematic review of 23 RCTs which concluded that omega-3 PUFAs reduce joint pain and have anti-inflammatory drug sparing potential [7].

Our aim was to investigate the use of omega-3 PUFAs on pain in RA in order to provide a more current and robust evidence base in this field. As there is general consensus that a higher dose of omega-3 PUFAs for a period of at least 4-6 weeks is likely to be more effective in achieving an anti-inflammatory effect [12,14], we restricted the focus of our systematic review to RCTs which used omega-3 PUFA dosage levels of >2 g/d and for a minimum duration of > 1 month.

Methodology

Literature search

This systematic review was conducted according to the principles of "Preferred Reporting for Systematic Reviews and meta-analysis" (PRISMA) [15] (Figure 1). Literature searches were performed from September to December 2015 in PubMed, Embase, Delphis, Web of Science, Google Scholar and Google, Medline and Cochrane Library using search terms including "omega-3 fatty acid", "fish oil", "EPA" and "DHA", "rheumatoid arthritis" and "patient or physician assessment of pain". In addition, reference lists of included studies were manually checked to identify further relevant publications.

Study selection

Studies which met the following criteria were included in this review: RCT involving human patients with RA; peer-reviewed publication; published in full in the English language; oral administration of marine omega-3 fatty acids at dosage > 2 g/day; duration > 1 month; sample size >10 participants; and reporting patient or physician assessment of pain. Studies were excluded if they were not published in the English language; if the abstract did not mention outcomes of interest; if they were not RCTs; if they used < 2 g/day omega-3 fatty acid; if the omega-3 fatty acids used were not EPA and DHA; or if they were non-human studies. All articles of potential relevance were retrieved and evaluated for inclusion.

Publication bias

Publication bias was minimised by utilising multiple online databases in combination with manual reference searches. However, a degree of bias may have been introduced through exclusion of papers not written in the English language. This search strategy may also have missed studies that have yet to be published on electronic databases.

Data extraction

The information considered during data extraction was based on the PICO principle, and included trial design, sample size, dose of omega-3 PUFAs used and duration, outcome regarding pain associated with RA, and Jadad score (see Quality assessment).

Quality assessment

Studies that met the inclusion criteria were assessed for methodological quality and validity using the Jadad scale [16]. The risk of bias (including selection bias, performance bias, detection bias and attrition bias) was assessed using the Cochrane Risk of Bias tool as outlined in the Cochrane Handbook for Systemic Reviews of Interventions [17].

Results

Search results

From the electronic literature search, 531 publications of potential relevance were identified (Figure 1). An additional 13 publications were identified through the manual searching of bibliography of the articles. Of these 544 articles, 410 were excluded due to not meeting the eligibility criteria and 103 were excluded due to duplication. Upon reading the abstracts of the remaining 31 articles, 9 were excluded due to lack of intervention (n=3), not being about RA (n=4), or using supplements other than omega-3 PUFAs (n=2). Upon reading the full text of the remaining 22 articles [18-39], another 4 were excluded because the outcome in relation to the patient and physician assessment of pain was unknown [37,38], the total amount of omega-3 PUFAs used was < 2 g/day [39], or the trial was not randomised [18]. Finally 18 RCTs published between 1985 and 2013 were included in this systematic review (Figure 1; Table 1).

Characteristics of included studies

Table 1 summarises the characteristics of the 18 RCTs included in this review, including trial design, sample size, dose and duration of omega-3 PUFAs used, outcome regarding pain associated with RA, and Jadad score.

The sample size of the studies varied between 16 and 109, giving a total of 1,143 participants. For the majority of the RCTs, the study population comprised patients with active RA, stabilised through the use of NSAIDs and/or DMARDs and glucocorticoids. RA medications were continued throughout the majority of trials, with the exception of those studies which investigated the effect of omega-3 PUFA intake on reducing NSAID consumption [19-22]. The dose range of omega-3 PUFAs used was between 2.1 and 9.1 g/d with study durations between 12 and 52 weeks. All studies provided omega-3 PUFAs as fish oil capsules.

