

Influence of marine *n*-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of the joints and bones. The *n*-6 polyunsaturated fatty acid (PUFA) arachidonic acid (ARA) is the precursor of inflammatory eicosanoids which are involved in RA. Some therapies used in RA target ARA metabolism. Marine *n*-3 PUFAs (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) found in oily fish and fish oils decrease the ARA content of cells involved in immune responses and decrease the production of inflammatory eicosanoids from ARA. EPA gives rise to eicosanoid mediators that are less inflammatory than those produced from ARA and both EPA and DHA give rise to resolvins that are anti-inflammatory and inflammation resolving, although little is known about these latter mediators in RA. Marine *n*-3 PUFAs can affect other aspects of immunity and inflammation relevant to RA, including dendritic cell and T cell function and production of inflammatory cytokines and reactive oxygen species, although findings for these outcomes are not consistent. Fish oil has been shown to slow the development of arthritis in animal models and to reduce disease severity. A number of randomised controlled trials of marine *n*-3 PUFAs have been performed in patients with RA. A systematic review included 23 studies. Evidence is seen for a fairly consistent, but modest, benefit of marine *n*-3 PUFAs on joint swelling and pain, duration of morning stiffness, global assessments of pain and disease activity, and use of non-steroidal anti-inflammatory drugs.

Key words: Arachidonic acid: Fish oil: Eicosapentaenoic acid: Docosahexaenoic acid: Lipid mediator: Cytokine

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of the joints and bones⁽¹⁾. Joint inflammation is manifested by swelling, pain, functional impairment, morning stiffness, osteoporosis, and muscle wasting. Bone erosion commonly occurs in the joints of the hands and feet. The joint lesions are characterised by infiltration of immune cells and contain high concentrations of many of the chemical mediators they produce⁽²⁾. One pharmaceutical treatment for the inflammation involved in RA has involved the use of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs target the metabolism of the *n*-6 fatty acid arachidonic acid (ARA) to prostaglandins (PGs) by cyclooxygenase (COX) enzymes, suggesting a key involvement of these mediators in the pathology of RA. *N*-3 fatty acids from oily fish and fish oils target ARA availability and metabolism and also influence several other immuno-inflammatory responses involved in RA. Thus, marine *n*-3 fatty acids could be useful in treating

RA. This article will describe the effects of marine *n*-3 fatty acids on different aspects of immune responses of relevance to RA, and will then describe the effects of marine *n*-3 fatty acids in animal models of RA. The article will then report on a systematic evaluation of marine *n*-3 fatty acids in clinical trials with RA patients. Finally, the findings of the systematic evaluation will be compared with the conclusions of meta-analyses of the efficacy of marine *n*-3 fatty acids in RA. Parts of this article are updated from an earlier one on this topic⁽³⁾.

Rheumatoid arthritis

RA is a chronic inflammatory autoimmune disease that affects about 1% of adults. It is more common in women than in men. The joint lesions show infiltration of activated T lymphocytes, macrophages and antibody-secreting B lymphocytes into the synovium (the tissue lining the joints) and there is

Abbreviations: ARA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HLA, human leukocyte antigen; IL, interleukin; LT, leukotriene; LOX, lipoxygenase; MHC, major histocompatibility class; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

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proliferation of fibroblast-like synovial cells called synoviocytes⁽⁴⁾. These cells and new blood vessels form a tissue termed pannus which leads to progressive destruction of cartilage and bone. This is most likely due to cytokine- and eicosanoid-mediated induction of destructive enzymes such as matrix metalloproteinases. Synovial fluid from patients with RA contains high levels of pro-inflammatory cytokines including tumour necrosis factor (TNF)- α , IL-1 β , IL-6, IL-8 and granulocyte/macrophage colony stimulating factor⁽²⁾. Synovial cells cultured ex vivo spontaneously produce these cytokines for extended periods of time⁽²⁾. RA is also characterised by signs of systemic inflammation, such as elevated plasma concentrations of some cytokines (e.g. interleukin (IL)-6), acute phase proteins, and rheumatoid factors.

Genetic studies have linked susceptibility to, and severity of, RA to genes in the major histocompatibility class (MHC) II locus⁽⁵⁾; in humans these genes encode the human leukocyte antigen (HLA) II proteins involved in antigen presentation. RA is associated with specific alleles of the HLA-DRB1 gene, although other HLA-DR alleles may also play a role⁽⁵⁾. Because the function of HLA-DR is antigen presentation to T lymphocytes, the genetic association indicates a role for T cells in RA⁽⁶⁾. In total, the HLA region contributes 30 to 50% of the genetic component of RA. The second largest genetic risk for RA lies with a variant in the protein tyrosine phosphatase

non-receptor 22 gene, which encodes an intracellular protein tyrosine phosphatase⁽⁵⁾. The variant may act to reduce the ability to down-regulate activated T cells.

Arachidonic acid, eicosanoids and the links with inflammation and RA

Eicosanoids are amongst the most important mediators and regulators of inflammation⁽⁷⁾. They are formed from 20 carbon polyunsaturated fatty acids (PUFAs). Because immune cells usually contain a high proportion of the *n*-6 PUFA ARA and low proportions of other 20-carbon PUFAs, ARA is considered to be the major substrate for synthesis of eicosanoids. Eicosanoids include PGs, thromboxanes, leukotrienes (LTs) and other oxidised derivatives. Fig. 1 summarises the pathway of synthesis of these mediators from ARA⁽⁸⁾. COX is key to synthesis of PGs and there are two principal COX isoforms. These are COX-1, which is constitutively expressed, and COX-2, which is up-regulated by inflammatory stimuli. Expression of both COX isoforms is increased in the synovium of patients with RA and in joint tissues in rat models of arthritis⁽⁹⁾. Eicosanoids produced by both the COX and lipoxygenase (LOX) pathways are found in the synovial fluid of patients with active RA⁽¹⁰⁾. Infiltrating leukocytes such as neutrophils, monocytes and synoviocytes are the key sources of

