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3rd International Immunonutrition Workshop

Session 3: Fatty acids and the immune system
Fish oil and rheumatoid arthritis: past, present and future

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Meta- and mega-analysis of randomised controlled trials indicate reduction in tender joint counts and decreased use of non-steroidal anti-inflammatory drugs with fish-oil supplementation in long-standing rheumatoid arthritis (RA). Since non-steroidal anti-inflammatory drugs confer cardiovascular risk and there is increased cardiovascular mortality in RA, an additional benefit of fish oil in RA may be reduced cardiovascular risk via direct mechanisms and decreased non-steroidal anti-inflammatory drug use. Potential mechanisms for anti-inflammatory effects of fish oil include inhibition of inflammatory mediators (eicosanoids and cytokines), and provision of substrates for synthesis of lipid suppressors of inflammation (resolvins). Future studies need progress in clinical trial design and need to shift from long-standing disease to examination of recent-onset RA. We are addressing these issues in a current randomised controlled trial of fish oil in recent-onset RA, where the aim is to intervene before joint damage has occurred. Unlike previous studies, the trial occurs on a background of drug regimens determined by an algorithm that is responsive to disease activity and drug intolerance. This allows drug use to be an outcome measure whereas in previous trial designs, clinical need to alter drug use was a 'problem'. Despite evidence for efficacy and plausible biological mechanisms, the limited clinical use of fish oil indicates there are barriers to its use. These probably include the pharmaceutical dominance of RA therapies and the perception that fish oil has relatively modest effects. However, when collateral benefits of fish oil are included within efficacy, the argument for its adjunctive use in RA is strong.

Rheumatoid arthritis: Fish oil: Pain: Non-steroidal anti-inflammatory drugs

Efficacy: different outcome measures and the evidence

The main reason that patients with rheumatoid arthritis (RA) seek medical treatment is for alleviation of pain and discomfort. Meta- and mega-analysis of ten double-blind, placebo-controlled trials showed that fish oil supplying 2·9– >6 g long-chain *n*-3 fatty acids daily for 3 months was associated with decreased number of tender joints and duration of morning stiffness in patients with RA of 10–11 years' duration[1,2]. It was concluded that there was little difference in the magnitude of effect between 2·9 and 7·1 g/d long-chain *n*-3 fats[3].

Another symptomatic outcome measure is overall pain experience, which is measured most commonly in clinical trials by use of a visual linear analogue scale or categorical scales. A meta-analysis of fish oil trials that measured inflammatory joint pain, mainly with RA patients, reported a beneficial effect of fish oil on patient-reported joint pain intensity, number of painful or tender joints, duration of morning stiffness and non-steroidal anti-inflammatory drug (NSAID) use[4]. However, another meta-analysis that examined the effect of fish oil on pain scores in RA reported that 'There were no significant effects in twelve studies'[5]. However, this latter meta-analysis did not take

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DMARD, disease-modifying anti-rheumatic drugs; LOX, lipoxygenase; LTB, leukotriene B; NSAID, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; RCT, randomised controlled trials.

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Table 1. Influence of non-steroidal anti-inflammatory drugs (NSAID) on outcomes in studies with fish oil in patients with rheumatoid arthritis (RA)

References	Design issues	Outcomes/comments
Skoldstam *et al.*[6], Belch *et al.*[7] and Lau *et al.*[8]	Participants were instructed to titrate NSAID use according to the pain level	NSAID sparing effect of fish oil
Kremer *et al.*[3]	The trial included an NSAID cessation period as provocation	NSAID sparing effect of fish oil
Kremer *et al.* [9]	Change of NSAID or DMARD was a withdrawal criterion	% Withdrawn due to need to change drugs: olive oil, 26%; fish oil in low dose, 4%; fish oil in high dose, 0%. ($P = 0{\cdot}008$ for olive *v.* fish oil), i.e. fish oil decreased clinical need for drug changes
Kremer *et al.* [47], Tulleken *et al.*[48], van der Tempel *et al.*[49] and Volker *et al.*[50]	All or most trial participants were taking NSAID at baseline and either were instructed not to change them during the study or there is no information on NSAID management during the study	NSAID use expected to make pain measures insensitive, i.e. the conditions are not suited to examination of fish oil and pain

DMARD, disease-modifying anti-rheumatic drugs.

account of the influence and management of NSAID or disease-modifying anti-rheumatic drugs (DMARD) in nine of those trials (Table 1).

