ROLE OF ω-3 POLYUNSATURATED FATTY ACIDS IN INFLAMMATION AND RHEUMATOID ARTHRITIS DISORDERS

Mohammad Asif
Department of Pharmacy, GRD (PG) IMT, Dehradun, (Uttarakhand), 248009, India

Corresponding author email: aasif321@gmail.com

Abstract

Reviewing the relationships between polyunsaturated FAs (PUFAs) with inflammation and rheumatoid arthritis disorders, the PUFAs containing ω-3, ω-6 and ω-9, these ω-3FAs levels were correlated with ω-6: ω-3 ratios including arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Based on previously-reports, the levels of ω-3 FAs considered being as a 'lower risk' category for inflammation and rheumatoid arthritis. Certain PUFAs ratios may aid in inflammation and rheumatoid arthritis-related risk assessment. PUFAs are the most effective for the production of oil with high concentration of DHA and EPA content significantly.

Key words: Inflammatory diseases, rheumatoid arthritis, polyunsaturated fatty acids.

Introduction

Polyunsaturated fatty acids (PUFAs), α-linolenic acid (ALA) is an essential ω-3 FAs (ω-3FAs) which are important in human nutrition. Other acids such as α-linolenic acid (18:3, ω-3; ALA), eicosapentaenoic acid (20:5, ω-3; EPA), and docosahexaenoic acid (22:6, ω-3; DHA) are also important for human nutrition. Mostly naturally-produced FAs are in cis-configuration where they are more easily transformable. The trans-configuration results in much more stable chains those are very difficult to further break or transform, forming longer chains that aggregate in tissues and lacking the necessary hydrophilic properties. However, ω-3 compounds are still more fragile than ω-6 because the last double bond is geometrically and electrically more exposed, notably in the natural cis configuration. A number of dietary factors have irritant or immunological effects in the gut, which allow an anti-inflammatory effect. There are also factors that can be enriched in the diet to achieve anti-inflammatory effects, such as ω-3-PUFAs. The ethylesterized ω-3-FAs, such as E-EPA and combinations of E-EPA and E-DHA, have drawn attention as more effective products than the traditional ones. The health benefits of ω-3FAs (DHA and EPA) are the best known. The high level of ω-3FAs in diet may reduce triglycerides, heart rate, blood pressure, and atherosclerosis. The health claim status to EPA and DHA, stating that consumption of EPA and DHA ω-3FAs may reduce the risk of coronary heart disease (US FDA, 2004). It also has recognized the importance of DHA ω-3FAs, helps the normal development of the brain, eyes and nerves. PUFAs have also been reported as other health benefits. ω-3FAs have been known as essential to normal growth and health, awareness of their health benefits (Holman, 1998)

Table 1: ω-3 content as the percentage of ALA in the seed oil.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Common name</th>
<th>Alternative name</th>
<th>Linnaean name</th>
<th>% ω-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chia</td>
<td>chia sage</td>
<td>Salvia hispanica</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>Kiwifruit</td>
<td>Chinese gooseberry</td>
<td>Actinidia chinensis</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>Perilla</td>
<td>Shiso</td>
<td>Perilla frutescens</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>Flax</td>
<td>linseed</td>
<td>Linum usitatissimum</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>Lingonberry</td>
<td>Cowberry</td>
<td>Vaccinium vitis-idaea</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Camelina</td>
<td>Gold-of-pleasure</td>
<td>Camelina sativa</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>Purslane</td>
<td>Portulaca</td>
<td>Portulaca oleracea</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Black Raspberry</td>
<td></td>
<td>Rubus occidentalis</td>
<td>33</td>
</tr>
</tbody>
</table>
An intense interest in the health benefits of ω-3 PUFAs, their active component, cis-EPA and cis-DHA. Both EPA and DHA have several benefits on cardiovascular disorders, autoimmune, inflammatory diseases and cancer. The beneficial effects of ω3 PUFA are attributed to eicosanoid synthesis such as prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs). The importance of these FAs to infant nutrition is particularly relevant because DHA is important for fetal and term-infant neural development. Although PUFAs can be synthesized in the body by elongation and desaturation of ALA, ingestion of the preformed molecules usually is more effective. Lipases are known to have little reactivity on PUFA (e.g., γ-linolenic acid, AA, EPA and DHA), and these acids can be enriched by selective hydrolysis, direct esterification of glycerol with EPA and DHA, and interesterification (Bastos & de Oliveira, 2002).

**Biological sources of PUFAs**

Flax seeds produce linseed oil, which has very high ω-3 content. Six times richer than most fish oils in ω-3, flax (or linseed) (*Linum usitatissimum*) and its oil are perhaps the most widely available botanical source of ω-3. Flaxseed oil consists of approximately 55% ALA (α-linolenic acid). Flax, like chia, contains approximately three times as much ω-3 as ω-6. 15 grams of flaxseed oil provides ca. 8 grams of ALA, which is converted in the body to EPA and then DHA at an efficiency of 5–10% and 2–5%, respectively. The most widely available source of EPA and DHA is cold water oily fish such as salmon, herring, mackerel, anchovies and sardines. Oils from these fish have a profile of around seven times as much ω-3 as ω-6. Other oily fish such as tuna also contain ω-3 in somewhat lesser amounts. Consumers of oily fish should be aware of the potential presence of heavy metals and fat-soluble pollutants which may accumulate up the food chain. Although fish is a dietary source of ω-3 FAs, fish do not synthesize them; they obtain them from the algae or plankton in their diet (DeFilippis, and Laurence, 2007). Cold water fish are the highest source of ω-3 FAs. Other foods contain these FAs as well, however, in smaller amounts. The recommendations are to have 7 to 11 grams of ω-3 FAs each week. Milk and cheese may also be good sources of ω-3. One study showed that half a pint of milk provides 10% of the recommended daily intake (RDI) of ALA, while a piece of organic cheese the size of a matchbox may provide up to 88%. The microalgae *Crypthecodinium cohnii* and *Schizochytrium* are rich sources of DHA (22:6 ω-3) and can be produced commercially in bioreactors. This is the only source of DHA acceptable to vegans. Oil from brown algae is a source of EPA. Walnuts are one of few nuts that contain appreciable ω-3 fat, with approximately a 1:4 ratio of ω-3 to ω-6. Acai palm fruit also contains ω-3 FAs. It is also found in combination with ω-6, ω-9 and shark liver oil. Some vegetables contains noteworthy amount of ω-3, including strawberries and broccoli (Okuyama, 2001; Griffin, 2008).