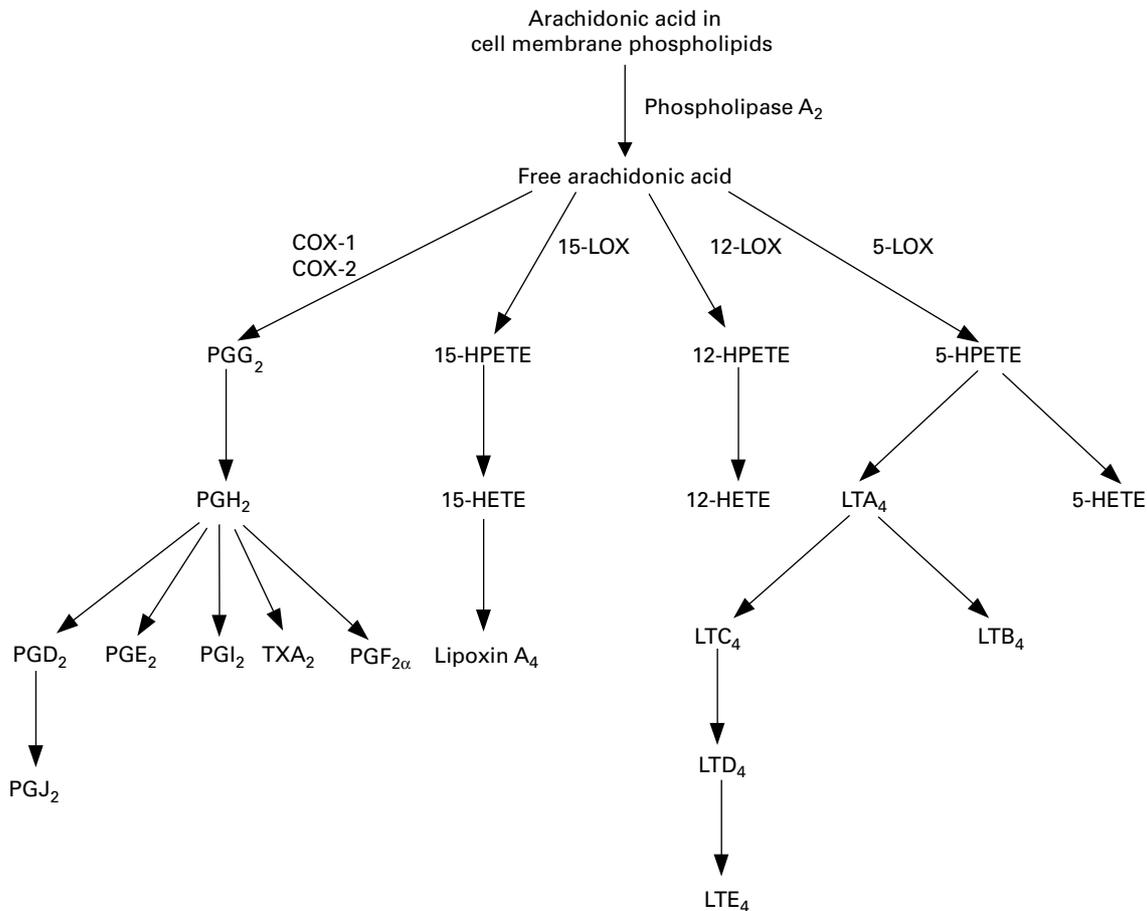


Fig. 1. Outline of the pathway of eicosanoid synthesis from arachidonic acid. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane. Taken from Calder⁽⁸⁾ with permission.

eicosanoids in RA⁽¹⁰⁾. PGE₂ has a number of proinflammatory effects including increasing vascular permeability, vasodilation, blood flow and local pyrexia, and potentiating pain caused by other agents. It also promotes the production of some of the destructive matrix metalloproteinases and stimulates bone resorption. The efficacy of NSAIDs, which are COX inhibitors, in RA indicates the importance of this pathway in the pathophysiology of the disease. These drugs provide rapid relief of pain and stiffness by inhibiting joint inflammation. LTB₄ increases vascular permeability, enhances local blood flow, is a potent chemotactic agent for leukocytes, induces release of lysosomal enzymes, and enhances release of reactive oxygen species and inflammatory cytokines like TNF- α , IL-1 β and IL-6.

Fatty acid modification of immune cell fatty acid composition and of eicosanoid profiles

Fatty acids are constituents of phospholipids and phospholipids are components of cell membranes. The bulk phospholipids of immune cells (e.g. neutrophils, lymphocytes, monocytes) isolated from the blood of healthy people consuming typical Western diets have been reported to contain about 10 to 20% of fatty acids as ARA, with about 0.5-1% of the *n*-3 PUFA eicosapentaenoic acid (EPA) and about 1.5-3% of another *n*-3 PUFA docosahexaenoic acid (DHA)⁽¹¹⁻¹⁷⁾. There are, however, differences between the different phospholipid classes in terms of the content of these fatty acids⁽¹³⁾. EPA and DHA are found in seafood, especially oily fish, and in fish oil-type supplements. Thus EPA and DHA may be referred to as marine *n*-3 PUFAs. The fatty acid composition of human blood leukocytes can be modified by increasing the oral intake of marine *n*-3 PUFAs. This results in increased proportions of EPA and DHA in blood monocytes, mononuclear cells and neutrophils⁽¹¹⁻²¹⁾. Typically the increase in content of marine *n*-3 PUFAs occurs at the expense of *n*-6 PUFAs, including ARA. Time-course studies suggest that the incorporation of EPA and DHA into human blood leukocytes begins within days and reaches its peak within one or two weeks of commencing increased intake^(16-18,20-22). Studies using multiple doses of fish oil show that the incorporation of EPA and DHA into human blood leukocytes occurs in a dose-response manner^(17,21,23).

There are many reports of decreased production of PGE₂ and of 4 series-LTs by immune cells following a period of

fish oil supplementation of the diet of healthy volunteers^(11-13,15,24,25). Similar effects are seen in patients with RA, where fish oil supplements decreased LTB₄ production by neutrophils⁽²⁶⁻³¹⁾ and monocytes⁽³⁰⁾, 5-hydroxyeicosatetraenoic acid production by neutrophils⁽³⁰⁾, and PGE₂ production by mononuclear cells⁽³²⁾.

The studies in humans demonstrating a reduction in ARA-derived eicosanoid production by oral marine *n*-3 fatty acids have typically used fairly high intakes (several g/day). A dose-response study in healthy volunteers reported that an EPA intake of 1.35 g/d for 3 months was not sufficient to influence *ex vivo* PGE₂ production by endotoxin stimulated mononuclear cells, whereas an EPA intake of 2.7 g/day significantly decreased PGE₂ production⁽²¹⁾. These data suggest a threshold of intake of EPA to elicit an anti-inflammatory effect; this threshold would be between 1.35 and 2.7 g/day.

EPA is also a substrate for the COX and LOX enzymes that produce eicosanoids (Fig. 2), but the mediators formed have a different structure from the ARA-derived mediators. Neutrophils from healthy volunteers supplemented orally with fish oil for several weeks produced much increased amounts of 5-series LTs⁽¹¹⁻¹³⁾. In patients with RA given marine *n*-3 PUFAs, there was generation of the usually undetectable LTB₅ and 5-hydroxyeicosapentaenoic acid by stimulated neutrophils⁽²⁹⁻³¹⁾ and monocytes⁽²⁹⁾. The functional significance of generation of eicosanoids from EPA is that EPA-derived mediators are often much less biologically active than those produced from ARA⁽³³⁻³⁵⁾ and they may even act as antagonists of the ARA-derived mediators⁽³⁶⁾.