Regarding assessment of bias (Table 2), some studies presented a high risk in the areas of attrition, detection and performance bias. Regarding attrition bias, four studies scored high risk due to significant participant withdrawals including Geusens et al. (30 of 60 participants), Volker et al. (24 of 50 participants), Galarraga et al. (39 of 97 participants) and Sundrarjun et al. (25 of 60 participants) [22,29,31,34]. Regarding detection bias, Berbert et al. reported that clinical investigators were not blinded to the treatment allocation and hence this study was scored as high risk in this category [24]. Three studies scored high risk for performance bias due to inadequate participant blinding. These included Das Gupta et al. in which participants were given fish oil capsules in addition to indomethacin vs indomethacin alone [20], and Lau et al. and Galarraga et al. which both used air-filled capsules as placebo [19,22]. In contrast, two studies that scored low risk in this category took steps to mask placebo oil capsules with fish oil / fish oil aroma to ensure participant blinding [30,36].

Effect of omega-3 PUFAs on patient / physician assessment of pain in rheumatoid arthritis

Of the 18 studies included, ten supported the hypothesis that there is a reduction in patient or physician assessment of pain associated with RA after intake of omega-3 PUFAs [20,22,24-31] (Table 3). Eight studies found no significant effect of omega-3 PUFAs on arthritic pain [19,21,23,32-36] (Table 3).

Of the ten studies that found a significant benefit in pain reduction with intake of omega-3 PUFAs, four studies (n = 237 participants) observed a significant improvement in pain vs placebo [22,24,27,30], with the remaining six studies reporting improvement in pain vs baseline levels only [20,25,26,28,29,31] (Table 3). Berbert et al., Nielsen et al. and Tulleken et al. all used similar doses of omega-3 PUFAs (3 to 3.6 g/d for 12 or 24 weeks) in patients with active RA that were taking NSAIDs and/or DMARDs [24,27,30]. Statistically significant reduction in pain parameters such as joint pain intensity / index or VAS pain score vs placebo was found where placebo was soybean oil [24], oil with a composition representative of the typical Danish diet [30], or coconut oil [27]. Galarraga et al. used a

lower omega-3 PUFA dose (2.2 g/d) for a longer period of time (36 weeks) in a similar patient population and also observed a significant improvement in patient assessment of pain vs placebo [22].

Of the six studies that reported statistically significant improvements in pain vs baseline levels only (i.e. not compared with placebo), omega-3 PUFA doses ranged between 2.1 and 9.1 g/d with duration of intervention between 12 and 52 weeks [20,25,26,28,29,45]. Kremer et al. used the highest omega-3 PUFA dose of all RCTs in two separate studies (estimated at ~ 6.3 g/d for 24 weeks and ~ 9.1 g/d for 26-30 weeks respectively; based on a 70 kg person) and reported a significant reduction in patient and physician evaluation of pain vs baseline levels [25,26].

Eight studies failed to show a statistically significant effect of omega-3 PUFAs on pain in patients with RA stabilised with NSAIDs and/or DMARDs [19,21,23,32-36]. With the exception of Cleland et al. and Kjeldsen-Kragh et al., all these RCTs used omega-3 PUFA dosage levels of ~ 3 g/d, including Lau et al. which also had the longest duration of all studies, at 52 weeks. Despite a lack of significant improvement in pain, Lau et al. did report a significant reduction in NSAID use in the treatment group vs placebo which is suggestive of a degree of pain improvement [19]. Skoldstam et al. also observed a statistically significant reduction in NSAID use at a dose of 3.2 g/d omega-3 PUFAs, although patient assessment of pain remained unchanged [21]. Cleland et al. and Kjeldsen-Kragh et al. used higher doses of 5.2 g/d and 5.8 g/d omega-3 PUFAs for 12 weeks and 16 weeks respectively, but failed to see a statistically significant improvement in pain score [23,35].

