Ω-3 Polyunsaturated Fatty Acids and Immune-Mediated Diseases: Inflammatory Bowel Disease and Rheumatoid Arthritis

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Abstract: Inflammation is part of the normal host response to infection and injury. However, inappropriate inflammation contributes to several diseases, including inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). Both conditions are characterized by the excessive production of inflammatory cytokines, arachidonic acid (AA)-derived eicosanoids, and other inflammatory agents (e.g., reactive oxygen species, adhesion molecules). By virtue of their anti-inflammatory action, ω-3 polyunsaturated fatty acids (PUFA) may be beneficial in inflammatory diseases. A large body of evidence supports a protective effect of ω-3 PUFA in experimental animal and ex-vivo models of Crohn's disease (CD), Ulcerative colitis (UC) and Rheumatoid arthritis (RA). Although fish oil supplementation in patients with IBD results in ω-3 PUFA incorporation into gut mucosal tissue and modification of inflammatory mediator profiles, the evidence of clinical benefits of ω-3 PUFA is weak. On the other hand, more convincing data support the efficacy of ω-3 PUFA in reducing pain, number of tender joints, duration of morning stiffness, use of non-steroidal anti-inflammatory drugs and improving physical performance in RA patients. In both IBD and RA further clinical trials with large sample size are needed to clarify the efficacy of ω-3 PUFA as a treatment.

Keywords: Polyunsaturated fatty acids, inflammation, reumathoid arthritis, inflammatory bowel disease, Crohn disease, Ulcerative colitis.

1. INTRODUCTION

Chronic autoimmune diseases, such as inflammatory bowel diseases (IBD) and rheumatoid arthrirtis (RA), are inflammatory-mediated conditions characterized by an uncontrolled inflammatory response causing an excessive damage to the host tissues and resulting in a disease status. Autoimmune diseases affect up to 5-8% of the United States population and are among the most important conditions causing disability and death in Western societies [1, 2].

A common characteristic of IBD and RA is the excessive production of inflammatory mediators, including eicosanoids and cytokines, that are destructive for body's tissues. Eicosanoids are the most important mediators and regulators of the immune responses. They represent the key link between dietary PUFA and autoimmune diseases because they are directly generated from ω-6 or ω-3 PUFA phospholipids of the immune cell membranes whose composition reflects PUFA dietary intake [3].

Both the increasing prevalence of autoimmune diseases and the switch from ω-3 to ω-6 PUFA dietary intake observed in Western countries during the past decades support the hypothesis that PUFA may be an explanatory and modifiable environmental factor in the pathogenesis of autoimmune diseases. Indeed, high ω-6 compared to ω-3 PUFA dietary intake may increase the amount of ω-6 PUFA membrane phospholipids of immune cells and, therefore, protract the inflammatory processes predisposing to or exacerbating inflammatory diseases [4].

A large number of preclinical and clinical studies have been performed on the relationship between PUFA, inflammatory biomarkers and clinical outcomes of patients with autoimmune-mediated disorders [4]. Although a clear association between the shifted balance of ω-6 and ω-3 PUFA intake and the risk of autoimmune diseases is lacking, many investigators recognised the potential of ω-3 PUFA in dampening excessive inflammatory responses in most inflammatory chronic diseases and conditions [3].

This review summarizes the preclinical and clinical scientific evidence concerning the effects of PUFA, either from diet or supplements, on inflammatory biomarkers, clinical symptoms and clinical outcomes of patients with IBD and RA.

2. SELECTION CRITERIA

The studies included in this review were collected by means of a systematic Medline search from 1966 to July 2009, using the following keywords: (IBD OR Crohn's disease OR Ulcerative colitis) AND (ω-3 OR polyunsaturated fatty acids OR PUFA OR DHA OR EPA OR fish oil), as well as (Rheumatoid arthritis) AND (ω-3 OR polyunsaturated fatty acids OR PUFA OR DHA OR EPA OR fish oil). Reference lists from identified articles were also scrutinized for pertinent studies that had not been indexed in

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the Medline electronic database. Inclusion criteria were animal and human studies. The latter included case reports and case series, ecological, cross-sectional, case-control, cohort, randomized controlled studies published in peer-reviewed English journals. Two authors independently reviewed the literature to identify eligible studies and assessed their suitability for inclusion, resolving disagreements in discussion.

3. THE LINK BETWEEN INFLAMMATION AND POLYUNSATURATED FATTY ACIDS

Inflammation is the body response to cellular injury. It occurs to begin the immunologic process of elimination of invading agents, e.g. microbiological, immunological and toxic ones, in order to protect the tissues from further damage. The inflammatory response usually results in increased blood flow, higher permeability across blood capillaries, increased movement of large molecules (e.g. complement, antibodies, and cytokines) and leukocytes from the bloodstream into the surrounding of damaged tissue.