Resolvins: Novel anti-inflammatory and inflammation resolving mediators produced from EPA and DHA

EPA and DHA both give rise to a family of lipid mediators termed resolvins (Fig. 2). The pathways of synthesis of resolvins are complex and not fully elucidated yet, but they involve the action of both COX and LOX enzymes and may be modified in the presence of aspirin. E-series resolvins are produced from EPA and D-series resolvins from DHA. DHA is also a substrate for similar molecules called protectins, also known as neuroprotectins. A large number of studies using cell culture and animal models have convincingly shown that E- and D-series resolvins and protectins are anti-inflammatory, inflammation resolving

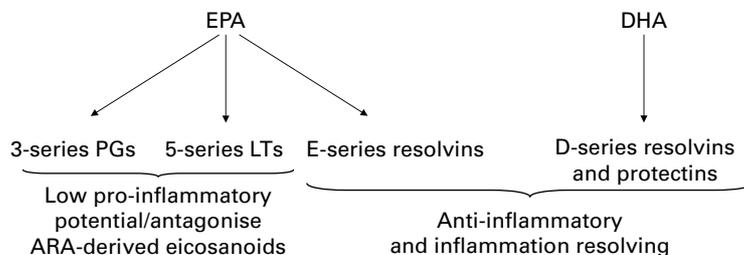


Fig. 2. Overview of eicosanoid and resolvin synthesis from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Abbreviations used: LT, leukotriene; PG, prostaglandin.

and immunomodulatory^(37–41). There is very limited human data on resolvins and they have not yet been studied in the context of RA.

Influence of marine *n*-3 fatty acids on production of inflammatory cytokines

Cell culture studies report that EPA and DHA can inhibit the production of the classic pro-inflammatory cytokines (TNF- α , IL-1, IL-6) by several cell types^(42–47), effects supported by animal feeding studies⁽⁸⁾. Several studies in healthy human volunteers involving supplementation of the diet with fish oil have demonstrated decreased production of TNF- α , IL-1 β and IL-6 by endotoxin-stimulated monocytes or mononuclear cells (a mixture of lymphocytes and monocytes)^(12,15,24,48–52), although not all studies confirm this effect^(16,20,21,53–58). Reasons for the different findings include the low *n*-3 PUFA dose, the short duration and the small sample size of some of the studies failing to find an effect, but these are unlikely to be the sole explanations. Studies using fish oil in patients with RA report decreased IL-1 production by monocytes⁽²⁸⁾, and decreased circulating concentrations of IL-1 β ^(59,60), TNF- α ^(61,62) and soluble receptor activator of nuclear factor kappa B ligand⁽⁶²⁾.

Influence of marine *n*-3 fatty acids on production of reactive oxygen species

Providing high doses (>3 g/day) of marine *n*-3 PUFAs to healthy volunteers resulted in decreased production of reactive oxygen species (superoxide or hydrogen peroxide) by blood neutrophils stimulated with different agents^(63–65) and high dose marine *n*-3 PUFAs decreased hydrogen peroxide production by human monocytes⁽⁶⁶⁾. Studies using lower doses of marine *n*-3 PUFAs (< 2.3 g/day) did not see effects on reactive oxygen species production by either neutrophils or monocytes^(17,53–55,67). Rees *et al.*⁽²¹⁾ identified an EPA dose-dependent decrease in the number of blood neutrophils producing superoxide in elderly subjects, but there was no effect in younger subjects. Fish oil supplements decreased reactive oxygen species production by neutrophils from the blood of RA patients⁽⁶⁸⁾.

Influence of marine *n*-3 fatty acids on T cells

Cell culture studies report that EPA and DHA inhibit proliferation of human T cells and their production of IL-2⁽⁶⁹⁾. Animal feeding studies support these observations⁽⁸⁾, but human data in this area are inconsistent. Some studies in healthy humans report that increased intake of marine *n*-3 PUFAs decreases human T cell proliferation^(18,24,57) and IL-2 production^(24,52), but several other studies show no effect on these outcomes^(16,48,49,54,55,70). Again the reasons for these different findings may include the low *n*-3 PUFA dose, the short duration and the small sample size of some of the studies; differences in the age of the subjects studied might also contribute to the variation in findings⁽²⁴⁾.

Influence of marine *n*-3 fatty acids on antigen presentation

A small number of cell culture studies have found that MHC II expression and antigen presentation via MHC II are decreased following exposure of antigen presenting cells to EPA or DHA^(71,72). These findings are supported by work in animals fed fish oil⁽⁷³⁾, but there is limited information on *n*-3 PUFAs and antigen presentation in humans⁽⁷⁴⁾.

Marine *n*-3 PUFAs and animal models of RA

The effects of marine *n*-3 PUFAs on antigen presentation, T cell reactivity, and inflammatory lipid, peptide and oxygen-derived mediator production suggest that these fatty acids might have a role both in decreasing the risk of development of RA and in decreasing severity in those patients with the disease. This has been explored using animal models of arthritis. In an early study, compared with vegetable oil, fish oil fed mice had delayed onset (mean 34 days vs. 25 days) and reduced incidence (69% vs. 93%) and severity (mean peak severity score 6.7 vs. 9.8) of type II collagen-induced arthritis⁽⁷⁵⁾. In another study, both EPA and DHA suppressed Streptococcal cell wall-induced arthritis in rats, although EPA was more effective⁽⁷⁶⁾. A recent study compared fish oil, which provides marine *n*-3 PUFAs in triglyceride form, and krill oil, which provides marine *n*-3 PUFAs partly in the form of phospholipids, in collagen-induced arthritis in the susceptible DBA/1 mouse strain⁽⁷⁷⁾. Both chemical formulations of marine *n*-3 PUFAs slowed the onset of arthritis, decreased its severity, reduced paw swelling, and decreased knee joint pathology compared with the control group; for some outcomes krill oil appeared superior to fish oil.

A systematic review of randomized controlled trials of orally administered marine *n*-3 PUFAs in RA

Introduction

The studies outlined above indicate that oral marine *n*-3 PUFAs can modulate a range of immunological reactions that are associated with the immunological dysfunction or inflammation-induced pathology associated with RA; aspects reported to be modified by marine *n*-3 PUFAs in some studies in healthy volunteers or in RA patients include antigen presentation, T cell reactivity, reactive oxygen species production by leukocytes, inflammatory cytokine production by macrophages, and inflammatory eicosanoid production by various cells. These effects have been demonstrated mainly in healthy volunteers, but similar findings are made in a small number of studies in patients with RA^(26–32,59–62,68). Animal models of arthritis have been used to demonstrate a benefit from marine *n*-3 PUFAs^(75–77). These observations, particularly the early finding that marine *n*-3 PUFAs decrease eicosanoid formation from ARA, have led to a number of clinical trials of oral marine *n*-3 PUFAs, usually in the form of fish oil, in patients with RA. The rest of this article is given over to a systematic review of randomized, controlled trials of oral marine *n*-3 PUFAs in adults with RA.