Discussion

The majority of studies (10 of 18) included in this review suggest that use of the omega-3 PUFAs EPA and DHA has beneficial effects in reducing patient or physician assessment of pain in RA, either compared to baseline or to placebo (Table 3). However, the studies included in this review have several limitations which might mitigate the overall conclusion. Although the studies were all RCTs, some did not have effective randomisation to eliminate all selection bias [19], and some studies failed to state whether the investigators assessing the outcome were blinded to treatment allocation [24]. Furthermore, in some studies participant blinding was questionable [19,20,22]. Two studies utilised air-filled capsules as placebo, which are easily distinguishable from fish oil capsules [19,22], whilst one study investigated fish oil capsules in addition to indomethacin versus indomethacin alone thereby enabling the participants to know which group they had been allocated to [20]. Just two studies took steps to preserve participant blinding through the masking of placebo oil capsules with fish oil / fish oil aroma [30,36]. A further limitation may be the potential of concurrent NSAID use to reduce the anti-inflammatory effect observed from the omega-3 PUFA intervention. Both NSAIDs and EPA inhibit COX enzymes and reduce the production of AA-derived inflammatory mediators. However, studies are not conclusive regarding this and have reported conflicting outcomes. In one study, Geusens et al. noted that intake of omega-3 PUFAs with NSAIDs caused a greater improvement in RA disease activity than NSAIDs alone, despite the low dosage of omega-3 PUFAs used [29]. It is important to note that several studies (e.g. [19,21]) reported a reduction in use of NSAIDs when omega-3 PUFAs were given, suggesting a reduction in pain. Given that long-term use of NSAIDs is associated with adverse effects, this NSAID-sparing effect of omega-3 PUFAs is important.

Whilst all studies used participants with stable, active RA, the participants may have had differing severity of pain. For example, Cleland et al. used patients with long standing disease, some had more advanced joint damage compared to other studies, and this may have caused variation in the pain levels [23]. This may have contributed to the failure of the

reasonably high amount of omega-3 PUFAs used (5.2 g/d) to demonstrate any significant improvement in pain [23]. Conversely, Park et al. used participants with mild RA and already high baseline EPA and DHA levels due to high dietary consumption of fish [33]. Both factors may have contributed to the failure of omega-3 PUFA consumption (3.25 g/d for 16 weeks) to exert a beneficial pain-reducing effect in this patient population.

Regarding the smaller number of studies (n=4) that found a pain-reducing effect of omega-3 PUFAs vs placebo compared to those that found an effect compared to baseline (n=6), there may be several reasons for this. Patient assessment of pain is a subjective parameter and may be influenced by the 'placebo effect'. Furthermore, sample sizes were relatively small and some studies may have lacked statistical power to detect a difference between treatment and placebo groups. There may also be potential for the placebo itself to exert an anti-inflammatory and pain-reducing effect, making inter-group differences harder to identify. This is evidenced by several studies observing a pain-reducing effect in the placebo group, where placebo was olive oil [23,25,29], and one trial finding a greater reduction in joint pain intensity in the olive-oil supplemented fish oil group vs fish oil alone [24]. This may have been a contributing factor in the statistically significant treatment effect vs placebo observed in those four RCTs that used placebo oils other than olive oil [22,24,27,30]. These observations suggest that olive oil is not an appropriate placebo for studies of omega-3 PUFAs.

With respect to doses of omega-3 PUFAs used in the studies, the trial outcomes are inconsistent. Some RCTs used relatively modest levels of omega-3 PUFAs (~3 g/d) and observed a clear pain-reducing effect [20,22,24,27,29,30], whilst others failed to show a benefit at this dose [19,21,32-34]. For those studies using omega-3 PUFAs > 3 g/d, Cleland et al. and Kjeldsen-Kragh et al. failed to observe a significant reduction in pain at 5.2 g/d and 5.8 g/d omega-3 PUFAs respectively [23,35], whilst both the higher-dose Kremer et al. studies (~ 6 g/d and ~ 9 g/d) found a significant improvement in pain vs baseline [25,26]. Interestingly, the extent of the pain-reducing effect found in Kremer's higher dose study (~

9.1 g/d) was no greater than that observed in the more moderate (~ 6.3 g/d) study, suggesting that omega-3 PUFA intake > 6 g/d does not bring additional benefit with regard to pain associated with RA.