In the early course of inflammatory activation, the up-regulation of adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin on the surface of endothelial cells induces the movement of immune cells into the inflammatory site. Activated monocytes and macrophages migrate into the target area releasing cytokines, nitric oxide compounds, enzymes and other mediators that regulate the whole-body response to injury, while activated leukocytes release a family of lipid inflammatory mediators and regulators, termed eicosanoids, which include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), and other oxidized derivatives.

Eicosanoids are generated from 20-carbon PUFA phospholipids of immune cell membranes and their synthesis depends on the specific cell, stimulus and ω-6/ω-3 PUFA balance in the membrane phospholipids of immune cells. The type and the amount of eicosanoids produced during the inflammatory response, i.e. PGs, TXs, LTs, modulate the intensity and duration of it as well as several cellular functions, including nociception, hemodynamic and blood clotting, renal function, reproductive activity [4,5].

The membrane phospholipids of human immune cells typically contain high proportion of ω-6 arachidonic acid (AA) (20%) and low proportions of both ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (1% and 2.5%, respectively) [6,7]. AA, EPA and DHA are the substrates for the synthesis of eicosanoids, such as PGs, TXs, LTs and hydroxyeicosapentaenoic acid (HEPEs) which are locally produced through pathways involving cycloxygenases (COX-1 and COX-2) and lipoxygenases (LOX) [4]. Since the composition of PUFA phospholipids of immune cell membranes may be influenced by the diet, a high intake of ω-3 PUFA leads to a shift in ω-6/ω-3 PUFA balance which causes the incorporation of more EPA and DHA compared to AA. The consequence is a reduced synthesis of AA-derived eicosanoids, i.e. PGE2, PGD2, TXB4, LTB4 and LTE4, in favour of EPA and DHA-derived eicosanoids, such as LTB5, LTE5 and 5-HEPE, which are less potent compared to the former and contribute to a less pro-inflammatory environment [8,9]. Additionally, the COX- and LOX-mediated metabolism of EPA and DHA may contribute to the activation of the resolution program of inflammatory-mediated disease, which mainly consists in a lipid mediator class switching from pro-inflammatory PGs and LTs to the biosynthesis of anti-inflammatory agents, such as lipoxins, resolvins and protectins [10, 11]. Resolvins and protectins have been demonstrated to be anti-inflammatory, inflammation resolving and immunomodulatory in cell culture and animal feeding studies [11-13].

The findings that AA-derived metabolites may predispose to or exacerbate inflammatory response, while EPA- and DHA-derived compounds may control the pro-inflammatory environment at tissue levels sustain the hypothesis that dietary ω-3 PUFA may be of clinical benefit in patients with autoimmune diseases. Furtheremore, in autoimmune diseases the self-reactive T helper 1 (TH1) and TH17 cells enter into the target tissue, release proinflammatory cytokines and chemokines that promote the recruitment and activation of inflammatory cells, matrix-degrading enzymes and free radicals causing tissue damage. In addition, TH1-mediated production of autoantibodies by B cells, which form immune complexes and activate complement and neutrophils, contribute to the autoimmune pathology and disease propagation [2,13,14]. In this context, ω-3 PUFA may exert beneficial effects via lipid mediator-related and nonlipid mediator-related mechanisms through decreased activation of the pro-inflammatory transcription factor NFkB and perhaps through increased activation of the anti-inflammatory transcription factor PPAR-γ [14].

In animal models of autoimmune diseases, resolvins and protectins promote resolution of the inflammatory process by reducing the recruitment of leukocytes to the inflammatory exudate and the expression of proinflammatory genes, by promoting phagocyte removal of apoptotic neutrophils and microbial products and by enhancing the efflux of the phagocytes from inflamed peritoneum to draining lymph nodes and spleen [15].

4. INFLAMMATORY BOWEL DISEASES

Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are related but distinct entities resulting from the interplay of genetic, environmental, and immunological factors. The main difference between these conditions is the nature and the location of the lesions that are restricted to the epithelial lining of the gut (mucosa) in UC, while affects the whole intestinal wall in CD.

CD is a long-term illness that causes inflammation in the gut. It can affect any part of the digestive system from the mouth to the anus, mainly the ileum and the colon. Symptoms of CD include diarrhoea, abdominal pain, fever, weight loss, and a general feeling of being unwell. CD affects both adults and older persons, while UC is most common in young and middle-aged adults.

UC is mainly localized at colon and rectum. UC is characterised by chronic inflammation and ulceration of the gastrointestinal lining that can lead to anaemia, toxic megacolon (a life-threatening complication of intestinal conditions characterized by a very inflated colon), or colorectal cancer.