The fatty acid (FA) composition of serum (or plasma) phospholipid has become established as a valid biochemical marker for assessing the physiological status of various FAs including predictive correlations with the dietary intakes of fish-derived ω-3 FAs including EPA (20:5 ω-3) and DHA (22:6 ω-3) (Hjartaker et al., 1997; Kobayashi et al., 2001). Population studies have shown an inverse relation between total ω-3 FAs in blood serum phospholipid and the risk for coronary heart disease with percentages of total ω-3 ≥ 7.2 being associated with a 31% lower risk (Grimble et al., 2002; Curtis et al., 2000). Furthermore, DHA levels (as percent of total FAs in serum phospholipid) of ≥ 4.5 have been associated with a 34% lower risk for coronary heart disease (Simon et al., 1995; Holub and Holub. 2004). With respect to the risk of fatal ischemic heart disease, EPA+DHA (summed) levels amounting to at least 4.6% of total FAs in the serum phospholipid were associated with a 70% lower risk as compared to those with much lower levels of these FAs (Holub and Holub. 2004; Lemaître et al., 2003). Since the ω-6 FA, AA (20:4 n-6), found in abundance in various cells and tissues including serum phospholipid can be readily converted into pro-inflammatory eicosanoids and other products associated with inflammatory processes and chronic disorders in contrast to EPA (Cleland et al., 2005), the AA: EPA ratio in serum phospholipid has been studied in relation to the risk of chronic disorders. These studies have, as an example, indicated that the AA/EPA ratio in serum (or plasma) phospholipid correlates positively with clinical symptoms of depression; furthermore, higher ratios of AA: DHA were associated with greater neuroticism. Others have implicated the abundance of the summed ω-6 relative to the ω-3 FAs in human plasma phospholipid with respect to chronic disorders (Trebonová, et al., 2007; Holub, et al., 2009).

**Table 2**: ω-3 content as the percentage of ALA in the whole food.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Common name</th>
<th>Linnaean name</th>
<th>% ω-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flaxseed</td>
<td><em>Linum usitatissimum</em></td>
<td>18.1</td>
</tr>
<tr>
<td>2</td>
<td>Butternuts</td>
<td><em>Juglans cinerea</em></td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>Hempseed</td>
<td><em>Cannabis sativa</em></td>
<td>8.7</td>
</tr>
<tr>
<td>4</td>
<td>Walnuts</td>
<td><em>Juglans regia</em></td>
<td>6.3</td>
</tr>
<tr>
<td>5</td>
<td>Pecan nuts</td>
<td><em>Carya illinoinensis</em></td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>Hazel nuts</td>
<td>* Corylus avellana*</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Complete FA profiles of their serum phospholipid, the following were of interest:

- to determine the relationship between the percentage of total FAs in serum phospholipid as total ω-3, DHA, and (EPA+ DHA)-risk factors for coronary heart disease and fatal ischemic heart disease (plus other chronic disorders) to the various ratios (ω-6:ω-3, AA:EPA, AA:DHA, and AA:(EPA+DHA));
- to compare the relative strengths of these correlations (fatty acid percentages with ratios);
- to determine the corresponding cut-points (95 percentile) for the (ω-6:ω-3), AA:EPA, AA:DHA, and AA:(EPA+DHA) ratios which are associated with a 95% likelihood of the percentage of total FAs in serum phospholipid as ω-3, DHA, and EPA+DHA being in the aforementioned 'lower-risk' category (Simon et al., 1995; Holub and Holub. 2004; Lemaître et al., 2003).

With regard to anti-inflammatory effects of diet away from the gut, altering the balance of dietary PUFA in favour of ω-3 PUFA provides the best documented examples of effective dietary intervention. PUFA are essential macronutrients of which there are two non-interchangeable classes, n-6 and ω-3. These FAs are metabolized to mediators that regulate cardiovascular homeostasis and inflammation. n-6 rich diets tend to be pro-inflammatory and, by comparison diets rich in ω-3 PUFA are antiinflammatory. The difference is explained by the action of ω-3 PUFA as competitive inhibitors of enzymes that metabolize n-6 fats and by the lesser biological activities of most ω-3 mediators, compared with their n-6 counterparts. Fish oils are a particularly rich source of desirable long chain ω-3 PUFA. Fish oil has been used with benefit in the treatment of inflammatory diseases of joints and other organs and tissues. A potential collateral benefit is reduced risk for adverse cardiovascular events, which are increased in rheumatoid arthritis (RA). Lack of knowledge amongst physicians of relevant biochemistry, evidence of efficacy, latency in effect, availability of affordable preparations and tactics for discussing issues efficiently with patients appears to be a barrier to broader clinical use (Cleland et al., 2005).