One aspect that need to be considered in studies of omega-3 PUFAs is compliance to the treatment. Omega-3 capsules can cause problems of taste, nausea and gastrointestinal upset, especially when given at high doses and this may limit compliance especially in long term studies. Of the 18 studies included in this review, the majority (13 studies) reported measurement of participant compliance through either capsule counting, participant questioning, or blood/blood cell fatty acid analysis [19,22-26,28,29,30-34]. In cases where participant compliance was questionable, some studies reported subsequent participant exclusion. However, this may not have been the case for all studies, so that whether the lack of effect of omega-3 PUFAs reported in some trials is due to poor compliance cannot be easily assessed.

In general, from this systematic review it appears that dosage levels of omega-3 PUFAs between 3 and 6 g/d are most likely to have a pain-reducing effect in RA. This is broadly in agreement with Goldberg & Katz's systematic review of 17 RCTs in 823 patients investigating the analgesic effects of omega-3 PUFAs in inflammatory joint pain [12]. The authors found that whilst significant effects were not found for physician-assessed pain, significant reduction in patient-reported joint pain intensity was found following 3-4 months supplementation of omega-3 PUFAs of at least ~3 g/d [12].

Conclusion

The findings of this systematic review support the hypothesis that intake of omega-3 PUFAs (EPA and DHA) at a dose of > 3 g/day for duration of 12 weeks or more reduce patient and physician assessment of pain in RA. Although a relatively high number of studies (10 out of 18) confirmed the beneficial effect of omega-3 PUFA supplementation on pain, a firm conclusion cannot be made with certainty because there are many limitations identified in

the studies included in the review. Therefore, in order to investigate the beneficial effect of omega-3 fatty acids in arthritic pain, future studies need to be conducted over extended periods and in larger populations. Such studies should include investigation of different intakes of omega-3 PUFAs in order to identify the minimum dose required for an effect to be seen and the maximum dose beyond which there is no further enhancement of effect. Assessment of patient compliance, including unused capsule counting and measurement of omega-3 PUFAs in blood compartments should be included. The placebo to be used should be carefully considered. Finally, future studies are required to clarify the exact mechanisms by which omega-3 PUFAs exert their beneficial effect on pain associated with RA; inclusion of measurements of eicosanoids, cytokines and omega-3 PUFA-derived pro-resolving mediators would contribute to such clarification.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Table 1. Summary of the characteristics of the included studies

Reference	Study design	Sample size (n) at study entry / completion	Dose of omega-3 PUFAs; EPA + DHA (g/day)	Duration (weeks)	Effect of omega-3 PUFAs on pain (Further details are given in Table 3)	Jadad score
Adam et al. (2003) [28]	Random order cross over (8 week washout)	Total: 68 (60 completed). Control (corn oil) vs. omega-3 PUFAs each against a background Western diet (n=34; 30 completed) or anti-inflammatory diet (n=34; 30 completed).	Estimation for 70 kg person: ~ 2.1 g/d omega-3 PUFAs: ~900 mg/d EPA + 670 mg/d DHA	12	Statistically significant reduction in patient assessment of pain from baseline, with greatest reduction in anti-inflammatory diet + omega-3 PUFAs group	3
Berbert et al. (2005) [24]	Parallel; 3 arm	Total: 55 (43 completed). Control (soybean oil): 17 (13 completed). Omega-3 PUFAs: 18 (13 completed). Omega-3 PUFAs + olive oil: 20 (17 completed).	3.0 g/d omega-3 PUFAs	24	Statistically significant reduction in joint pain intensity vs control	2
Cleland et al. (1988) [23]	Parallel; 2 arm	Total: 60 (46 completed). Control (olive oil): 30 (23 completed). Omega-3 PUFAs: 30 (23 completed).	5.2 g/d omega-3 PUFAs with 3·2 g/d EPA + 2·0 g/d DHA	12	Non statistically-significant improvement in analogue pain score	3
Das Gupta et	Parallel; 2 arm	Total: 100 (81 completed). Control	3.0 g/d omega-3 PUFAs	12	Statistically significant improvement in	1

