Dietary fatty acids and arthritis
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Abstract
Musculoskeletal complaints are the second most frequent reason for medical treatments. Within these diseases rheumatoid arthritis (RA) and, especially, osteoarthritis (OA) are common. Although the causes of arthritis are multifactorial and not fully understood, clinical trials have generally shown benefit from dietary n-3 polyunsaturated fatty acids. This has usually been attributed to their anti-inflammatory properties. Recently we have used in vitro model systems to study the molecular mechanism(s) by which n-3 PUFAs may act to alleviate the symptoms of arthritis. These experiments showed that n-3 PUFAs reduce expression of cartilage-degrading proteinases, cyclooxygenase-2 and inflammatory cytokines. Eicosapentaenoic acid (EPA) was more effective than docosahexaenoic acid (DHA) or alpha-linolenic acid. The data provide a scientific rationale for the consumption of n-3 fatty acids as part of a healthy diet and perhaps in treating arthritis.

1. Arthritis is a common complaint
Musculoskeletal complaints are the second most frequent reason for going to a doctor and represent 10–20% of primary care consultations [1]. Within these diseases, arthritis and rheumatism are prominent. Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory, autoimmune disorder which is not well understood and which requires precise matching to specific criteria in order to be diagnosed initially (see [2]). Major characteristics are listed in Table 1.

The most common articular disorder is osteoarthritis (OA) and this accounts for more disability in the elderly than any other disease [3]. It is a slowly progressing chronic disease which is characterised by loss of articular cartilage within synovial joints. Typical clinical manifestations are joint pain, restriction of movement and local inflammation [2]. A summary of major characteristics is given in Table 2.

An indication of the prevalence (and associated costs of arthritis—OA and RA mainly) is shown in Table 3. Partly because there is a lack of regular data collection, the incidence of OA and RA is difficult to estimate and reports of the prevalence vary somewhat [2]. OA is much more common than RA, which in turn affects women more than men. In the UK, incidence of RA (in 2001) has been estimated as 1.16% for women and 0.44% for men [4]. For OA worldwide, the incidence is 9.6% for men and 18.0% for women aged 60 years and over [1]. Overall in the USA, 30% of the population have symptoms of arthritis (2/3rds of whom need medication), 5–10% have disability which is related to the disease and 0.5% are totally disabled as a result of the disease [3]. Similar figures apply to the UK [2]. While the prevalence and severity of OA and RA increase inexorably with age [1], there is some evidence that the absolute incidence of RA is reducing, particularly in women. The reason(s) for this is unclear but may be due to environmental factors, a protective effect from oral contraceptive pills and/or a general reduction in bacterial infections [2].

Although limited mortality is attributed to arthritis, the diseases cause intense suffering. Joint stiffness and pain are the usual symptoms and the morbidity associated with arthritis has a massive economic cost (see Table 3). For the UK approaching a quarter of the total working days lost through illness were due to arthritis, costing about £18 billion in 2000 [2]. However one looks at it – prevalence, economic cost, suffering – arthritis is a huge problem.

2. Risk factors for arthritis
OA and RA have multiple risk factors which, to a large extent, overlap. These are discussed well by Rayman and Callagher [2]. Genetic factors have been implicated in both the susceptibility and severity of RA while they play a major role for the incidence of OA in different tissues (e.g. 60% in hip, 70% in spine). As mentioned before, age is a major factor with OA starting to increase markedly beyond age 50 and RA increasing after 40 in

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Inuits and the Japanese (who have an increased level of a high containing n-3 PUFAs derived from the observations that the decrepitude''. Much more recently, further interest in fish oils sciaticas of long standing and in those cases of premature liver oil should be given in ''obstinate chronic rheumatisms, arthritis are long standing. In 1783 Percival[5] wrote that cod 3. Clinical evidence for a benefit of dietary n-3 PUFAs

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chronic, systemic, inflammatory autoimmune disorder</td>
<td>[3,4]</td>
</tr>
<tr>
<td>Usually begins in small joints of the hands and feet</td>
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</tr>
<tr>
<td>Spreads to larger joints</td>
<td></td>
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<tr>
<td>Inflamed joint lining extends and erodes articular cartilage and bone</td>
<td></td>
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<tr>
<td>Joint deformity</td>
<td></td>
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<td>Progressive physical disability</td>
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Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tbody>
<tr>
<td>Most common articular disorder</td>
<td></td>
</tr>
<tr>
<td>Accounts for more disability amongst elderly than any other disease</td>
<td></td>
</tr>
<tr>
<td>Affects hands or weight bearing joints (hips, knees, spine)</td>
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<tr>
<td>Slowly progressing chronic disease</td>
<td></td>
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<tr>
<td>Loss of articular cartilage</td>
<td></td>
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<tr>
<td>Joint pain, tenderness, limitation of movement</td>
<td></td>
</tr>
<tr>
<td>Local inflammation</td>
<td></td>
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</table>