Identification of articles to include in the systematic review

A PubMed search was performed on 25 November 2011 using the MeSH terms (Fatty acids, Omega-3 OR Fish oils) AND Arthritis, Rheumatoid AND Human. The search identified 155 articles. The *a priori* inclusion criteria for the systematic review were:

- randomized, controlled trial
- use of marine *n*-3 PUFAs
- oral administration through supplements or foods
- published in full as a research paper
- published in English
- reporting clinical outcomes

Examination of the titles and short PubMed descriptions of the articles resulted in exclusion of 80 review, discussion, opinion, and comment articles, of two meta-analyses, of 16 articles not written in English, of four articles in which *n*-3 PUFAs were administered by a non-oral route (e.g. intravenously),

and of two articles not about *n*-3 PUFAs (Fig. 3). The abstracts of the remaining 51 articles were read. This led to exclusion of a further 28 articles (Fig. 3). The full text of the remaining 23 articles was read; based on this two articles were excluded (Fig. 3). Reference lists in three meta-analyses^(78–80) were read to identify further relevant studies; two were included after reading their full text. Thus, the systematic review included 23 articles (Fig. 3). Studies of plant *n*-3 PUFAs are not included.

Description of the included studies

Table 1 describes the studies included in the systematic review including aspects of study design, sample size, dose of EPA plus DHA used, duration, nature of the placebo, JADAD score⁽⁸¹⁾ and whether the study was included in a previous meta-analysis of *n*-3 PUFAs and RA. Included studies were published between 1985 and 2009^(26–28,30,31,60,61,68,82–95). Most studies adopted a parallel design, although four adopted a random order, cross-over design. This latter design is not

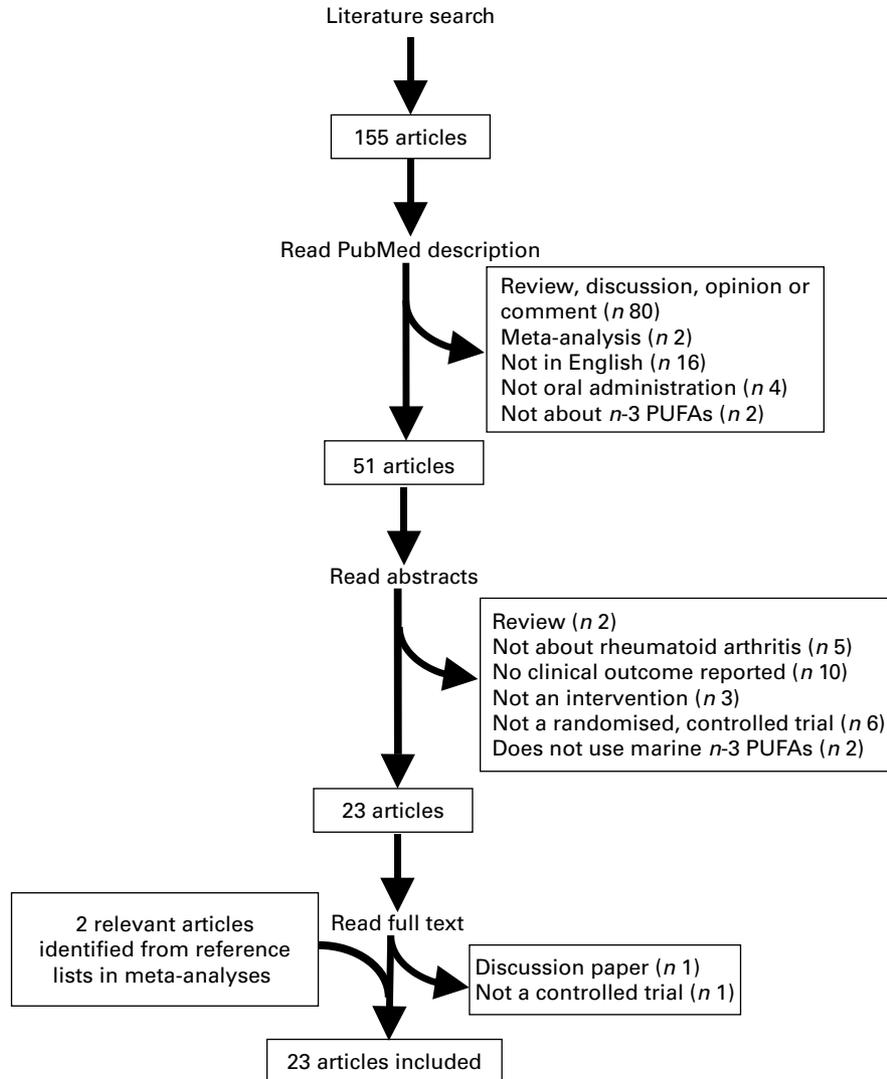


Fig. 3. Overview of the selection of articles for inclusion in the systematic review.

Table 1. Summary of the studies included in the systematic review of marine *n-3* PUFAs and clinical outcomes in rheumatoid arthritis