The meta-analysis that concluded that fish oil had no effect on pain did not consider that the extent of patient-determined NSAID use can be considered a measure of pain[5]. It is clear that fish oil had an NSAID sparing effect in the four trials designed to examine that issue[3,6–8]. In another trial where need to change drug use was a withdrawal criterion, there were ≥6 times the number of trial participants withdrawn in the placebo group compared with the fish-oil groups, an indication of lesser pain/discomfort in the fish oil groups[9]. Of the remainder of the 12 studies cited, one did not measure pain[10], one did not use fish oil[11] and the other did show a decrease in pain scores[12].

There was no effect of fish oil on disease activity as measured by erythrocyte sedimentation rate[5].

Efficacy of fish oil in RA includes collateral benefits that extend beyond symptomatic effects. RA is associated with an approximate 2-fold increased standardised mortality ratio and the excess mortality is due mainly to cardiovascular deaths[13]. Acute and unrecognised myocardial infarction are 3–6-fold increased in RA and sudden cardiac death is approximately 2-fold increased[14]. The increased cardiovascular risk is not explained by traditional (i.e. Framingham) risk factors, and it has been postulated that chronic systemic inflammation is a contributor, perhaps via altered endothelial function[13]. The magnitude of this issue had led to management of cardiovascular risk being recommended as an integral component of RA treatment[15]. Additional to the disease-associated increased cardiovascular risk is that further added by NSAID use. A comprehensive review suggests slightly increased cardiovascular risk with non-selective NSAID, with possibly naproxen being the safest and diclofenac conferring increased risk similar to that of the cyclooxygenase (COX)-2 selective drug, celecoxib[16]. It is well established that fish oil decreases cardiovascular risk due to the protective effect of fish and fish oil on coronary mortality[17], including sudden cardiac death[18,19]. The latter is concordant with the anti-arrhythmic effects of *n*-3 fatty acids, including fish oil[20–24]. A protective effect seems evident at doses of long-chain *n*-3 fats >250 mg[17], much lower than those needed for symptomatic effects in RA. Fish oil may reduce cardiovascular mortality in RA via direct myocardial actions and possibly via anti-thrombotic actions, evidence for which has been reported in an RA clinic setting with fish oil[25]. In addition, there is a further possible protective effect due to the NSAID sparing effect of fish oil in RA[6–8]. The potential for this latter effect is underlined by comparison of the use of NSAID by 77% of patients with established RA in a large US and Canada database with 22% NSAID use in our early arthritis patients taking fish oil[25,26]. The latter also had an improved blood lipid profile[25].

Consideration of what constitutes 'efficacy' of fish oil should be sufficiently broad to encompass all that will benefit an RA patient. This extends beyond symptomatic relief.

Potential mechanisms for anti-inflammatory effects of fish oil

The pro-inflammatory lipids, PGE_2 and leukotriene $B(LTB)_4$, are products of the *n*-6 PUFA, arachidonic acid (AA). This is prevalent in cell membranes, in part due to the high intake of *n*-6 relative to *n*-3 fats. AA is released from membrane phospholipids in response to inflammatory stimuli, whereupon the free AA is a substrate for COX and 5-lipoxygenase (5-LOX) with the production of PGE_2 and LTB_4, respectively. PGE_2 synthesised peripherally by COX-1 and COX-2 results in swelling[27], and PGE_2 produced in the central nervous system by constitutive/inducible COX-2 and inducible PGE synthase results in hyperalgesia[28–30]. LTB_4 is a chemoattractant and activator of neutrophils, which are essential for inflammatory arthritis expression in animal models[31] and which are the most prominent leucocyte type in rheumatoid synovial fluid.

Fish oil contains the *n*-3 fatty acids EPA and DHA. These are homologues of AA (Fig. 1). AA has 20 C and

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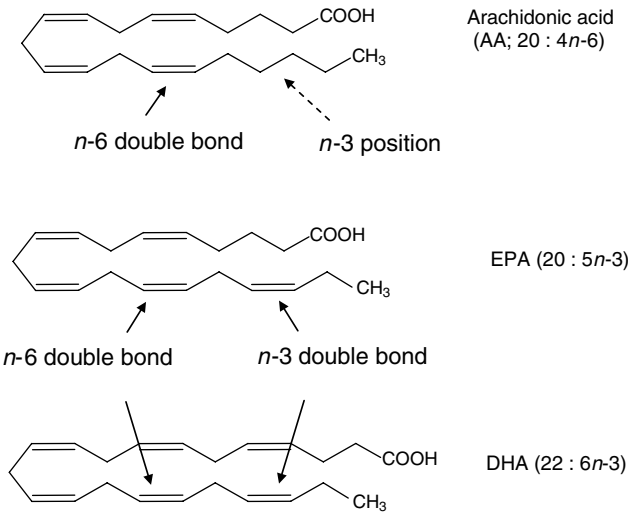