al. (2009) [20]		(indomethacin alone): 50 (41 completed). Omega-3 PUFAs + indomethacin: 50 (40 completed).			patient assessment of pain from baseline	
Galarraga et al. (2008) [22]	Parallel; 2 arm	Total: 97 (58 completed). Control (air-filled capsules): 48 (26 completed). Omega-3 PUFAs: 49 (32 completed).	2.2 g/d omega-3 PUFAs; 1·5 g/d EPA + 0·7 g/d DHA.	36	Statistically significant improvement in patient assessment of pain vs placebo.	3
Geusens at al. (1994) [29]	Parallel; 3 arm	Total: 90 (60 completed). Control (olive oil): 30 (20 completed). Low dose omega-3 PUFAs + olive oil: 30 (21 completed). High dose omega-3 PUFAs: 30 (19 completed).	High dose: 2.6 g/d omega-3 PUFAs; 1·7 g/d EPA + 0·4 g/d DHA. Low dose: 1.3 g/d omega-3 PUFAs; 0·85 g EPA + 0·2 g DHA.	52	Statistically significant improvement in physician assessment of pain from baseline in high dose group	4
Kjeldsen-Kragh et al. (1992) [35]	Parallel; 3 arm	Total: 79 (67 completed). Declining NSAIDs + control: 28 (24 completed). NSAIDs + omega-3 PUFAs: 25 (20 completed). Declining NSAIDs + omega-3 PUFAs: 26 (23 completed).	5.8 g/day omega-3 PUFAs; 3·8 g/d EPA + 2·0 g/d DHA.	16	No significant improvement in pain score	3

Kremer et al. (1985) [32]	Parallel; 2 arm	Total: 52 (37 completed). Control (paraffin oil) + high SFA/low PUFA diet: 25 (20 completed). Omega-3 PUFAS + high PUFA/low SFA diet: 27 (17 completed).	3 g/d omega-3 PUFAs; 1.8 g/d EPA + 1.2 g/d DHA	12	No change in patient or physician assessment of pain vs baseline or control	3
Kremer et al. (1990) [25]	Parallel; 3 arm	Total: 60 (49 completed). Control (olive oil): 20 (12 completed). Low dose omega-3 PUFAs: 20 (20 completed). High dose omega-3 PUFAs: 20 (17 completed).	Estimation for 70 kg person: High dose: ~6.3 g/d omega-3 PUFAs; ~3.8 g/d EPA + ~2.5 g/d DHA. Low dose: ~3.2 g/d omega-3 PUFAs; ~1.9 g/d EPA + ~1.3 g/d DHA.	24	Statistically significant improvement in patient and physician evaluation of pain in high dose group vs baseline.	3
Kremer et al. (1995) [26]	Parallel; 2 arm	Total: 66 (49 completed). Control (corn oil): 33 (26 completed). Omega-3 PUFAs: 33 (23 completed).	Estimation for a 70 kg person: ~ 9.1 g/d omega-3 PUFAs; ~ 4.0 g/d EPA + 2.1 g/d DHA.	26-30	Statistically significant decrease in physician's evaluation of pain vs baseline	3
Lau et al. (1993) [19]	Parallel; 2 arm	Total: 64 (unknown completers). Control (air-filled capsules): 32 (completers not explicitly stated)	2.9 g/d omega-3 PUFAs; 1·7 g/d EPA + 1·1 g/d DHA.	52	No significant difference in patient or physician-assessed pain	2