Fish oil is rich in the long chain ω-3 FAs EPA (20:5 ω-3) and DHA (22:5 ω-3), which can displace AA (20:4 ω-6) from cell membranes. These ω-3 FAs are also released with AA by phospholipases and act as substrate inhibitors of conversion of AA by COX and the terminal synthases to the pro-inflammatory oxygenated inflammatory mediators known as eicosanoids. EPA is structurally identical to AA with the exception of its additional ω-3 double bond and can be converted to eicosanoids that resemble ω-6 eicosanoids but have the additional ω-3 double bond. This structural difference is sufficient to confer substantial differences in activity between ω-6 LTB4, a very potent chemo-toxin and leucocyte agonist and ω-3 LTB5, which is a weak chemotoxin and weak agonist. ω-3 TXA3 appears to lack the potency of ω-6 TXA2, which is an aggregator of platelets and vasoconstrictor. ω-3 prostaglandin E3 (PGE3) seems to have similar oedemogenic activity to ω-6 PGE2, but very little PGE3 is produced by monocytes either from endogenous or exogenous EPA in vitro. ω-6 prostacyclin (PGI2) and ω-3 PGI3 are thought to have similar activities as agents for vascular patency. In addition to these effects on inflammatory eicosanoid synthesis, dietary fish oils have been shown to reduce the production of the inflammatory cytokines IL-1β and TNF-α by monocytes stimulated in vitro. These cytokines are important effector molecules in inflammatory responses and TNF α blocking agents are now used widely to treat rheumatoid disease that has proven refractory to less expensive therapies. In vitro studies have also shown inhibition of release of the metalloproteinases that are implicated in the tissue damage that is the hallmark of RA and other inflammatory diseases (Caughey et al., 1996; Grimble et al., 2002; Curtis et al., 2000).

Epidemiological studies, the possibility of an anti-inflammatory effect of dietary long chain ω-3 FAs (Kromann and Green. 1980). In this regard, they contrast with continental Inuits, for whom meat from grazing animals, such as caribou, contribute to the diet. Thus, extreme case in which the diet is comprised of foods very rich in long chain ω-3 fats (7-10g/day) and poor in ω-6 fats. With the introduction of market foods, the ω-3 dominance of their aboriginal diet has been diluted by Western products with a higher ω-6 FA content. The very high ω-3 content of the Greenland Inuit diet needs to be recognized when extrapolating the putative bleeding tendency of the Inuits to the possible risks of fish oil supplements taken against the background of an ω-6 abundant Western diet. Immunogenetic studies of circumpolar Inuits have shown a high frequency of alleles of human leucocyte antigens (HLA) HLA DRB1 0401 and HLA-B27 that in other populations have been associated with increased risk for a variety of arthritides and other inflammatory conditions (Welinder et al., 2000; Harvald. 1989).

For example, HLA DR B1 0404 is associated with increased risk for and severity of RA. HLA B27 is associated with spondyloarthritides, seronegative arthritis, uveitis and the peripheral arthritis, conjunctivitis and urethritis of Reiter's syndrome. Since these genotypes have been found in a high proportion of Inuits, one can speculate that they may, through strong antigen presentation of certain peptides, provide a selective advantage in relation to defence against particular infections, which may be critical in the context of an anti-inflammatory ω-3 dominant diet. These same antigen presentation phenotypes may lead to unwanted inflammation and auto-immunity when the diet is rich in pro-inflammatory ω-6 fats. Epidemiological studies of the Japanese, whose traditional diet contains about 3G long chain ω-3 fats, equivalent to an anti-inflammatory dose of...
The effects of fish oil feeding has yielded mixed results in animal models of inflammation. Fish oil diets have been shown to have a striking protective effect when used prophylactically in mice genetically predisposed to systemic lupus (Prickett et al., 1981). A fish oil diet is also effective when introduced after the emergence of signs of murine lupus, but less effective than when used prophylactically (Robinson et al., 1986). A fish oil diet increased the frequency but not the severity of collagen-induced arthritis in mice (Pritchett et al., 1984) and results in rats with adjuvant-induced arthritis were strain specific (Robinson, 1991). This latter model causes universally severe disease in susceptible strains and is resistant to a number of other treatments that are effective in all but the most severe forms of RA in humans. Collectively, the animal studies support an anti-inflammatory effect of dietary long chain ω-3 fats, but caution that this effect cannot be generalized to all inflammatory diseases.

**Rheumatoid Arthritis**

Multiple studies have shown symptomatic benefit in RA with fish oil treatment. The anti-inflammatory dose appears to be at least 2.6g long chain ω-3 FAs per day (Cleland et al., 2003). This dose requires 9 standard fish oil capsules per day. Most studies have used 15–20 standard fish oil capsules per day to deliver 4.5–6g long chain ω-3 FAs per day. The salient benefits have been reduced joint pain and tenderness. There is usually a latency of six to twelve weeks from the introduction of fish oil to symptomatic response, which appears shorter with higher doses. The need for the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs) is reduced by fish oil treatment. Fish oil has been mainly tested as an adjunct to long acting therapies for RA, known as the disease modifying anti-inflammatory drugs (DMARDs) (James et al., 1997).

**Safety of fish oil with long term use in anti-inflammatory doses**

As there is no previously documented experience with long term use of fish oils in anti-inflammatory doses, this question requires special attention. Through our Pilot Early Arthritis Study (open label fish oil), we have an experience with anti-inflammatory doses of fish oil extending over a period of more than 6 years for some patients. In the quest of cost efficiency, we have developed a method for giving bottled fish oil on juice in a way that masks the fishy taste of the oil. Initially, we used a commercially available cod liver oil preparation that contained EPA 100mg, DHA 100mg, vitamin D 80u, vitamin A 610u per mL. The recommended dose was 20mL daily, which delivers 4g long chain ω-3 FAs per day, 1600u cholecalciferol and 12,500u vitamin A. Compliance with the regimen was reflected in increased plasma phospholipids EPA (Cleland et al., 2003). A mean increase in both vitamin D and vitamin A levels was seen at 12 months (Fig 3). Some vitamin D levels approached or slightly exceeded the upper limit of the normal reference range, but all were far below levels at which vitamin D toxicity has been reported (Mason et al., 1980). A single patient who displayed hypercalcaemia was found to have primary hyperparathyroidism. In some cases slight elevations of vitamin A above the reference range were also seen. Patients were monitored for bone mineral density and no difference in bone loss was seen relative to RA patients no taking fish oil. Notwithstanding, because recent reports show higher levels of vitamin A supplementation correlate inversely with bone mineral density (Promislow et al.,
2002), we decided to switch from use of cod liver oil to a fish body oil, since fish body oils contain trace amounts only of fat soluble vitamins. As there was no available retail supply of fish oil other than in capsules, we purchased fish oil in bulk and arranged bottling in our hospital pharmacy. We advised a 15mL dose of this preparation, which delivers 4.5g long chain ω-3 FAs daily. Long term use of fish oil raises concerns regarding the possible ingestion of industrial toxins found in fish. The presence of mercury in the meat of carnivorous fish has attracted considerable attention. Mercuric chloride is not lipophilic and mercury is not present in fish oils. A greater concern is polychlorinated biphenyls (PCBs), which are lipophilic and are present in trace amounts in fish oil. Components of this family are produced as byproducts of chemical synthetic reactions and are not biodegradable. Processes that generate these compounds have been outlawed but PCBs persist to varying degrees in the environment. Since they are relatively volatile they can be removed using standard fractionation processes such as molecular distillation. Notwithstanding, taking anti-inflammatory doses of fish oils harvested from industrial regions without adequate processing could involve ingestion of PCBs at or above currently recommended intakes, albeit below intakes prior to institution of avoidance measures.