Fig. 1. Basis for *n*-3 and *n*-6 fatty acid designation.

four double bonds with the double bond proximal to the methyl terminus being in the *n*-6 position. This is designated 20:4*n*-6. EPA is 20:5*n*-3 and DHA is 22:6*n*-3. EPA and DHA are effective competitive inhibitors of AA metabolism by COX, having K_i values of approximately 2 μM, which is similar to that of ibuprofen[32] (Fig. 2). EPA could be potentially metabolised by COX to the *n*-3 eicosanoid, PGE_3. However, EPA is a poor COX substrate and little, if any PGE_3, is formed by leucocytes[33]. EPA is a good substrate for 5-LOX and both LTB_5 and LTB_4 are synthesised in relation to the amounts of EPA/AA substrates[34]. However, LTB_5 has little pro-inflammatory activity on neutrophils relative to LTB_4[35]. Thus, the overall effect of EPA is production of a less inflammatory mix of eicosanoids compared with those derived from AA.

Fish oil also has been shown capable of inhibiting the peptide mediators of inflammation, TNFα and IL-1β (Fig. 2). Fish oil suppressed *ex vivo* monocyte TNFα and IL-1β production in healthy volunteers at 2·4 to 4·7 g/d long-chain *n*-3 fats[36–38] and in patients with RA at 2·9–5·9 g/d long-chain *n*-3 fats[9]. However, a review of studies of this phenomenon shows considerable variation in outcome[39]. Some of this variation may be due to genetic factors because the extent of suppression of TNFα appears to be a function of the basal level of synthesis and a polymorphism in the TNFα gene[40]. It is possible that other variability in the effect of dietary fish oil on cytokine synthesis is due to the large inter-individual variation in blood levels of EPA arising from a fixed oral dose of EPA (Fig. 3). This source of variation is rarely considered and may be larger in the community than that shown in Fig. 3 where healthy trial participants received intensive dietary advice and were provided with monounsaturated cooking oil, spread and salad dressing, all with the aim of achieving a uniform dietary background[41].

In addition to suppression of lipid and peptide inflammatory mediator production, EPA and DHA are substrates for a class of anti-inflammatory lipids which are proposed as being promoters of inflammation resolution (Fig. 2). The discovery and elucidation of these compounds has led to the suggestion that chronic inflammation is a failure of resolution[42]. DHA can be metabolised by 15-LOX or aspirin-treated COX-2 to 17(S)- and 17(R)-hydroxy derivatives, respectively, and these are metabolised by 5-LOX to resolvin D1 and aspirin-triggered resolvin D1, respectively[43]. Likewise, EPA can be metabolised to a tri-hydroxy derivative, resolvin E1, via 5-LOX and aspirin-treated COX-2 or perhaps cytochrome P450 enzymes[42,44,45]. These resolvins suppress dermal inflammation, murine peritonitis and colitis, and a receptor that mediates resolvin E1 activity has been identified[43,45,46]. It is proposed that the cellular interactions that occur between neutrophils and endothelium or cells within an inflammatory focus with the development of an inflammatory reaction serve to up-regulate 15-LOX which, combined with neutrophil 5-LOX, generate resolvins that lead to resolution of inflammation[42]. The production of lipids with pro-resolution properties is not limited to EPA and DHA. AA is a substrate for the production of tri-hydroxy derivatives known as lipoxins or aspirin-triggered lipoxins via 5-LOX and 15-LOX or aspirin-treated COX-2[42]. The lipoxins and resolvins have overlapping activities and it is not clear whether there are distinct roles, e.g. produced in different tissues or different leucocyte targets or act at different times after the initiation of inflammation, or whether there is simple redundancy.