Reference	Location	Design	Sample size (<i>n</i>)	Dose of EPA + DHA (g/day)	Duration (wk)	Placebo	JADAD score	Included in meta-analysis of Fortin <i>et al.</i> ⁽⁷⁸⁾	Included in meta-analysis of Maclean <i>et al.</i> ⁽⁷⁹⁾	Included in meta-analysis of Goldberg and Katz ⁽⁸⁰⁾	Comments
Kremer <i>et al.</i> ⁽²⁶⁾	USA	Parallel; 2 arm	Control: 25 (21 completed) <i>n-3</i> : 27 (23 completed; data analysed for 17 patients only because of non-compliance)	1.8 + 1.2	12	Paraffin oil	3	Y	Y	Y	
Kremer <i>et al.</i> ⁽²⁷⁾	USA	Random order cross-over; 4 week washout	40 (33 completed both arms of the cross-over study)	2.7 + 1.8	14	Olive oil	3	Y	N	N	
Belch <i>et al.</i> ⁽⁸²⁾	UK	Parallel; 3 arm	Control 18 (8 completed) <i>n-3</i> (+ γ -linolenic acid): 15 (13 completed) γ -linolenic acid: 16 (13 completed)	0.24 (DHA not specified) + 0.54 of γ -linolenic acid	52 (+ a further 12 weeks on placebo)	Paraffin oil	2	N	N	N	Patients asked to reduce or stop NSAID use
Cleland <i>et al.</i> ⁽³⁰⁾	Australia	Parallel; 2 arm	Control: 30 (23 completed) <i>n-3</i> : 30 (23 completed)	3.2 + 2.0	12	Olive oil	3	Y	Y	Y	
Magaro <i>et al.</i> ⁽⁶⁸⁾	Italy	Parallel; 2 arms	<i>n</i> 12, but allocation to groups not specified	1.6 + 1.1	4	Control group did not receive a placebo	2	N	Y	Y	Not properly controlled; Patients cannot have been blind to treatment; No mention of number of dropouts/completers
Van der Tempel <i>et al.</i> ⁽³¹⁾	The Netherlands	Random order, cross-over (no wash out in between)	<i>n</i> 16 entered study; <i>n</i> 14 completed	2.0 + 1.3	12	Coconut oil	3	Y	Y	Y	
Kremer <i>et al.</i> ⁽²⁸⁾	USA	Parallel; 3 arm	Control: 20 (12 completed) Low dose <i>n-3</i> : 20 (20 completed) High dose <i>n-3</i> : 20 (17 completed)	1.7+1.2 or 3.5 + 2.4	24	Olive oil	3	Y	Y	Y	
Tullekan <i>et al.</i> ⁽⁸³⁾	The Netherlands	Parallel; 2 arm	Control: 14 (14 completed) <i>n-3</i> : 14 (13 completed)	2.0 + 1.3	12	Coconut oil	2	Y	Y	Y	
Skoldstam <i>et al.</i> ⁽⁸⁴⁾	Sweden	Parallel; 2 arm	Control: 23 (21 completed) <i>n-3</i> : 23 (22 completed)	1.8 + 1.2	24	Mixed oils	3	N	Y	Y	
Esperson <i>et al.</i> ⁽⁵⁹⁾	Denmark	Parallel; 2 arm	Control: 14 <i>n-3</i> : 18	2.0 + 1.2	12	Mixed oils	2	N	N	N	
Nielsen <i>et al.</i> ⁽⁸⁵⁾	Denmark	Parallel; 2 arm	Control: 28 (24 completed) <i>n-3</i> : 29 (27 completed)	2.0 + 1.2	12	Vegetable oil	3	Y	Y	Y	
Kjeldsen-Kragh <i>et al.</i> ⁽⁸⁶⁾	Norway	Parallel; 3 arm	Declining NSAID + control: 28 (24 completed) NSAID + <i>n-3</i> : 25 (20 completed) Declining NSAID + <i>n-3</i> : 26 (23 completed)	3.8 + 2.0	16	Corn oil	3	Y	Y	N	Required patients to reduce NSAID use
Lau <i>et al.</i> ⁽⁸⁷⁾	UK	Parallel; 2 arm	Control: 32 (completers not explicitly stated) <i>n-3</i> : 32 (completers not explicitly stated)	1.7 + 1.1	52	Air	2	N	Y	Y	Required patients to reduce NSAID use

Table 1. Continued

Reference	Location	Design	Sample size (n)	Dose of EPA + DHA (g/day)	Duration (wk)	Placebo	JADAD score	Included in meta-analysis of Fortin <i>et al.</i> ⁽⁷⁸⁾	Included in meta-analysis of Maclean <i>et al.</i> ⁽⁷⁹⁾	Included in meta-analysis of Goldberg and Katz ⁽⁸⁰⁾	Comments
Geusens <i>et al.</i> ⁽⁸⁸⁾	Belgium	Parallel; 3 arm	Control: 30 (20 completed) Low dose <i>n</i> -3: 30 (21 completed) High dose <i>n</i> -3: 30 (19 completed)	0.85 + 0.2 or 1.7 + 0.4	52	Olive oil	3	N	Y	Y	
Kremer <i>et al.</i> ⁽⁶⁰⁾	USA	Parallel; 2 arm	Control: 33 (26 completed) <i>n</i> -3: 33 (23 completed)	4.6 + 2.5	26 to 30	Corn oil	3	N	Y	N	
Volker <i>et al.</i> ⁽⁸⁹⁾	Australia	Parallel; 2 arm	Control: 25 (13 completed) <i>n</i> -3: 25 (13 completed)	Total 40 mg/kg body weight (~ 2.2 to 3.0)	15	Mixed oils	3	N	Y	N	
Adam <i>et al.</i> ⁽⁶¹⁾	Germany	Random order cross over (8 week washout)	Cross-over design: control vs. <i>n</i> -3 each against a background Western diet (<i>n</i> 34; 30 completed) or "anti-inflammatory diet" (<i>n</i> 34; 30 completed) in random order	Approx. 2.4 + 1.8	12	Corn oil	3	N	Y	Y	
Remens <i>et al.</i> ⁽⁹⁰⁾	The Netherlands	Parallel; 2 arm	Control: 33 (29 completed) <i>n</i> -3: 33 (26 completed)	1.4 + 0.2 (+0.5 γ -linolenic acid) in a liquid supplement also providing vitamins, minerals, amino acids, and other nutrients	16	Liquid supplement without added PUFA	3	N	N	Y	
Sundrarajun <i>et al.</i> ⁽⁸¹⁾	Thailand	Parallel; 2 arm	Control: 23 (13 completed) <i>n</i> -3: 23 (13 completed)	1.9 + 1.5	24	Not stated	3	N	N	Y	
Berbert <i>et al.</i> ⁽⁹²⁾	Brazil	Parallel; 3 arm	Control: 17 (13 completed) <i>n</i> -3: 18 (13 completed) <i>n</i> -3 + olive oil: 20 (17 completed)	Total 3.0	24	Soybean oil	3	N	N	Y	Some patients could not have been blind to treatment
Galarraga <i>et al.</i> ⁽⁹³⁾	UK	Parallel; 2 arm	Control: 48 (26 completed) <i>n</i> -3: 49 (32 completed)	1.5 + 0.7	36	Air	3	N	N	N	Required patients to reduce NSAID use
Dawczynski <i>et al.</i> ⁽⁹⁴⁾	Germany	Random order cross-over (8 week washout)	Cross-over design (<i>n</i> 55; 21 completed)	0.7 + 0.4 through modified foods	12	Standard foods	3	N	N	N	
Das Gupta <i>et al.</i> ⁽⁹⁵⁾	Bangladesh	Parallel; 2 arm	Control: 100 (91 completed) <i>n</i> -3: 100 (90 completed)	Not specified	12	Control group did not receive placebo capsules	1	N	N	N	Patients not blind to treatment