Table 3

<table>
<thead>
<tr>
<th>Incidence, morbidity and economic costs of arthritis.</th>
<th>Reference</th>
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<tbody>
<tr>
<td>In USA, 30% of the population have some symptoms of arthritis</td>
<td>[21,22]</td>
</tr>
<tr>
<td>RA more prevalent in women</td>
<td></td>
</tr>
<tr>
<td>RA affects 0.3–1% of population in USA and Europe</td>
<td></td>
</tr>
<tr>
<td>In UK, OA affects 7–13 million</td>
<td></td>
</tr>
<tr>
<td>10% World’s population &gt; 60 years of age have symptomatic problems</td>
<td></td>
</tr>
<tr>
<td>Quality of life severely impaired</td>
<td></td>
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<tr>
<td>206 million working days lost annually in the UK (46 million due to RA)</td>
<td></td>
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<tr>
<td>In UK, £1.1 billion annual expenditure on prescribed medications and operations</td>
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both men and women. While sports injuries and trauma are associated with OA, obesity increases the risk of both RA and OA, while smoking (associated with the production of rheumatoid factor) is a significant risk factor for RA.

Inconsistent and often conflicting evidence for different dietary constituents as risk factors for OA and RA have been found[2]. These include coffee, tea and alcohol. On the other hand, positive benefits have been associated with vitamin C and, especially, vitamin D. For dietary fats, lower incidences of RA were found in various studies for olive oil and fish consumption. The beneficial effect of fish was related to its n-3 polyunsaturated fatty acid (PUFA) content[2].

3. Clinical evidence for a benefit of dietary n-3 PUFAs

Our awareness of the potential benefits of n-3 PUFAs for arthritis are long standing. In 1783 Percival [5] wrote that cod liver oil should be given in "obstinate chronic rheumatisms, sciaticas of long standing and in those cases of premature decrepitude". Much more recently, further interest in fish oils containing n-3 PUFAs derived from the observations that the Inuits and the Japanese (who have an increased level of a high susceptibility gene for RA) [6,7] both have low incidence of arthritis.

The subject of the importance of dietary PUFAs in the treatment of arthritis has been excellently covered by Rayman and Callaghan [2] and we will only make summary observations here.

Almost all of the clinical studies have focussed on RA, which is a pity since OA is much more prevalent. There have been around 20 studies of the use of fish oil (and (oily) fish) intake on RA. For most of these studies improvement in at least two clinical outcomes was achieved (see Table 4 for a summary of some trials). Apart from subjective assessments by patients (e.g. grip strength, stiffness, joint pain), intake of non-steroidal anti-inflammatory drugs (NSAIDs) and biochemical measurements of inflammation (e.g. interleukin-1, leukotriene B4) were amongst the outcomes included in some studies. Dietary fish oil intakes and RA have been reviewed [8–11]. Because not all n-3 PUFAs are equally effective [12], due in part to metabolic restrictions (which will be commented on later), there has been a focus on the content of n-3 eicosapentaenoic acid (EPA) and n-3 docosahexaenoic acid (DHA) in evaluating clinical effectiveness. For the studies summarised above the doses of EPA and DHA were in the range 1–7 g/day. This variability in experimental design is compounded by different durations of supplementation, ignorance of other confounding dietary constituents (e.g. n-6 PUFAs), lack of recognition of genetic variation, as well as a frequent lack of plasma measurements to estimate both the level of compliance and incorporation of the assumed biologically active fatty acids.

Meta-analyses of the trial data have been carried out. Fortin et al. [23] included trials that were double-blind, placebo-controlled and showed randomisation as well as parallel or crossover design. They examined data from seven published trials and included additional data from a further three unpublished trials. Improvements in morning stiffness and tender joints were statistically significant, but not other outcomes such as grip strength or joint swelling. MacLean et al. [24] examined 21 studies that met US Department of Health and Human Services inclusion criteria. From these they used 5–9 sets of data for four outcomes (pain, joint swelling, disease activity and overall patient assessment). Improvement was found for all four evaluations on fish oil treatment but none reached statistical significance. The use of NSAIDs in 4 of the above studies was also assessed. Fish oil lowered NSAID use significantly in 3 of these [24]. In another study measuring the effect of fish oil alone or with olive oil, the latter combination proved more effective – perhaps because olive oil is thought to decrease expression of intercellular adhesion molecule-1 and, also may increase incorporation of n-3 PUFAs into membranes [25]. Moreover, oleic acid (which is highly enriched in olive oil) can be metabolised to n-9 eicosatrienoic acid and this can, in turn, reduce the production of the pro-inflammatory leukotriene B4 from arachidonic acid [25,26].