**Effectiveness of fish oil in other inflammatory disease**

Fish oil supplements have been shown to reduce relapse in Crohn’s disease by more than 60% and to reduce substantially loss of renal function and progression to end stage renal failure in IgA nephropathy (Belluzzi et al., 1996; Donadio et al., 1994). Some, but not all, variants of psoriasis have been shown to respond to fish oil treatment (Mayer et al., 2002). Control of systemic lupus has been shown to improve with fish oil supplements (Walton et al., 1991; Ioannou and Isenberg, 2002). Dietary fish oil has been shown to improve outcomes in ischaemic heart disease, to which patients with RA are especially prone (Burr et al., 1989; GISSI, 1999). Cardiovascular benefits of fish oil include a myocardial membrane stabilizing effect, reduced incidence of malignant arrhythmias and sudden death, improved blood pressure control, reduction in raised plasma triglycerides and, in experimental animals, and anti-atherogenic effect (Leaf et al., 1999).

**Bleeding tendency and fish oil supplements**

Fish oil supplementation may lead to an increased bleeding tendency, this has not been our experience. The concerns centre on an extrapolation from a putatively increased bleeding times in Greenland Inuits (Dyerberg and Bang, 1979) and somewhat increased incidence of apoplexy (Kromann and Green, 1980). The latter is likely multifactorial with high dietary salt intake potentially a factor. The bleeding time data from Greenland Inuit studies show a moderate increase in bleeding time (Dyerberg and Bang, 1979). Whether this putative effect seen in Inuits with very high dietary long chain ω-3 intake and low n-6 diet will translate to Westerners in whom an ω-3 rich fish oil supplement is being taken against the background of a Western diet abundant in n-6 FAs is doubtful. In any case, we have compared competitor AA and EPA in platelets of patients with RA on long term therapy with fish oil (>3 years) with those reported for Greenland Inuits. The AA is far more suppressed and the EPA higher in the Inuits than the fish oil treated patients. Thus, on biochemical grounds a lesser effect on platelet function in patients on fish oil can be expected than seen in Inuits. Also, it has been reported that consumption of 3.4g/day of ω-3 fats in conjunction with 300 mg/day of aspirin had no effect on bleeding time, or episodes of bleeding in patients undergoing coronary artery bypass surgery (Eritsland et al., 1995).

**Favourable interactions between fish oil and anti-inflammatory drugs**

As discussed above, fish oil reduces recourse to NSAIDs for analgesia in RA and thereby reduces risk for upper GI haemorrhage. Fish oil contrasts with the highly selective COX-2 inhibitor rofecoxib, which has been associated with increased serious cardiovascular events, by reducing risk for these events (Bombardier et al., 2000; Calder, 2004). Anti-inflammatory doses of fish oil have been shown to reduce the hypertensive and nephrotoxic effects of cyclosporine (Duralametos and Varonos, 2001).

**Oils containing n-6 gamma linolenic acid (GLA)**

Oils rich in GLA may have anti-inflammatory effects. The putative biochemical basis for this effect is relative accumulation of the elongation product of GLA, dihomogamma linolenic acid (DGLA) which, like EPA, can compete in metabolic pathway that are usually dominated by AA. The result is fewer AA derived eicosanoids with production of homologous metabolites products of DGLA such as PGE1 (one less double bond than AA derived PGE2). GLA rich oils appear to reduce symptoms in RA but available evidence is far less than that for fish oil in RA (Zurier et al., 1996).

**Anti-inflammatory and rheumatoid arthritis benefits**

Perilla oil is rich in the ω-3 FAs, on metabolism gives EPA and DHA, which can displace AA from cell membranes. These ω-3 FAs are also released with AA by phospholipases and act as substrate inhibitors of conversion of AA by COX and the terminal synthases to the pro-inflammatory oxygenated inflammatory mediators known as eicosanoids. EPA is structurally identical to AA with the exception of its additional ω-3 double bond and can be converted to eicosanoids that resemble eicosanoids. In addition to these effects on inflammatory eicosanoid synthesis, perilla oils have been shown to reduce the production of the inflammatory cytokines IL-1β and TNFα by monocytes stimulated in vitro. These cytokines are important effector molecules in inflammatory responses and TNFα blocking agents are now used widely to treat rheumatoid disease that has proven refractory to less expensive therapies. In vitro
studies have also shown inhibition of release of the metalloproteinasises that are implicated in the tissue damage that is the hallmark of RA and other inflammatory diseases (Osakabe et al. 2005; Osakabe, et al. 2004; Banno, et al. 2004; James, et al. 2000). It has been reported that conversion of ALA to EPA and further to DHA in humans is limited, but varies with individuals. Women have higher ALA conversion efficiency than men, probably due to the lower rate of utilization of dietary ALA for β-oxidation. PUFAs reduce recourse to NSAIDs for analgesia in RA and thereby reduces risk for upper GI haemorrhage. Perilla oil contrasts with the highly selective COX-2 inhibitor rofecoxib, which has been associated with increased serious cardiovascular events, by reducing risk for these events. The result is fewer AA derived eicosanoids with production of homologous metabolites products such as PGE1 (one less double bond than AA derived PGE2). ALA rich oils appear to reduce symptoms in RA but available evidence is far less than that for perilla oil in RA (Osakabe, et al. 2005; Banno, et al. 2004; Calder, 2004; James, et al. 2000; Borchers, et al. 1997) Faeces of rats before dry Faeces of rats.