Clinical trial design for rheumatoid arthritis studies: past and future

Clinical trials of fish oil in RA have been conducted as double-blind, randomised, placebo-controlled trials[3,6,8,9,12,47–54]. This is a standard design for examination of the effects of agents, mainly drugs, in clinical medicine. In these trials, fish-oil was examined as an addition to other medications already being taken by the patients at baseline. The medications were a combination of NSAID and DMARD that were mainly methotrexate, hydroxychloroquine, D-penicillamine and gold. In general, the need to change DMARD dose during the trials due to disease activity or drug toxicity was a trial withdrawal criterion[6,9,12,48–50,53]. The need for this is understandable within that type of design, which could not evaluate the effects of fish oil against a changing drug background. Thus, the medical need to change medications during the trial was seen as a problem. However, the need for drug changes can be informative. In one of the studies where DMARD change or need for steroids was a withdrawal criterion, the number withdrawn for this reason was reported and analysed with significantly more withdrawals in the placebo group compared with the fish-oil group[9]. In one study where patients with longstanding RA took fish oil supplying 2·6 g/d long-chain *n*-3 fats, medication adjustment was allowed and was reported as an outcome measure; 47% of those in the fish oil group had their medication decreased compared with 15% in the placebo group, a statistically significant difference[54]. In the remaining studies, it was stated that DMARD and NSAID were continued, presumably without change but with no further information given[47,51,52].

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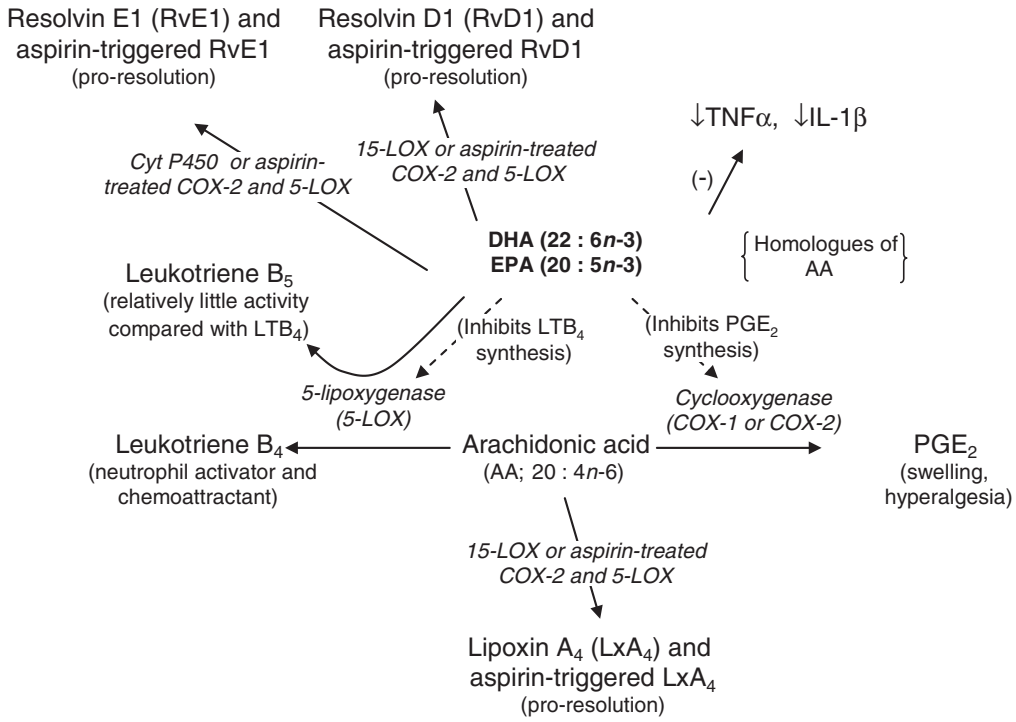

Fig. 2. Possible metabolic pathways for anti-inflammatory effects of the long-chain *n*-3 fatty acids, EPA and DHA. Cyt P450, cytochrome P450; LTB_4, leukotriene B_4; LOX, lipoxygenase; COX, cyclooxygenase.

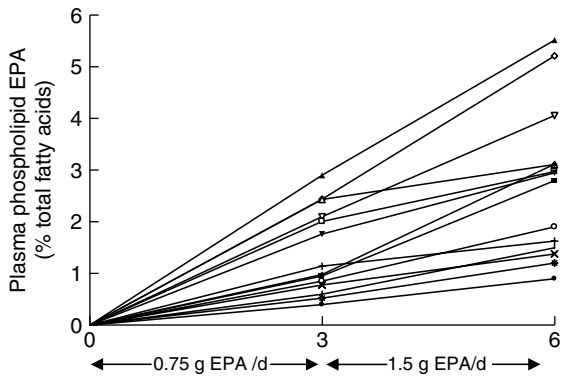

Fig. 3. Change in plasma phospholipid EPA arising from the ingestion of purified EPA at doses of 0·75 g/d for 0–3 weeks and 1·5 g/d for 3–6 weeks in healthy volunteers. Each line represents one subject. Mean data were reported previously[41].