Marine *n*-3 fatty acids and arthritis

optimal for studying effects of marine *n*-3 PUFAs because of their slow turnover which requires a significant wash-out phase. Wash-out periods of 4, 4 and 8 weeks were used in three of the studies employing this design; there was no wash-out phase in a fourth study of this type. Sample size was typically modest and only four studies appear to have included a formal power calculation^(31,61,84,90). Most articles did not mention a specific primary outcome. The dose of EPA plus DHA used in these studies varied from <1 to >7 g/day and averaged about 3 g/day; one study did not specify intake of either EPA or DHA⁽⁹⁵⁾. Twenty-one of the studies provided marine *n*-3 PUFAs as fish oil type supplements. One study used a liquid formula containing EPA plus DHA in addition to other nutrients⁽⁹⁰⁾, while one used modified foods containing marine *n*-3 PUFAs⁽⁹⁴⁾. Two studies^(28,88) evaluated two doses of marine *n*-3 PUFAs. Duration of the included studies varied from 4 to 52 weeks; some studies reported data at several intermediate time points. The placebo used in the studies was highly variable and commonly included another “nutritional” oil (e.g. coconut, olive or corn oil) or a mixture of such oils. In some cases the inert oil paraffin oil was used. The JADAD score⁽⁸¹⁾ of the included studies was typically 3, although some studies scored lower than this. None of the included studies provided information about how patients were randomised or how the blinding was performed. For some studies it seems unlikely that patients were blind to their treatment. For example in the studies by Magaro *et al.*⁽⁶⁸⁾ and Das Gupta *et al.*⁽⁹⁵⁾, the control group did not receive any placebo supplement. Most studies provided relevant information about drop-outs and withdrawals. Statistical analysis of the results of most of the studies was poor, often relying upon multiple pairwise comparisons with no correction. The designs of many of the studies would merit a more sophisticated analytical approach than was used; for example for many of the studies a two-factor analysis of variance (factors: time and treatment) with appropriate covariates would have been appropriate. In many cases the focus of the analysis was on comparison to baseline within a group, rather than on comparison between the treatment and control group. This restricts the interpretation of some of the findings.

A range of clinical outcomes was reported in the included studies (Table 2). The most commonly reported outcomes were related to tender or swollen joints, duration of morning stiffness, grip strength, physician or patient assessment of pain or disease severity, and use of NSAIDs. All studies reported multiple clinical outcomes (Table 2).

Findings of the included studies

Almost all of the trials included here showed some clinical benefit of marine *n*-3 PUFAs (Table 3). Commonly reported benefits include reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue, increased grip strength, reduced pain or disease activity (assessed by physician or patient) and decreased use of NSAIDs (Table 3). These effects are frequently reported within the *n*-3 PUFA group comparing

back to the baseline value. Much less frequent benefits are reported compared with the placebo group (Table 3). Kremer *et al.*⁽²⁸⁾ reported that both a “low” and a “high” dose of marine *n*-3 PUFAs brought about a similar clinical benefit, but that the effect of the high dose became apparent (i.e. significant) sooner. An interesting approach was used in the study of Adam *et al.*⁽⁶¹⁾ where marine *n*-3 PUFAs or placebo were given against a background of a Western diet or a so-called anti-inflammatory diet. The latter aimed to reduce the intake of *n*-6 PUFAs, especially ARA, on the basis that the effects of marine *n*-3 PUFAs might be stronger if *n*-6 PUFA intake (and status) was decreased simultaneously with the increased *n*-3 PUFA intake. Indeed *n*-3 PUFAs had benefits irrespective of background diet, but the effect was greater when intake of ARA was decreased. Three studies used fairly low intakes (< 1.5 g EPA + DHA/day) of marine *n*-3 PUFAs^(82,90,94), which may explain why those studies are the only ones that fail to report any clinical benefit from marine *n*-3 PUFAs. Using LPS-stimulated PGE₂ production from blood mononuclear cells as a model, Rees *et al.*⁽²¹⁾ identified an “anti-inflammatory threshold” for EPA intake in healthy volunteers of between 1.35 and 2.7 g/day.

Comparison between the findings of the systematic review and the conclusions of previous meta-analyses

Table 4 summarizes the findings by indicating the number of studies that demonstrated a significant benefit (either vs. baseline or vs. placebo, the latter being more important) for the commonly reported outcomes. This organization of the outcomes emphasizes the lack of consistent findings across all of the studies conducted, but also shows the fairly high number of studies that have demonstrated a given clinical benefit. The most likely reasons for lack of consistency of findings relate to the dose of EPA + DHA used, which is probably too low in some of the studies; the small sample size of many of the studies, which in many cases has probably limited the ability to identify an effect; and the sub-optimal approaches to statistical analysis used in many of the studies. Overall it seems that marine *n*-3 PUFAs do reduce joint pain and swelling, decrease the duration of morning stiffness, and spare the need for some anti-inflammatory medications.

These conclusions can be compared with those of three meta-analyses^(78–80). The meta-analysis of Fortin *et al.*⁽⁷⁸⁾ included data from nine trials published between 1985 and 1992 inclusive and from one unpublished trial; 8 of the published trials are included in the current systematic review (Table 1). Fortin *et al.* concluded that “dietary fish oil supplementation for three months significantly reduces tender joint count (mean difference –2.9; *P*=0.001) and morning stiffness (mean difference –25.9 minutes; *P*=0.01)”. Maclean *et al.*⁽⁷⁹⁾ conducted a meta-analysis that included data from trials published between 1985 and 2002, although these included one study of flaxseed oil, one study that did not use a control for fish oil, and one study in which transdermal administration of *n*-3 PUFAs by ultrasound, rather than the oral route, was used. Maclean *et al.* concluded that fish oil supplementation has no effect on “patient report of pain,



Table 2. Clinical outcomes assessed in the studies included in the systematic review of marine *n-3* PUFAs in rheumatoid arthritis