The impairment of the barrier function of the gut is the common characteristic of both CD and UC which may be due to changes of bacterial microflora, excessive immune cell-mediated response, infiltration of activated T cells and monocytes/macrophages in the intestinal wall. Indeed, the intestinal mucosa of patients with IBD contains elevated levels of inflammatory eicosanoids, such as LTB4 [16] and cytokines [17]. In particular, the activation of IL-2 and IFN-γ producing Th1 cells in the lamina propria of the CD-affected gut may play a pivotal role in the pathogenesis [18].

The aetiology of CD and UC and the factors that might trigger the autoimmune response are unknown. An early hypothesis attributes the condition to a particular form of Mycobacterium, while more recently the concept of a "leak gut" has been proposed, as intestinal permeability appears to be significantly increased in IBD patients and relatives compared to controls [19].

Some progress has been made in identifying potential genetic and environmental risk factors [20], and among them biological plausibility is coming out for diet-related risk factors [21]. The main hypothesis is that the diet composition, in particular dietary fats, may affect immune responsiveness of the gut-associated lymphoid tissue. Specifically, ω-3 PUFA at high concentrations have a local and systemic suppressive effect on cell-mediated immunity via cytokine release, including TNF-α, changes in the receptor affinity or interactions with intracellular signal transduction [22].

4.1. *In Vitro* and Animal Studies

Several experimental animal models and ex-vivo cellular organ cultures have been used to investigate the relationship between dietary fats and IBD. The main reason for these studies relays on the evidence that animal models of IBD have similar patterns of PUFA membrane composition, eicosanoids and cytokine expression as compared with patients affected from IBD [23,24]. The induction of colitis in animal models results in the appearance of inflammatory eicosanoids, such as PGE2 and LTB4, in the colonic mucosa and similarly to patients with UC have increased amounts of LTB4 and IL-1ß [25,26]. LTB4 has distinct pro-inflammatory actions, i.e. inducing leukocyte infiltration and subsequent releasing of inflammatory mediators [3], and it is highly likely to be playing a pathologic role in animal models of IBD as compared with human disease. Consistently, the role of PGE2 in IBD is less certain, and there is evidence that it may be protective [17, 27].

Several evidence from experimental animal models of IBD and ex-vivo tissues of IBD patients support the benefits of fish oil enriched diet because of the strong anti-inflammatory properties of ω-3 PUFA [3, 27-29].

In 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitis rats, dietary fish oil decreases chemically induced colonic damage and inflammation as well as the progression of chronic inflammatory lesions compared with ω-6 PUFA rich diet [24, 26, 30-32]. Dietary ω-3 PUFA led to a lower mucosal levels of PGE2 and LTB4, a minimum stenosis score, a higher histological recovery, lower colon alkaline phosphatase and gamma-glutamyltranspeptidase activities in TNBS-induced colitis rats [24].

In particular, the decreased colonic damage and inflammation has been attribuited to the high content of n-3 α-linolenic acid (ALA) compared with ω-6 PUFA rich diets [32,33]. Shoda demonstrated a better suppressive effect of ALA on intestinal inflammation and LTB4 than that of EPA and DHA [34,35]. Whether these effects are due to ALA itself or are relate to the conversion of ALA to its longer chain, more unsaturated derivatives, like EPA, is not clear. However, the main point is that the protective effects of dietary fish oil on chemically induced colonic damage and inflammation compared with ω-6 PUFA rich diet were, in all cases, associated with a reduction in production of AA-derived eicosanoids [26,30,31,33].

Compared to wild-type with dextran sulfate sodium (DSS)-induced colitis, the fat-1 mice, which are able to produce ω-3 PUFA from ω-6 PUFA due to the presence of an ω-3 PUFA desaturase gene, had lower body weight loss, colon shortening, colonic damage and inflammation. Additionally, marked changes in the pattern of inflammatory mediators present in colonic tissue of fat-1 mice. i.e. high LTB5, PGE3, resolving E1, resolvin D3 and neuroprotectin DI, and low colonic tissue TNF-a, IL-1ß, and inducible NOS mRNA levels and NFkB activation, were observed as compared with wild-type mice [29]. The benefits of ω-3 PUFA fish oil have been recently confirmed in IL-10 knock-out mice that spontaneously develop colitis. The colonic inflammation of IL-10 knock-out mice feeded with fish oil was lower than that observed in those with ω-6 PUFA rich corn oil [28].

However, the PUFA supplementation had no effect on neutrophil function, neutrophil-mediated ileal inflammation and permeability in animal models of IBD compared with controls [36], and ω-3 PUFA did not protect pigs from DSS-induced IBD, but accelerated clinical remission [37]. Guinea pigs fed with EPA-Ethyl ester for 3 weeks had a significant increase in EPA content and a decrease in the level of AA in peritoneal macrophages with a prophylactic effect on the development of carrageenan-induced colitis [38]. A greater reduction of inflammatory markers, i.e. IL-1ra/Il-beta cytokine ratio, was observed in UC colon biopsies versus CD following their incubation in fish oil enriched solution [39].