**Rheumatoid arthritis**

The in-vitro anti-inflammatory activity of ω-3 acids translates into clinical benefits. Cohorts of neck pain patients and of RA sufferers have demonstrated benefits comparable to those receiving standard NSAIDs. Those who follow a Mediterranean-style diet tend to have less heart disease, higher HDL ("good") cholesterol levels (Kris-Etherton, et al., 2001), and higher proportions of ω-3 in tissue highly unsaturated FAs (Lands, William E.M. 2003).

**ω Fatty Acids: ω-3; ω-6; ω-9 fatty acid**

The ω-3 FAs (popularly known as ω-3 FAs or ω-3 FAs) are a family of unsaturated FAs that have in common a final carbon–carbon double bond in the ω-3 position; that is, the third bond from the methyl end of the FA. Important nutritionally essential ω-3 FAs include α-ALA, EPA, and DHA, all of which are polyunsaturated. The human body cannot synthesise ω-3 FAs de novo, but it can form 20-carbon unsaturated ω-3 FAs (like EPA) and 22-carbon unsaturated ω-3 FAs (like DHA) from the eighteen-carbon ω-3 FA α-linolenic acid. These conversions occur competitively with ω-6 FAs, which are essential closely related chemical analogues that are derived from linoleic acid. Both the ω-3 α-linolenic acid and ω-6 linoleic acid are essential nutrients which must be obtained from food. Synthesis of the longer ω-3 FAs from linolenic acid within the body is competitively slowed by the ω-6 analogues. Thus accumulation of long-chain ω-3 FAs in tissues is more effective when they are obtained directly from food or when competing amounts of ω-6 analogs do not greatly exceed the amounts of ω-3.

**Biological significance**

**Health benefits**

The α-linolenic acid has not been shown to have the same cardiovascular benefits as DHA or EPA. Currently there are many products on the market which claim to contain health promoting ω-3, but contain only α-linolenic acid (ALA), not EPA or DHA. These products contain mainly higher plant oils and must be converted by the body to create DHA and therefore considered less efficient. DHA and EPA are made by microalgae that live in seawater. These are then consumed by fish and accumulate to high levels in their internal organs. In fish, DHA can be produced directly from microalgae as a vegetarian source. People with certain circulatory problems, such as varicose veins, benefit from such supplements containing EPA and DHA which stimulate blood circulation, increase the breakdown of fibrin, a compound involved in clot and scar formation, and additionally have been shown to reduce blood pressure (Morris, et al., 1993; Mori, et al., 1993). There is strong scientific evidence that ω-3 FAs reduce bloodtriglyceride levels (Sanders, et al., 1997; Davidson et al., 2007) and regular intake reduces the risk of secondary and primary heart attack (Bucher et al., 2002; Willett, et al., 1993; Stone, 1996). Some benefits have been reported in conditions such as RA (Fortin et al., 1995), and cardiac arrhythmias (Pignier, et al., 2007). There is preliminary evidence that ω-3 FAs supplementation might be helpful in cases of depression (Su, et al., 2003; Naliwaiko, et al., 2004) and anxiety (Green, et al., 2006; Yehuda et al., 2005). Studies report highly significant improvement from ω-3 FAs supplementation alone and in conjunction with medication (Nemets, et al., 2002). The study, has found no connection between depression in heart patients and supplements containing ω-3 FAs (Caryn Rabin, 2009). Some research showed that PUFAs intake may reduce the risk of ischemic and thrombotic stroke (Iso, et al., 2001). However, very large amounts may actually increase the risk of hemorrhagic stroke. Lower amounts are not related to this risk (Iso, et al., 2001), 3 grams of total EPA/DHA daily are considered safe with no increased risk of bleeding involved and many studies used substantially higher doses without major side effects (for example: 4.4 grams EPA/2.2 grams DHA in 2003 study) (Su, et al., 2003; Catherine et al., 2006; Hooper et al. 2006).

**Cancer prevention**

Several studies report possible anti-cancer effects of ω-3 FAs (particularly breast, colon and prostate cancer) (Augustsson, Katarina; et al. 2003; De Deckere, E.A. 1999). Ω-3 FAs reduced prostate tumor growth, slowed histopathological progression, and increased survival (Yong Q. Berquin, et al. 2007). Among ω-3 FAs (ω-3), neither long-chain nor short-chain forms were consistently associated with breast cancer risk. High levels of DHA, however, the most abundant ω-3 PUFA (ω-3) in erythrocyte membranes, were associated with a reduced risk of breast
cancer (Pala V, et al. 2001). A trial found that a supplement of EPA helped cancer patients retain muscle mass (Ryan et al., 2009).