Reference	Clinical outcomes assessed
Kremer <i>et al.</i> ⁽²⁶⁾	Number of tender joints; Number of swollen joints; Proximal interphalangeal joint circumference; Duration of morning stiffness; Time to onset of fatigue after waking; Grip strength; Physician global assessment of pain and disease activity; Patient global assessment of pain and disease activity; American Rheumatism Association functional class
Kremer <i>et al.</i> ⁽²⁷⁾	Number of tender joints; Number of swollen joints; Proximal interphalangeal joint circumference; Duration of morning stiffness; Time to onset of fatigue after waking; Grip strength; Physician global assessment of pain and disease activity; Patient global assessment of pain and disease activity; American Rheumatism Association functional class
Belch <i>et al.</i> ⁽⁸²⁾	Ritchie's articular index; Duration of morning stiffness; Grip strength; Patient global assessment of pain
Cleland <i>et al.</i> ⁽³⁰⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Grip strength; 15 min walk time; Physician global assessment of disease activity; Patient global assessment of pain and disease activity; American Rheumatism Association functional class
Magaro <i>et al.</i> ⁽⁶⁸⁾	Ritchie's articular index; Duration of morning stiffness; Grip strength
Van der Tempel <i>et al.</i> ⁽³¹⁾	Joint pain; Joint swelling; Duration of morning stiffness; Grip strength; Patient global assessment of pain
Kremer <i>et al.</i> ⁽²⁸⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Time to onset of fatigue after waking; Grip strength; Physician global assessment of pain and disease activity; Patient global assessment of pain and disease activity
Tullekan <i>et al.</i> ⁽⁸³⁾	Number of tender joints; Number of swollen joints; Ritchie's articular index; Duration of morning stiffness; Grip strength; Patient global assessment of pain
Skoldstam <i>et al.</i> ⁽⁸⁴⁾	Joint tenderness (Ritchie's articular index); Duration of morning stiffness; Grip strength; Physician global assessment of disease activity; Patient global assessment of pain; Patient global assessment of disease activity; Use of NSAIDs
Esperson <i>et al.</i> ⁽⁵⁹⁾	Ritchie's articular index
Nielsen <i>et al.</i> ⁽⁸⁵⁾	Joint pain; Joint swelling; Duration of morning stiffness; Grip strength; Patient global assessment of pain; Use of NSAIDs
Kjeldsen-Kragh <i>et al.</i> ⁽⁸⁶⁾	Number of tender joints; Number of swollen joints; Joint tenderness (Ritchie's articular index); Duration of morning stiffness; Grip strength; Physician global assessment of functional ability; Patient global assessment of pain
Lau <i>et al.</i> ⁽⁸⁷⁾	Joint tenderness (Ritchie's articular index); Duration of morning stiffness; Grip strength; Physician global assessment of pain; Patient global assessment of pain; Use of NSAIDs
Geusens <i>et al.</i> ⁽⁸⁸⁾	Number of tender joints; Number of swollen joints; Ritchie's articular index; Duration of morning stiffness; Grip strength; Physician global assessment of pain and disease activity; Patient global assessment of pain and disease activity; Use of NSAIDs &/or Disease modifying anti-rheumatic drugs
Kremer <i>et al.</i> ⁽⁶⁰⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Time to onset of fatigue after waking; Grip strength; Physician global assessment of pain and disease activity; Patient global assessment of pain and disease activity; Use of NSAIDs; Paulus Index; OMERACT score
Volker <i>et al.</i> ⁽⁸⁹⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Physician global assessment of disease activity; Patient global assessment of pain and disease activity
Adam <i>et al.</i> ⁽⁶¹⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Grip strength; Physician global assessment of disease activity; Patient global assessment of pain and disease activity; Use of NSAIDs
Remens <i>et al.</i> ⁽⁹⁰⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Grip strength; Physician global assessment of disease activity; Patient global assessment of pain and disease activity; Use of NSAIDs
Sundrarjun <i>et al.</i> ⁽⁹¹⁾	Number of tender joints; Number of swollen joints; Patient global assessment of pain and disease activity
Berbert <i>et al.</i> ⁽⁹²⁾	Joint pain; Ritchie's articular index; Duration of morning stiffness; Time to onset of fatigue after walking; Grip strength; Patient global assessment of disease activity; American College of Rheumatology functional status
Galarraga <i>et al.</i> ⁽⁹³⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Grip strength; Patient global assessment of pain; Use of NSAIDs
Dawczynski <i>et al.</i> ⁽⁹⁴⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Disease activity
Das Gupta <i>et al.</i> ⁽⁹⁵⁾	Number of tender joints; Number of swollen joints; Duration of morning stiffness; Disease activity; Patient global assessment of pain

swollen joint count, disease activity, or patient's global assessment". However, this conclusion may be flawed, because of the inappropriate manner in which studies were combined and because of a poor understanding of the study designs used. For example, the meta-analysis fails to recognize that patients' ability to reduce the need for using NSAIDs or their ability to be withdrawn from NSAID use, as was done in some designs, must indicate a reduction in pain with *n-3* PUFA use. This meta-analysis does state that "in a qualitative analysis of seven studies that assessed the effect of *n-3* fatty acids on anti-inflammatory drug or corticosteroid requirement, six demonstrated reduced requirement for these drugs" and concluded that "*n-3* fatty acids may reduce requirements for corticosteroids". The effect of marine *n-3* PUFAs on tender joint count was not assessed by Maclean *et al.*, who simply reiterated the findings of Fortin *et al.* that "*n-3* fatty acids

reduce tender joint counts". Goldberg and Katz⁽⁸⁰⁾ published a meta-analysis of 17 trials of *n-3* PUFAs in the context of joint pain, including one trial in RA with flaxseed oil and two trials of fish oil not in RA patients. Data on six outcomes were analysed. This analysis indicated that fish oil reduces patient assessed joint pain (*n* 13 studies; 26% reduction; *P*=0.03), duration of morning stiffness (*n* 8 studies; 57% reduction; *P*=0.003), number of painful and/or tender joints (*n* 10 studies; 71% reduction; *P*=0.003), and consumption of NSAIDs (*n* 3 studies; 60% reduction; *P*=0.01). However, this meta-analysis also found that there was no effect of marine *n-3* PUFAs on Ritchie's articular index (*n* 4 studies) or on patient assessed disease activity (*n* 5 studies). Nevertheless, the meta-analysis of Goldberg and Katz provides good evidence for the clinical efficacy of *n-3* PUFAs in RA, and the conclusions of the current systematic review are similar.

Table 3. Summary of the findings of randomised, controlled studies using marine *n*-3 PUFAs in patients with rheumatoid arthritis