These trials mainly spanned the period 1985–1995 with one being conducted in 2000. Modern rheumatology practice has changed since that era, which generally concentrated only on relief of symptoms. While that is critically important for each patient, it tended to ignore the underlying disease process that determines long-term patient outcomes. Serial monotherapy with DMARD was common and this gave a remission rate of <20%[55]. It is now recognised that combination DMARD therapy can achieve greater disease suppression and this may delay the progression of joint damage[56,57]. It is also accepted that outcomes are better if there is intervention during a 'window of opportunity' in early disease when joint damage is still absent or minimal[58]. This knowledge has framed modern RA treatment, which has implications for future clinical trial design.

With the aim for remission rather than 'merely' symptom relief, there is a frequent need to change medication in response to disease activity or drug intolerance or toxicity. While a changing medication background against which fish oil is tested makes analysis difficult, not allowing medication changes is a distortion that dissociates the trial results from applicability to standard rheumatology practice. While this was a feature of previous fish oil in RA trials, it is not unique to them. It is also a condition for trials with 'biological agent' therapies[59–61] and we have pointed out the ethical problem of avoiding the drug complexities by deliberately under-treating trial participants in RA[62]. These citations provide examples of randomised controlled trials (RCT) in RA where the conduct does not reflect 'real life' clinical practice because treatments should be adjusted in response to disease activity.

To address this deficiency, we have established a structured approach to the treatment of early RA that uses triple DMARD therapy from the outset, but with rules-based dose adjustments of DMARD and additions of leflunomide and anti-TNF agents if needed. The rules are responsive to signs of disease activity and drug intolerance or toxicity. The approach is directed by an explicit set of algorithms that we have published[63] and this results in a systematised series of allowable drug changes (Fig. 4).

We are conducting an RCT of fish oil in early RA in this treatment framework. Patients will have different drug trajectories toward remission or disease control. However, because the drug regimen is systematised and involves a

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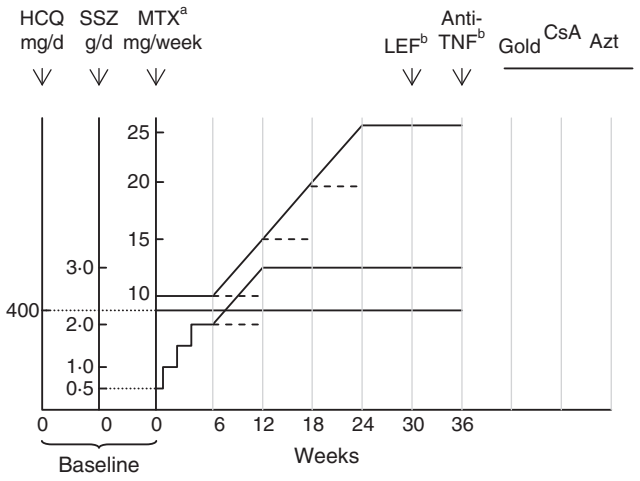

Fig. 4. Allowed drug changes that result from the treatment algorithm described[63]. Allowed dosing escalations can be made every 3–6 weeks according to disease activity and toxicity. Disease-modifying anti-rheumatic drug (DMARD): HCQ, hydroxychloroquine; SSZ, sulfasalazine; MTX, methotrexate; LEF, leflunomide; Anti-TNF, anti-TNF biological agent therapy; CsA, cyclosporine A; Azt, azathioprine. —— If active disease, drug doses escalated as shown. ---- If remission/low disease activity, drug doses maintained. [a]Oral MTX is used unless intolerable gastro-intestinal side effects, in which case subcutaneous (sc) MTX is used. If the max oral dose (25 mg) is reached, 25 mg sc MTX is used if dose adjustment is still needed. [b]If there is still active disease after the DMARD HCQ, SSZ, MTX have reached their max allowed doses, leflunomide (LEF) is added. If active disease is still present, then an anti-TNF agent (usually adalimumab) is added. N.B. Addition of LEF at 30 weeks and anti-TNF at 36 weeks is illustrative only. The requirement and timing are determined by disease activity.

pre-determined hierarchy of responses to persistent disease activity and drug intolerance or toxicity, variations in drug use can be employed as measures of disease activity, as modified by tolerance/toxicity considerations, rather than being discarded due to withdrawal.