**Cardiovascular disease prevention**

The results of major clinical study, patients with myocardial infarction treatment, 1 gram per day of ω-3 FAs reduced the occurrence of cardiovascular death (Marchioli R. 2002). In April 2006, reported review studies into ω-3 FAs, found in abundance in oily fish. It concluded that they do not have a significant protective effect against cardiovascular disease (Trivedi, Bijal. 2006). This meta-analysis was controversial (Wang, et al., 2006; Mozaffarian, D. and Rimm, EB, 2006), that indicated decreases in total mortality and cardiovascular incidents (i.e. myocardial infarctions) associated with the regular consumption of fish and fish oil supplements. Several studies published, Japanese men with unhealthy blood sugar levels were randomly assigned to receive 1800 mg daily of EPA (an ω-3 essential FA from fish oil) with the other half being a control group. The thickness of the carotid arteries and certain measures of blood flow were measured before and after supplementation. This went on for approximately two years. A total of 60 patients (30 in the E-EPA group and 30 in the control group) completed the study. Those given the EPA had a statistically significant decrease in the thickness of the carotid arteries along with improvement in blood flow. The authors indicated that this was the first demonstration that administration of purified EPA improves the thickness of carotid arteries along with improving blood flow in patients with unhealthy blood sugar levels (Mita, et al., 2007). In another study published in 2007, patients with high triglycerides and poor coronary artery health were given 4 grams a day of a combination of EPA and DHA along with some monounsaturated FAs. Those patients with very unhealthy triglyceride levels (above 500 mg/dl) reduced their triglycerides on average 45% and their VLDL cholesterol by more than 50%. VLDL is a bad type of cholesterol and elevated triglycerides can also be deleterious for cardiovascular health (McKenney, JM, Sica, D 2007). Another study on the benefits of EPA was published in 2007. This study involved over 18,000 patients with unhealthy cholesterol levels. The patients were randomly assigned to receive either 1,800 mg a day of E-EPA with a statin drug or a statin drug alone. The trial went on for a total of five years. It was found at the end of the study those patients in the E-EPA group had superior cardiovascular function. Non-fatal coronary events were also significantly reduced in the E-EPA group. The authors concluded that EPA is a promising treatment for prevention of major coronary events, especially non-fatal coronary events (Yokoyama, et al., 2007). Similar to those who consume high amounts of ω-3 FAs from fatty fish - also tend to have higher proportions of ω-3, increased HDL cholesterol and decreased triglycerides (fatty material in the blood) and less heart disease. Eating walnuts (the ratio of ω-3 to ω-6 is circa 1:4 respectively was reported to lower total cholesterol by 4% relative to controls when people also ate 27% less cholesterol (Zambón, et al., 2000). A study showed serum levels of EPA is inversely related to the levels of anti-oxidized-LDL antibodies. Oxidative modification of LDL is thought to play an important role in the development of atherosclerosis (Garrido-Sánchez, et al., 2008).

**Immune function**

Another study regarding fish oil, Sixty four healthy Danish infants from nine to twelve months of age received either cow’s milk or infant formula alone or with fish oil. It was found that those infants supplemented with fish oil had improvement in immune function maturation with no apparent reduction in immune activation (Damsgaard, et al., 2007).

**Brain health**

The study on ω-3 FAs, a group of mice were genetically modified to develop accumulation of amyloid and tau proteins in the brain similar to that seen in people with poor memory. The mice were divided into four groups with one group receiving a typical American diet (with high ratio of ω-6 to ω-3 FAs being 10 to 1). The other three groups were given food with a balanced 1 to 1 ω-6 to ω-3 ratio and two additional groups supplemented with DHA plus long chain ω-6 FAs. After three months of feeding, all the DHA supplemented groups were noted to have a lower accumulation of β-amyloid and tau protein. Some research suggests that these abnormal proteins may contribute to the development of memory loss in later years (Green, et al., 2007). There is also a study published regarding ω-3 supplementation in children with learning and behavioral problems. For the first fifteen weeks of this study, the children were given polyunsaturated FAs (ω-3 and ω-6, 3000 mg a day), PUFAs plus multi-vitamins and minerals or placebo. After fifteen weeks, all groups crossed over to the PUFAs plus vitamins and mineral supplement. Parents were asked to rate their children’s condition after fifteen and thirty weeks. After thirty weeks, parental ratings of behavior improved significantly in nine out of fourteen scales. The study is the largest PUFA trial to date with children falling in the poor learning and focus range. The results support those of other studies that have found improvement in poor developmental health with essential FAs supplementation (Sinn, and Janet 2007; Lee, et al., 2007). A study (Bousquet, et al., 2008) examining whether ω-3 exerts neuroprotective action in Parkinson’s disease found that it did, using an experimental model, exhibit a protective effect (much like it did for Alzheimer’s disease as well). The scientists exposed mice to either a control or a high ω-3 diet from two to twelve months of age and then treated them with a neurotoxin commonly used as an experimental model for Parkinson’s. The scientists found that high doses of ω-3 given to the experimental group completely prevented the neurotoxin-induced decrease of
dopamine that ordinarily occurs. Since Parkinson’s is a disease caused by disruption of the dopamine system, this protective effect exhibited could show promise for future research in the prevention of Parkinson’s disease. However, fish oil has been shown to have no effect on cognitive performance in older individuals without dementia (van de Rest, et al., 2008).

Psychiatric disorders

ω-3 FAs are thought by some to have membrane-enhancing capabilities in brain cells. One medical explanation is that ω-3 FAs play a role in the fortification of the myelin sheaths. Not coincidentally, ω-3 FAs comprise approximately eight percent of the average human brain according to Dr. David Horrobin, a pioneer in FA research. Another major researcher in studying essential FAs, who gave ω-3 its name, surmised how ω-3 components are analogous to the human brain by stating that “DHA is structure, EPA is function.” A benefit of ω-3 FAs is helping the brain to repair damage by promoting neuronal growth (Trivedi, Bijal 2006). In a six-month study involving people with schizophrenia and Huntington's disease who were treated with E-EPA or a placebo, the placebo group had clearly lost cerebral tissue, while the patients given the supplements had a significant increase of grey and white matter (Nemets, et al., 2006). In the prefrontal cortex (PFC) of the brain, low brain ω-3 FAs are thought to lower the dopaminergic neurotransmission in this brain area, possibly contributing to the negative and neurocognitive symptoms in schizophrenia. This reduction in dopamine system function in the PFC may lead to an overactivity in dopaminergic function in the limbic system of the brain which is suppressively controlled by the PFC dopamine system, causing the positive symptoms of schizophrenia. This is called the ω-3 PUFA/dopamine hypothesis of schizophrenia (Ohara, 2007). This mechanism may explain why ω-3 supplementation shows effects against both positive, negative and neurocognitive symptoms in schizophrenia. Consequently, the past decade of ω-3 FA research has procured some Western interest in ω-3 FAs as being a legitimate ‘brain food.’ Still, recent claims that one’s intelligence quotient, psychological tests measuring certain cognitive skills, including numerical and verbal reasoning skills, are increased on account of ω-3 FAs consumed by pregnant mothers remain unreliable and controversial. An even more significant focus of research, however, lies in the role of ω-3 FAs as a non-prescription treatment for certain psychiatric and mental diagnoses and has become a topic of much research and speculation.