		Omega-3 PUFAs: 32 (completers not explicitly stated).				
Nielsen et al. (1992) [30]	Parallel; 2 arm	Total: 57 (51 completed). Control (oil representing typical Danish diet): 28 (24 completed). Omega-3 PUFAs: 29 (27 completed).	3.6 g/day omega-3 PUFAs; 2·0 g/d EPA + 1·2 g/d DHA.	12	Statistically significant improvement in VAS pain score vs placebo	4
Park et al. (2013) [33]	Parallel; 2 arm	Total: 109 (81 completed). Control (sunflower oil with oleic acid): 54 (40 completed): Omega-3 PUFAs: 55 (41 completed).	2.09 g/d EPA + 1.165 g/d DHA.	16	No difference in pain score	5
Skoldstam et al. (1992) [21]	Parallel; 2 arm	Total: 46 (43 completed). Control: 23 (21 completed). Omega-3 PUFAs: 23 (22 completed).	3.7 g/d omega-3 PUFAs; 1·8 g/d EPA + 1·2 g/d DHA	24	No change in patient assessment of pain	4
Sundrarjun et al. (2004) [34]	Parallel; 2 arm	Total: 46 (26 completed). Control (no intervention): 23 (13 completed). Omega-3 PUFAs + low omega-6 PUFA diet: 23 (13 completed).	3.4 g/d omega-3 PUFAs; 1·9 g/d EPA + 1·5 g/d DHA.	24	No significant difference in VAS pain score	2
Tulleken et al. (1990) [27]	Parallel; 2 arm	Total: 28 (27 completed). Control (coconut oil): 14 (14 completed).	3.4 g/d omega-3 PUFAs; 2.04 g/d EPA + 1.32 g/d DHA	12	Statistically significant reduction of joint pain index vs control. No significant	2

		Omega-3 PUFAs: 14 (13 completed).			lowering of VAS pain vs control.	
Van der Tempel et al. (1990) [36]	Random order, cross-over (no wash out in between)	Total: 16 (14 completed). Control (coconut oil).	3.49 g/d omega-3 PUFAs; 2·0 g/d EPA + 1·3 g/d DHA.	12	No statistically significant change in VAS pain score	3
Volker et al. (2000) [31]	Parallel; 2 arm	Total: 50 (26 completed). Control: 25 (13 completed). Omega-3 PUFAs: 25 (13 completed).	Estimation for a 70 kg person: ~ 2.8 g/d omega-3 PUFAs.	15	Significant improvement in VAS pain score vs baseline	3

Table 2. Risk of bias assessment determined using the Cochrane risk of bias tool [17]

Study	**Selection bias**		**Attrition bias** (Incomplete outcome data)	**Detection bias** (Blinding of outcome assessment)	**Performance bias** (Blinding of participants & personnel)	**Reporting bias** (Selective reporting)	**Other**
	Random sequence generation	Allocation concealment					
Adam et al. 2003 [28]							
Berbert et al. 2005 [24]							
Cleland et al. 1988 [23]							
Das Gupta et al. 2009 [20]							
Galarraga et al. 2008 [22]							
Geusens et al. 1994 [29]							
Kjeldsen- Kragh et al. 1992 [35]							
Kremer et al. 1985 [32]							
Kremer et al. 1990 [25]							
Kremer et al. 1995 [26]							
Lau et al. 1993 [19]							
Nielsen et al. 1992 [30]							
Park et al. 2013 [33]							
Skoldstam et al. 1992 [21]							
Sundrarjun et al. 2004 [34]							
Tulleken et al. 1990 [27]							
Van der Tempel et al. 1990 [36]							
Volker et al. 2000 [31]							

 = Low Risk of bias = High risk of bias = Unclear risk of bias

Table 3. Effects on pain outcomes reported in the included studies

Reference	How pain assessed	Units	Placebo group at study entry	Placebo group at study end	Omega-3 PUFA group at study entry	Omega-3 PUFA group at study end	Significant effect of Omega-3 PUFAs at study end compared with study entry	Significant effect of Omega-3 PUFAs compared with placebo
Adam et al. (2003) [28]	Patient assessment of pain via visual analogue scale[a]	cm	100% of initial value (AID and WD)[b].	~80% of initial value (AID)[b]. ~100% of initial value (WD)[b].	100% of initial value (AID and WD)[b]	~60% of initial value (AID)[b]. ~80% of initial value (WD)[b].	Significant effect of omega-3 PUFAs for both AID[b] and WD[b] (both p<0.001)	No significant effect reported
Berbert et al. (2005) [24]	Joint pain intensity via five-point scale (0-4)[c]	points	1.77 ± 0.93	1.85 ± 1.21	Omega-3 PUFAs: 2.31 ± 0.86. Olive-oil supplemented omega-3 PUFAs: 2.18 ± 0.73.	Omega-3 PUFAs: 1.23 ± 0.83. Olive-oil supplemented omega-3 PUFAs: 0.53 ± 0.80.	No significant within-group change for omega-3 PUFAs only group (but trend for improvement). Significant within-group change for olive-oil	Significant effect for percentage change with omega-3 PUFAs and olive-oil supplemented omega-3 PUFAs vs placebo (p<0.05).