Reference	Clinical outcomes improved from baseline with marine <i>n</i> -3 PUFAs	Clinical outcomes improved with marine <i>n</i> -3 PUFAs compared with placebo group
Kremer <i>et al.</i> ⁽²⁶⁾ Kremer <i>et al.</i> ⁽²⁷⁾	Number of tender joints Number of tender joints; Number of swollen joints; Time to fatigue; Physician global assessment; American Rheumatism Association functional class	Duration of morning stiffness Number of tender joints; Time to fatigue
Belch <i>et al.</i> ⁽⁸²⁾ Cleland <i>et al.</i> ⁽³⁰⁾ Magaro <i>et al.</i> ⁽⁶⁸⁾	– Number of tender joints; Grip strength Ritchie's articular index; Duration of morning stiffness; Grip strength	Use of NSAIDs – Ritchie's articular index; Duration of morning stiffness; Grip strength
Van der Tempel <i>et al.</i> ⁽³¹⁾ Kremer <i>et al.</i> ⁽²⁸⁾	Joint swelling; Duration of morning stiffness Number of tender joints; Number of swollen joints; Duration of morning stiffness; Grip strength; Patient global pain assessment; Physician global pain assessment; Physician global disease activity assessment	Joint swelling; Duration of morning stiffness Grip strength
Tullekan <i>et al.</i> ⁽⁸³⁾	Joint swelling index; Joint pain index; Ritchie's articular index	–
Skoldstam <i>et al.</i> ⁽⁸⁴⁾	Ritchie's articular index; Physician global assessment of disease activity; Use of NSAIDs	Use of NSAIDs
Esperson <i>et al.</i> ⁽⁵⁹⁾ Nielsen <i>et al.</i> ⁽⁸⁵⁾ Kjeldsen-Kragh <i>et al.</i> ⁽⁸⁶⁾	Ritchie's articular index Joint pain; Duration of morning stiffness Ritchie's articular index; Duration of morning stiffness; Physician global assessment of functional ability	– Patient global assessment of pain –
Lau <i>et al.</i> ⁽⁸⁷⁾ Geusens <i>et al.</i> ⁽⁸⁸⁾	Use of NSAIDs Grip strength; Physician's pain assessment; Patient's global assessment of disease activity	Use of NSAIDs Patient's global assessment of disease activity; Use of NSAIDs &/or Disease modifying anti-rheumatic drugs
Kremer <i>et al.</i> ⁽⁶⁰⁾	Number of tender joints; Duration of morning stiffness; Physician assessment of pain; Physician global assessment of disease activity; Patient global assessment of disease activity	Number of tender joints
Volker <i>et al.</i> ⁽⁸⁹⁾	Number of swollen joints; Duration of morning stiffness; Physician global assessment of disease activity; Patient global assessment of pain and disease activity	Duration of morning stiffness
Adam <i>et al.</i> ⁽⁶¹⁾	Number of swollen joints; Number of tender joints; Patient's global assessment of disease activity; Physician's global assessment of disease activity; Patient's assessment of pain; Use of NSAIDs and of corticosteroids	–
Remens <i>et al.</i> ⁽⁹⁰⁾ Sundrarjun <i>et al.</i> ⁽⁹¹⁾ Berbert <i>et al.</i> ⁽⁹²⁾	– – –	– – Duration of morning stiffness; Joint pain; Time to onset of fatigue; Ritchie's articular index; Grip strength, Patient's global assessment
Galarraga <i>et al.</i> ⁽⁹³⁾ Dawczynski <i>et al.</i> ⁽⁹⁴⁾ Das Gupta <i>et al.</i> ⁽⁹⁵⁾	– – Number of tender joints; Number of swollen joints; Disease activity; Patient global assessment of pain	Use of NSAIDs; Patient global assessment of pain – –

Overall conclusions

Eicosanoids derived from the *n*-6 PUFA ARA play a role in RA, and the efficacy of NSAIDs in RA indicates the importance of pro-inflammatory COX pathway products in the pathophysiology of the disease. At sufficiently high intakes, marine *n*-3 PUFAs decrease the production of inflammatory eicosanoids from ARA and promote the production of less inflammatory eicosanoids from EPA and of anti-inflammatory resolvins and related mediators from EPA and DHA. Marine *n*-3 PUFAs have other anti-inflammatory actions including decreasing antigen presentation via MHC II, decreasing T cell reactivity and Th1-type cytokine production, decreasing inflammatory cytokine production by monocyte/macrophages, and decreasing

reactive oxygen species production by various leukocytes, although these effects are not consistently reported. One reason behind the lack of consistency may be the dose of EPA plus DHA used in many studies which was probably below the "anti-inflammatory threshold". Other contributors would include differences in duration of the intervention, in sample size, and in the age of the subjects studied. Work with animal models of RA has demonstrated efficacy of fish oil. There have been a number of clinical trials of fish oil in patients with RA. Most of these trials report some clinical improvements (e.g. improved patient assessed pain, decreased morning stiffness, fewer painful or tender joints, decreased use of NSAIDs), and when the trials have been pooled in meta-analyses statistically significant clinical benefit has emerged.

Table 4. Summary of findings of this systematic review and comparison with the findings of previous meta-analyses^(78–80)

Outcome	Number of studies reporting outcome	Number of studies reporting an improvement in outcome with marine <i>n</i> -3 PUFAs compared with baseline	Number of studies reporting an improvement in outcome with marine <i>n</i> -3 PUFAs compared with placebo	Comparison with findings from meta-analyses
Number of tender joints	15	7	2	Fortin <i>et al.</i> : Number of tender joints significantly decreased (<i>n</i> 10; <i>P</i> =0.001) Maclean <i>et al.</i> : Number of swollen joints not altered (<i>n</i> 6) Goldberg & Katz: Number of painful and/or tender joints significantly decreased (<i>n</i> 10; <i>P</i> =0.003) Ritchie's articular index not altered (<i>n</i> 4)
Number of swollen joints	15	5	0	
Ritchie's articular index	8	5	2	
Joint pain	2	2	1	
Joint swelling	2	2	1	
Duration of morning stiffness	21	7	4	Fortin <i>et al.</i> : Duration of morning stiffness significantly decreased (<i>n</i> 9; <i>P</i> =0.01) Goldberg & Katz: Duration of morning stiffness significantly decreased (<i>n</i> 8; <i>P</i> =0.003)
Grip strength	17	3	2	Goldberg & Katz: Physician assessment of pain not altered (<i>n</i> 3) Maclean <i>et al.</i> : Patient assessment of pain not altered (<i>n</i> 9) Goldberg & Katz: Patient assessment of pain significantly decreased (<i>n</i> 13; <i>P</i> =0.03) Maclean <i>et al.</i> : Patient assessment of disease activity not altered (<i>n</i> 5) Goldberg & Katz: Use of NSAIDs significantly decreased (<i>n</i> 3; <i>P</i> =0.01)
Physician global assessment of pain	6	3	0	
Physician global assessment of disease activity	12	6	0	
Patient global assessment of pain	19	4	2	
Patient global assessment of disease activity	12	4	2	
Use of NSAIDs	8	3	4	

The current systematic review supports the conclusion that there is fairly consistent evidence for a modest clinical efficacy of marine *n*-3 PUFAs in RA.

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PCC conducted the literature search; both authors scored, interpreted and discussed the clinical trials; PCC drafted the article; both authors agreed the final version of the article. Conflicts of interest:

PCC serves on Scientific Advisory Boards of the Danone Research Centre in Specialised Nutrition and Aker Biomarine. He acts as a consultant to Mead Johnson Nutritionals, Vifor Pharma, and Amarin Corporation. He has received speaking honoraria from Solvay Healthcare, Solvay Pharmaceuticals, Pronova Biocare, Fresenius Kabi, B. Braun, Abbott Nutrition, Baxter Healthcare, Nestle, Unilever and DSM. He currently receives research funding from Vifor Pharma. He is elected President of the International Society for the Study of Fatty Acids and Lipids, an organisation that is partly supported by corporate membership fees, mainly the food and supplements industries. He is a member of the Board of Directors of ILSI Europe, the Board of Directors of the European Nutraceutical Association, and the Council of the British Nutrition Foundation; these organizations are each supported in part by

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