In 1998, a small double-blindplacebo-controlled study in thirty patients diagnosed with bipolar disorder. The study showed that subjects in the ω-3 group were less likely to experience a relapse of symptoms in the study. Moreover, the ω-3 group experienced significantly more recovery than the placebo group. However, the study notes that the improvement in the ω-3 group was too small to be clinically significant. Several epidemiological studies suggest covariation between seafood consumption and rates of mood disorders. Biological marker studies indicate deficits in ω-3 FAs in people with depressive disorders, while several treatment studies indicate therapeutic benefits from ω-3 supplementation. A similar contribution of ω-3 FAs to coronary artery disease may explain the well-described links between coronary artery disease and depression. Deficits in ω-3 FAs have been identified as a contributing factor to mood disorders and offer a potential rational treatment approach.” In 2004, a study found that 100 suicide attempt patients on average had significantly lower levels of EPA in their blood as compared to controls (Freeman, et al., 2006; Lin, and Kuan-Pin 2007; Mischoulon, et al., 2009). The preponderance of epidemiologic and tissue compositional studies supports a protective effect of ω-3 EFA intake, particularly EPA and DHA, in mood disorders. Meta-analyses of randomized controlled trials demonstrate a statistically significant benefit in unipolar and bipolar depression. The results were highly heterogeneous, indicating that it is important to examine the characteristics of each individual study to note the differences in design and execution. There is less evidence of benefit in schizophrenia. EPA and DHA appear to have negligible risks and some potential benefit in major depressive disorder and bipolar disorder, but results remain inconclusive in most areas of interest in psychiatry. Health benefits of ω-3 EFA may be especially important in patients with psychiatric disorders, due to high prevalence rates of smoking and obesity and the metabolic side effects of some psychotropic medications.” Another published report in 2007, based on clinical trials, found that ω-3 PUFAs significantly improved depression in patients with both unipolar and bipolar disorder. A small trial, suggests that E-EPA, has an advantage over placebo in major depressive disorder (Food and Nutrition Board, 2005).

ω-3 and ω-6 FAs

There are two types of PUFAs that must be obtained through the diet because they can not be made by the human body. ω-3 FAs may be important in preventing many health problems, including heart disease, RA, cancer and improving mood and memory enhancer.

The ω-6 to ω-3 ratio

The biological effects of the ω-3 are largely mediated by their interactions with the ω-6 FAs.The ratio is the ratio of grams ω-6 FAs to grams ω-3 FAs in a food. The ratio is helpful to use when choosing foods because it is important not to eat too many more ω-6 FAs than ω-3 FAs. The current recommendation is 4 grams or less of ω-6 FA for every 1 gram of ω-3 FA. Choose foods whose ω-6/ω-3 ration is less than 4. The Clinical studies indicate that the ratio of ω-6 to ω-3 (especially Linoleic vs α-Linolenic) FAs is important to maintaining cardiovascular health. Both ω-3 and ω-6 FAs are essential, i.e. humans must consume them in the diet. ω-3 and ω-6 compete for the same metabolic enzymes,
Thus the ω-6:ω-3 ratio will significantly influence the ratio of the ensuing eicosanoids (hormones), (e.g. PGs, Lks, TXs etc.), and will alter the body's metabolic function. Generally, grass-fed animals accumulate more ω-3 than do grain-fed animals which accumulate relatively more ω-6. Metabolites of ω-6 are significantly more inflammatory (esp. AA) than those of ω-3. This necessitates that ω-3 and ω-6 be consumed in a balanced proportion; healthy ratios of ω-6: ω-3 range from 1:1 to 4:1 (Tribole, 2006; Simopoulos, 2003; Simopoulos et al., 2000). Studies suggest that the evolutionary human diet, rich in game animals, seafood and other sources of ω-3, may have provided such a ratio. Here are the ratios of ω-6 to ω-3 FAs in some common oils: canola 2:1, soybean 7:1, olive 3–13:1, sunflower (no ω-3), flax 1:3, cottonseed (almost no ω-3), peanut (no ω-3), grapeseed oil (almost no ω-3) and corn oil 46 to 1 ratio of ω-6 to ω-3 (Goyens, et al. 1 2006).

Conversion efficiency of ALA to EPA and DHA
It has been reported that conversion of ALA to EPA and further to DHA in humans is limited, but varies with individuals. Women have higher ALA conversion efficiency than men, probably due to the lower rate of utilization of dietary ALA for β-oxidation. The absolute amount of ALA, rather than the ratio of ω-3 and ω-6 FAs, which affects the conversion.

Daily values of PUFA in diet
As macronutrients, fats are not assigned recommended daily allowances. The acceptable intake (AI) for ω-3 is 1.6 grams/day for men and 1.1 grams/day for women. A growing body require higher intakes of ALA, EPA, and DHA that may afford some degree of protection against coronary heart disease. The physiological potency of EPA and DHA is much greater than that for ALA. There was insufficient evidence as of 2005 to set a UL (upper tolerable limit) for ω-3 FAs. The FDA recommends that total dietary intake of ω-3 FAs from fish not exceed 3 grams per day, of which no more than 2 grams per day are from nutritional supplements.