							supplemented omega-3 PUFA group ($p<0.05$).	
Cleland et al. (1988) [23]	Patient assessment of pain via visual analogue scale[a]	cm	9.8 ± 4.6	7.1 ± 5.1	9.6 ± 5.8	7.0 ± 4.6	Non-significant improvement reported for both placebo and omega-3 PUFAsss groups	No significant effect reported
Das Gupta et al. (2009) [20]	Patient assessment of pain via visual analogue scale[a]	cm	6.75 ± 1.01	2.52 ± 0.99	6.58 ± 0.91	1.66 ± 0.55	Significant effect for indomethacin plus omega-3 PUFAs group ($p<0.05$)	No significant effect reported
Galarraga et al. (2008) [22]	Patient assessment of pain via visual analogue scale[a]	cm	3.1 ± 2.8	3.2 ± 3.0	3.8 ± 2.8	3.1 ± 3.2	Not reported	Significant effect for omega-3 PUFAs vs placebo ($p=0.029$)
Geusens at al. (1994) [29]	Physician and patient assessment of pain via five-point scale (0-4)[c]	points	1.85 ± 0.11 (physician assessment). 1.95 ± 0.15	-0.19 ± 0.15 change (physician assessment).	1.90 ± 0.19 (physician assessment). 2.00 ± 0.17	-0.61 ± 0.16 change (physician assessment).	Significant effect in high dose omega-3 PUFAs group for physician	No significant effect reported

			(patient assessment)	-0.11 ± 0.18 change (patient assessment).	(patient assessment).	-0.47 ± 0.20 change (patient assessment).	assessment of pain ($p<0.05$)	
Kjeldsen-Kragh et al. (1992) [35]	Pain score via five-point scale (1-5)[e]	points	3.2[d]	3.6[d]	3.9[d]	3.9[d]	No significant effect reported	No significant effect reported
Kremer et al. (1985) [32]	Physician and patient assessment of pain via five-point scale (1-5)[e]	points	2.6 (patient assessment). 3.0 (physician assessment).	2.8 (patient assessment). 3.0 (physician assessment).	2.8 (patient assessment). 2.9 (physician assessment).	2.5 (patient assessment). 2.6 (physician assessment).	No significant effect reported	No significant effect reported
Kremer et al. (1990) [25]	Physician and patient evaluation of pain via five-point scale (0-4)[c]	points	I.6 (CI:1.2,2.0) (patient evaluation). I.4 (CI:1.1,1.7) (physician evaluation).	-0.3 change (CI:-0.7,0.2) (patient evaluation). 0.1 change (CI:-0.3,0.5) (physician evaluation).	I.6 (CI:1.3,1.9) (patient evaluation). 1.5 (CI:1.1,1.9) (physician evaluation).	-0.2 change (CI: -0.6,0.2) (patient evaluation). -0.4 change (CI:-0.7,0.2) (physician evaluation).	Significant effect of omega-3 PUFAs on patient evaluation of pain at 18 weeks and on physician evaluation of pain at study end (24 weeks) ($p<0.05$)	No significant effect reported