The drug-based outcome measures that will be used for analysis of the effects of fish oil will include (a) the proportion of participants progressing from triple DMARD therapy to leflunomide at 12 months and (b) the number of DMARD 'step-ups' and 'step-downs' as well as an 'area under the curve' for individual drugs ingested. The progression to leflunomide is a salient event because it represents a failure of triple DMARD therapy at the maximum allowable or tolerable doses, and it is a transition from treatments costing AUD100–200 to approximately AUD3000 per patient per year, after which are biological agents costing >AUD20 000 per patient per year. The number of step-ups and step-downs may detect a suppressive effect of fish oil on disease activity or an effect on tolerance to DMARD. An area under the curve for drugs ingested, while not equivalent to area under the curve of blood levels, is a reflection of total exposure to each drug over a certain period.

Our RCT of fish oil in early RA is still in a 3-year follow-up phase and results are not available. However, because our early RA clinic recommends use of fish oil and because regular plasma and erythrocyte EPA and DHA

Table 2. Comparison of fish oil with adalimumab (Values are the standardised mean difference*)

	Tender or swollen joint count	Pain
Fish oil	−0·29†	−0·26†
Adalimumab (Humira)	−0·52 to −0·69‡	−0·27‡

*Hedges' g was used to calculate the standardised mean difference, which is the difference between means divided by the pooled standard deviation.
†Goldberg and Katz[4].
‡Calculated from data in FDA[67].

are measured, it is possible to undertake observational studies with patients from this clinic. As an example, patients were classified as fish oil users or not according to plasma phospholipid EPA levels over a period of 3 years. At 3 years, NSAID use was significantly lower (approximately half), and remission rate was significantly higher in fish oil users[25]. The OR for remission if in the fish oil user group was 2·14 (95% CI 1·01, 4·5)[25].

Examination of early or recent-onset RA and use of innovative study design provides both challenges and opportunities for determining the place of fish oil as adjunctive treatment in modern treatment regimens. To this end, we have developed a computer-based patient management system with a decision support engine that incorporates the algorithm described above (Fig. 4). This is suited to routine management as well as testing of new treatments against a background of best practice combination therapy in early RA.

Fish oil compared with anti-cytokine therapy

An important consideration for rheumatologists considering fish oil for their patients may be the perception that the effects of fish oil are modest, especially compared with the biological anti-cytokine agents. The primary end-point measure used to demonstrate efficacy of the anti-cytokine agents etanercept, infliximab, anakinra and adalimumab for US Food and Drug Administration registration was the ACR20[64–67]. This is a composite score, endorsed by the American College of Rheumatology, that requires a 20% improvement in tender or swollen joint counts as well as 20% improvement in three of five other criteria. Unfortunately, insufficient data are available from the fish oil RCT to calculate ACR20 values for comparison with the anti-cytokine agents. The meta-analysis by Goldberg and Katz reported the significant effects of fish oil as standardised mean differences, which is the difference between means divided by the pooled standard deviation[4]. It is possible to calculate standardised mean differences of some of the same parameters from an RCT with the anti-TNF monoclonal antibody, adalimumab[68]. While the effects of fish oil are numerically less, they are comparable (Table 2). In addition, there are collateral cardiovascular benefits with the use of fish oil, as discussed earlier.

Summary and conclusions

Meta-analysis provides high-level evidence for symptomatic benefits of fish oil in RA. In addition, there is

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biological plausibility for the effects of fish oil. However, the uptake of fish oil in clinical management of RA is limited. While there may be perceptions of relatively modest benefits compared with the expensive biological agents, some benefits may be comparable. It is probable that the main barrier to clinician acceptance is the promotion of pharmaceutical use as the dominant treatment modality by the pharmaceutical industry sales force that attends to the 'detailing' of doctors. In the absence of an equivalent marketing effort for fish oil, rheumatologists are not inclined to consider, or even be aware of fish oil as a potential component of routine therapy for RA patients, despite the efficacy for symptom relief, the NSAID sparing and the benefits for cardiovascular health, which is compromised in RA patients due to their disease. Future trials need to examine recent-onset RA and use designs that allow 'real-world' drug use in order to enhance the external validity of the findings for modern rheumatology treatment.

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