The modest effects seen for dietary fish oils in many trials may have an explanation in ‘biological variation’. In particular, genetic
Moreover, the dietary ratio of n-6:n-3 PUFAs may be important in vitro arthritis [32,33]. These culture systems used chondrocytes or degenerative features and inflammatory pathways involved in arthritis. In a series of experiments we tested the relative efficacy of three n-3 PUFAs (ω-3-linolenic acid, EPA, DHA) let alone the higher levels associated with demonstrable anti-inflammatory effects [2]. Moreover, the dietary ratio of n-6:n-3 PUFAs may be important (see [12]), and typical Western diets currently have a ratio of 10–15 where a ‘healthier’ ratio is thought generally to be in the range 3–4.

4. Probing the molecular mechanism by which n-3 PUFAs can be of benefit for arthritis

The way in which dietary n-3 PUFAs can be of benefit in arthritis is not entirely clear. Nevertheless, based on evidence from clinical studies as well as our knowledge of the metabolism and molecular effects of n-3 PUFAs, we can summarise these as being to lower inflammation and to reduce cartilage destruction. To do this n-3 PUFAs may have some specific actions which vary in extent according to individual cellular/tissue conditions, genetic predisposition and environmental (including diet-de-

Table 5
Possible limitations of human intervention trials on arthritis with n-3 PUFAs.

- Too short a duration
- Inappropriate (inflammatory or anti-inflammatory) placebo used
- Dose of n-3 PUFAs too low
- Inappropriate n-3 PUFAs used (18 carbon fatty acids much less effective)
- Other dietary components (e.g. n-6 PUFAs) not considered
- Patient genotype and medication can confound results

Based on [2].

differences may make some individuals more responsive to n-3 PUFAs because of their inherent inflammatory cytokine production [27,28]. Moreover, as pointed out before (and discussed in [2]), other components in the diet can influence the effectiveness of the n-3 PUFAs. Thus, although specific n-6 PUFAs, namely ω-6-linolenic acid/GLA; 18:3n-6) and dihomo-ω-6-linolenic acid (DGLA; 20:3n-6) may help alleviate some symptoms of RA (see [9]), it is usually thought that dietary n-6 PUFAs are pro-inflammatory and will, therefore, reduce the effectiveness of EPA and DHA or fish oils. Thus, there have been some trials where the intake of n-6 PUFAs was deliberately restricted. These tended to show an enhanced benefit for the dietary n-3 PUFAs [29–31].

Because clinical trials have been conducted over the last 20 years and our knowledge of the biochemistry and mechanisms of action of n-3 PUFAs has evolved over that period, limitations are clearly present for the evaluation of the data. These limitations are summarised in Table 5 and are discussed in [2]. In particular, inappropriate placebos have often been chosen and this is a problem with many animal studies using n-3 PUFAs also.

So far as current intakes of n-3 PUFAs are concerned, most European and North American populations consume considerably less than the current dietary recommendations for n-3 PUFAs (about 0.5 g/day of EPA plus DHA) let alone the higher levels associated with demonstrable anti-inflammatory effects [2]. Moreover, the dietary ratio of n-6:n-3 PUFAs may be important (see [12]), and typical Western diets currently have a ratio of 10–15 where a ‘healthier’ ratio is thought generally to be in the range 3–4.

Table 6
Molecular mechanisms by which n-3 PUFAs may act to reduce symptoms of arthritis.

- Compete with n-6 PUFAs in metabolism to eicosanoids
- Produce less (non-) inflammatory eicosanoids
- Produce anti-inflammatory resolvins, docosatrienes, etc.
- N-3 PUFA derived eicosanoids compete with n-6-derived compounds for receptors
- Reduce gene expression of cyclooxygenase-2 (COX-2) but not COX-1
- Reduce gene expression of inflammatory cytokines
- Reduce gene expression of cartilage-degrading proteinases
- Affect signalling pathways for transcription factors (e.g. NFκB)
- Reduce lymphocyte proliferation

4. Probing the molecular mechanism by which n-3 PUFAs can be of benefit for arthritis

The experimental results are shown in Refs. [34–36]. Abbreviations: MMP, matrix metalloproteinase; COX, cyclooxygenase; IL-1, interleukin-1; TNF, tumour necrosis factor; ALA, alpha-linolenic acid; ADAMTS, A Disintegrin And Metalloproteinase with ThromboSponin motifs (ADAMTS-4 and -5 were previously called aggrecanase-1 and -2, respectively).