Kremer et al. (1995) [26]	Physician and patient assessment of pain via five-point scale (0-4)[c]	points	1.7 ± 0.8 (patient assessment). 1.6 ± 0.6 (physician assessment).	Not reported	1.8 ± 0.7 (patient assessment). 1.8 ± 0.6 (physician assessment).	-0.38 ± 0.12 change up to maximum duration of diclofenac (physician assessment)	Significant effect of omega-3 PUFAs on physician assessment of pain up to maximum duration of diclofenac (p=0.004)	No significant effect reported
Lau et al. (1993) [19]	Metrologist and patient assessment of joint pain severity via visual analogue scale[a]	cm	1.42 [1.5 (0-6.8)][f] (patient assessment). 3.12 [0.27 (0.8-8.5)][f] (metrologist assessment)	Not reported	1.33 [0.85 (0-5.2)][f] (patient assessment). 3.11 [2.75 (0.5-8.2)][f] (metrologist assessment)	Not reported	No significant effect reported	No significant effect reported
Nielsen et al. (1992) [30]	Patient assessment of pain via unspecified visual pain score	arbitrary units	118 (81-42)[g]	136 (86-70)[g]	120 (90-143)[g]	104 (78-143)[g]	No significant effect reported	Significant effect of omega-3 PUFAs vs placebo (p=0.002)
Park et al.	Assessment of pain	points	33.63 ± 20.16	28.75 ± 20.90	33.41 ± 22.54	35.85 ± 26.41	No significant effect	No significant effect

(2013) [33]	via pain scale (0-100)						reported	reported
Skoldstam et al. (1992) [21]	Patient assessment of pain via visual analogue scale (0-3)	cm	1.29 (0.14)[h]	0.17 (0.17)[h] change from baseline	1.46 (0.13)[h]	0.02 (0.14)[h] change from baseline	No significant effect reported	No significant effect reported
Sundrarjun et al. (2004) [34]	Pain via unspecified visual analogue scale	cm	55.86 ± 5.48	33.44 ± 4.30	45.86 ± 4.07	47.14 ± 4.36	No significant effect reported	No significant effect reported
Tulleken et al. (1990) [27]	1. Joint pain index 2. Pain score via visual analogue scale[a]	1. points 2. cm	27 (5-52)[l] (joint pain index). 4.4 (1.4-8.0)[i] (pain score).	20 (4-48)[l] (joint pain index). 3.8 (0.5-8.1)[i] (pain score).	27 (3-103)[l] (joint pain index). 4.0 (0.5-6.I)[i] (pain score).	6 (0-49)[l] (joint pain index). 2.4 (0-7.4)[i] (pain score).	No significant effect reported	Significant effect of omega-3 PUFAs on joint pain index vs placebo ($p<0.05$)
Van der Tempel et al. (1990) [36]	1. Joint pain index on a four point scale (0-3)[j] 2. Pain score via visual analogue scale[a]	1. points 2. cm	33 (7)[h] (joint pain index). 3.1 (0.6)[h] (pain score).	42 (9)[h] (joint pain index). 4.0 (0.7)[h] (pain score).	33 (7)[h] (joint pain index). 3.1 (0.6)[h] (pain score).	29 (7)[h] (joint pain index). 2.7 (0-5)[h] (pain score).	No significant effect reported	No significant effect reported
Volker et al. (2000) [31]	Pain score via visual analogue scale[a]	cm	5 ± 1	-8.6 ± 13.8 percent change	5 ± 1	-10 ± 42.6 percent change	Significant effect of omega-3 PUFAs vs baseline (p value not given)	No significant effect reported

[a] VAS; Visual analog scale: 0–10 cm : 0 without symptoms, 10 severe.

[b] AID, Anti-inflammatory diet; WD, Western diet.

[c] Five point scale (0-4): 0, absent; 1, mild; 2, moderate; 3, severe; 4, very severe.

[d] Estimated from graph.

[e] Five point scale (1-5): 1, symptom-free; 2, mild; 3, moderate; 4, severe; 5, very severe.

[f] Data expressed as mean and median (range).

[g] Data expressed as median and 25th and 75th quartiles in brackets.

[h] Data expressed as mean (standard error).

[i] Data expressed as median (range)

[j] Four point scale (0-3): 0, absent; 1, mild; 2, moderate; 3, severe.

Figure 1. PRISMA flow diagram showing multistage search strategy and study selection [15]