Discussion
The ω-3 PUFA as DHA and EPA. These FAs compete with AA and inhibit its synthesis from LA by competing as substrates for COX enzyme as well as for the 2-position on membrane phospholipids. AA is the precursor of 2-series PG and TXA2 and 4-series leukotrienes (LTB4), while the products of EPA are 3-series of these eicosanoids, PGI3 and PGE3 (physiologically inactive) and LTB5, which is less potent than LTB4. Thus the net result is homeostatic balance towards a more vasodilatory state, less platelet aggregation and inflammation. Because of these effects, EPA has been implicated in the low incidence of atherosclerosis among Eskimos, who basically eat fish. The other sources of PUFA are marine mammals and vegetables like soyabean, butternuts and common beans. It has also been suggested that EPA plays a protective role in the progression of chronic renal failure (CRF). However, these findings are controversial since multifactorial mechanisms appear to be involved in its pathogenesis. Besides inflammatory responses, altered PG synthesis, coagulation abnormalities, and alterations in lipid metabolism observed in some models of CRF, the hemodynamic changes, such as increased glomerular pressure and flow are also important for progression of CRF. It has been proven that PUFA can prevent or slow down the decline in renal function in a variety of animal models of renal disease. In various studies, a positive effect of the use of linoleic acid on renal function had been described. However, this was not the case in all animal models studied. A more consistent pattern with positive effects could be found with the use of ω-3 PUFA mixtures, although one study had only reported unfavorable findings. Up to now, studies on the effect of fish oil on renal function in patients with chronic renal insufficiency are relatively rare. In all the studies, glomerular filtration rate increased, there was rise in effective renal plasma flow and a fall in filtration fraction. There was a tendency for proteinuria to fall. These changes suggest an efferent arteriolar vasodilatation. Nevertheless, it should be emphasized that the individual reaction was variable, with sometimes a considerable fall in renal function. The reaction could neither be predicted from either the underlying cause of the chronic renal insufficiency, nor from the initial severity of renal function loss. Long-term studies directed towards the possible preservation of renal function with fish oil have been reported in patients with IgA nephropathy. The results are contradictory. Therefore the verdict regarding the usefulness of fish oil on renal function in patients with chronic renal insufficiency remains open (Reddy et al., 2002). In view of the above considerations the present study was undertaken to evaluate the role of PUFA in the prevention of progression of chronic renal disease, effect on proteinuria and lipid metabolism in patients with chronic renal disease (Bastos & de Oliveira, 2002). In summary, patients on PUFA, the rate of rise of creatinine was slower and there was improvement in proteinuria and serum albumin levels though it did not reach statistical significance. In both the groups there was reduction in serum triglyceride and cholesterol levels on follow up but the reduction in triglyceride level was statistically significant only in non PUFA group. We conclude that the role of PUFA in prevention of progression of chronic renal disease is not conclusive and may need larger controlled studies (Cappelli et al. 1997; Raffaele et al., 1993; Leaf and Weber. 1988; Tsukamoto et al., 1982).

Conclusion
The ‘essential’ FAs were given their name when researchers found that they were essential to normal growth in young children and animals. A small amount of ω-3 in the diet (~1% of total calories) enabled normal growth, and increasing the amount had little to no additional effect on growth. Likewise, researchers found that ω-6 FAs (such as
γ-linolenic acid and AA) play a similar role in normal growth. However, they also found that ω-6 was “better” at supporting dermal integrity, renal function, and parturition. These preliminary findings led researchers to concentrate their studies on ω-6, and it was only in recent decades that ω-3 has become of interest. The ω-6 AA was converted by the body into pro-inflammatory agents called PGs. Eicosanoids: TX, prostacyclins and the LTs. The eicosanoids, which have important biological functions, typically have a short active lifetime in the body, starting with synthesis from FAs and ending with metabolism by enzymes. However, if the rate of synthesis exceeds the rate of metabolism, the excess eicosanoids may have deleterious effects. The ω-3 is also converted into eicosanoids, but at a much slower rate. Eicosanoids made from ω-3 fats are often referred to as anti-inflammatory, but in fact they are just less pro-inflammatory than those made from ω-6 fats. If both ω-3 and ω-6 are present, they will “compete” to be transformed, so the ratio of ω-3: ω-6 directly affects the type of eicosanoids that are produced. This competition was recognized as important when it was found that TX is a factor in the clumping of platelets, which leads to thrombosis. The LTs were similarly found to be important in immune/inflammatory-system response, and therefore relevant to arthritis, lupus, and asthma. These discoveries led to greater interest in finding ways to control the synthesis of ω-6 eicosanoids. The simplest way would be by consuming more ω-3 and fewer ω-6 FAs. The ω-3 FA EPA forms in the body potent antiinflamatory nanomolecules, called resolvins. The ω-3s also turn into other antiinflamatory molecules called maresins and ω-3-oxylipins, which partly explain the versatile health effects of PUFAs.

References

This paper can be downloaded online at http://ijasbt.org & http://nepjol.info/index.php/IJASBT


Food and Nutrition Board, Institute of Medicine of the National Academies (2005), pp.423and 770


GISSI Prevenzione Investigators. (1999) Dietary supplementation with ω-3 polysaturated FAs and vitamin E after...


Griffin BA (2008). "How relevant is the ratio of dietary ω-6 to ω-3 polyunsaturated FAs to cardiovascular disease risk? Evidence from the OPTILIP study". *Curr. Opin. Lipidol*, **19**(1): 57-62. DOI: 10.1097/MOL.0b013e3282f2e2a8


Prickett JD, Robinson DR and Steinberg AD (1981) Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZB x NZW F1 mice. *J. Clin. Invest, 68*: 556-559. DOI: 10.1172/JCI110288


Robinson DR, Prickett JD, Makoul GT, Steinberg AD and Colvin RB (1986) Dietary fish oil reduces progression of established renal disease in (NZB x NZW)F1 mice and delays renal disease in BXSB and MRL/1 strains. Arthritis Rheum. 29: 539–46. DOI: 10.1002/art.1780290412