Table 7
Summary of the effects of the in vitro action of n-3 PUFAs on factors associated with arthritis pathology.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effects</th>
<th>Comments</th>
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<tr>
<td>ADAMTS-4</td>
<td>mRNA levels reduced by n-3 PUFAs</td>
<td>Cause the initial proteolysis of cartilage</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>As above but EPA much more effective</td>
<td></td>
</tr>
<tr>
<td>MMP-3</td>
<td>EPA reduced mRNA levels</td>
<td>Responsible for further protein degradation. Elevated in arthritis</td>
</tr>
<tr>
<td>MMP-13</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>COX-1</td>
<td>No effect of any n-3 PUFA</td>
<td>The constitutive isoform</td>
</tr>
<tr>
<td>COX-2</td>
<td>mRNA, protein, activity reduced by n-3 PUFAs</td>
<td>Inflammation-induced isoform</td>
</tr>
<tr>
<td>IL-1α</td>
<td>Induced by inflammation and reduced by n-3 PUFAs (but not by n-6 PUFAs) with IL-1 and TNF-α</td>
<td>The main cytokines associated with the inflammation in arthritis</td>
</tr>
<tr>
<td>IL-1β</td>
<td>mRNA expression reduced by n-3 PUFAs</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>EPA &gt; DHA &gt; ALA</td>
<td></td>
</tr>
</tbody>
</table>

The way in which dietary n-3 PUFAs can be of benefit in arthritis is not entirely clear. Nevertheless, based on evidence from clinical studies as well as our knowledge of the metabolism and molecular effects of n-3 PUFAs, we can summarise these as being to lower inflammation and to reduce cartilage destruction. To do this n-3 PUFAs may have some specific actions which vary in extent according to individual cellular/tissue conditions, genetic predisposition and environmental (including diet-derived) factors. These are listed in Table 6.

In order to define the molecular mechanisms more fully we have used in vitro culture systems that mimic many of the degenerative features and inflammatory pathways involved in arthritis [32,33]. These culture systems used chondrocytes or cartilage explants derived from bovine metacarpophalangeal joints (7-day-old calves) or from human patients undergoing total knee replacement surgery for OA. The bovine systems were stimulated with interleukin-1α and the human tissues with interleukin-1β to elicit an inflammatory response. We then evaluated mRNA levels for proteinases, COX-1 and -2, lipoxygenases and inflammatory cytokines as well as protein levels and activities of some of the above. By using these in vitro model systems, we were able to test directly various PUFAs (including different n-6 and n-3 PUFAs) for their ability to change expression and/or activity of factors associated with the pathology of arthritis. The overall results are summarised in Table 7.

In a series of experiments we tested the relative efficacy of three n-3 PUFAs (ω-3-linolenic acid, EPA, DHA). Overall, the n-3 PUFAs reduced mRNA levels for the key initial cartilage-degrading proteinases ADAMTS-4 and -5 and the matrix metalloproteinases (MMP)-3 and -13, which are all known to be important in arthritis. They also reduced mRNA for COX-2 (but not the constitutive COX-1) and for the inflammatory cytokines, tumour necrosis factor-α (TNF-α), interleukin-1α (IL-1α) and interleukin-1β (IL-1β) [34]. EPA was the most effective of the three n-3 PUFAs tested [34].

We also showed that it was possible to modify palm oil (the world’s major edible oil) by esterification with EPA to produce a product that had effective anti-inflammatory properties. The EPA-enriched oil (but not the un-modified palm oil) reduced the IL-1-induced expression of ADAMTS-4, COX-2, TNF-α, IL-1α and IL-1β in a concentration-dependent manner back down to control (un-induced) levels [35].
Because COX-2 is a key enzyme for inflammatory responses, we examined its expression and activity in more detail. Using both bovine and human culture systems, we showed that EPA was able to reduce COX-2 mRNA and protein levels. Activity (assessed by prostaglandin E₂ production) was reduced in a concentration-dependent manner by EPA. These effects of EPA contrasted with those of arachidonic acid, which did not alter COX-2 expression or activity [36]. These data provide direct evidence that beneficial effects of dietary n-3 PUFAs (particularly EPA) can involve reduction in the expression and, hence, activity of the key COX-2 enzyme.

Further exploitation of these in vitro model systems should be able to elucidate additional molecular mechanisms of relevance to the progression of arthritis. Such mechanisms could include the role of transcription factors as well as cross-talk between different signalling pathways. Furthermore, they may also provide information concerning the competition between different individual PUFAs which may influence dietary advice for ‘healthy’ as well as arthritic patients.

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References