According to other authors, dietary ω-3 PUFA supplementation in mouse model of colitis may reduce clinical symptoms of colitis and colonic immunopathology by enhancing epithelial barrier integrity and function [28,40,41]. Based on in-vitro tissue models from CD patients, ω-3 PUFA supplementation effectively reduces the distortion of tight junction (TJ) barrier morphology by preventing the redistribution of TJs proteins (occludin and ZO-1), and reducing the transepithelial electrical resistance induced by IFN-γ and TNF-α [42]. Beneficial effects of DHA and EPA have been confirmed in animal models of colitis where ω-3 PUFA may positively affect the barrier defects by maintaining the affiliation of TJ proteins with the membrane microdomains [42]. However, to date, there is no information on whether altered expression of TJ proteins in membrane microdomains in UC patients is affected by ω-3 PUFA, and the molecular mechanism underlying this beneficial effect remains to be elucidated.

4.2. Ecological Studies

The prevalence of IBD is highest in North America, Northern Europe, and the United Kingdom, with averages ranging from 100 to 200 cases per 100.000 [43]. Since the mid-1900s, the incidence of IBD has been observed to be rapidly increasing in Northern European and North American populations [43,44] with a significant geographic variations from North to South and, to a lesser degree, from West to East countries. The prevalence of IBD is much lower in Asian countries, including Japan, than in Western countries [45]. Although the replacement of traditional diet with Western type diet may be related with the rapidly icrease of IBD in Japanese, the prevalence is still much lower in Asian countries than in Western ones [45].

IBD seems also to have a characteristic racial-ethnic distribution [46-48]. IBD is more common in European Americans compared with African Americans, and the lowest rates of IBD have been reported in Hispanics and Asians [49,50]. Within European ancestry populations, the rates of IBD are higher in persons of Jewish ancestry than other ethnic groups [51,52], and higher in Ashkenazi (Central and Eastern European) Jews than in Sephardic (Middle Eastern and Spanish ancestry) Jews [51,52]. Older studies have suggested that IBD occurs more frequently in populations of high socioeconomic status [53,54].

Several epidemiological studies suggest an association of dietary fat intake with the increasing prevalence of IBD in Western as well as in Eastern countries [55-57].

Historically, Kromann attributed the paucity of IBD in the Greenland populations to their marine diet rich in ω-3 PUFA [58]. The gradual replacement of a traditional diet high in fish-based ω-3 PUFA with a Western type diet, characterized by increasing ω-6 PUFA dietary consumption, has been linked with the increasing incidence of autoimmune disorders, including CD, in a relatively homogeneous sample of the Japanese population followed-up from 1966 to 1985 [55]. Therefore, it has been proposed that a diet rich in fish oil protected the Japanese from IBD and the dietary shift from ω-3 to ω-6 fat intake is related with the increasing rate of IBD [35].

In Japanese multicenter hospital-based case-control study, which included 111 UC patients and 128 CD patients, a lower intake of both ω-3 and ω-6 PUFA was positively associated with CD risk [59]. Compared to controls, 29 UC Japanese patients had lower intake of ω-6 PUFA with reduced plasma levels of linoleic acid (LA) and AA, but significantly higher LA and AA acid in neutrophil phospholipids [59].

Among 260,686 men and women aged 20-80 years, participating in a large European prospective cohort study (EPIC), no dietary association was detected in 139 incident case of UC, apart from a marginally significant positive association with an increasing percentage intake of energy from total PUFA [60].

Possible reasons for the inconsistencies among the studies depend from the fact that many reports are retrospective case-control studies which have difficulties in measuring pre-illness diet, recall bias, relative low incidence of IBD cases with inappropriate selection of control populations.

4.3. Plasma Levels and Phospholipid Fatty Acid Membrane Composition

Dietary ω-3 PUFA may play an important role in the pathogenesis of IBD by determining changes in the pattern of PUFA cellular membrane phospholipid composition [61] as well as PUFA plasma concentrations [62,63].

Several case-control studies investigated the total plasma lipid composition, which is a less reliable indicator of essential fatty acid status, the fatty acid composition of individual plasma lipid classes, and the phospholipids membrane composition of erythrocyte and immune cells.

As far as adult patients are concerned, Esteve-Comas reported elevated plasma levels of ALA and DHA with decreased levels of diomo-γ-linolenic acid in active IBD patients compared to controls [62]. Later on, ω-3 PUFA levels were found significantly increased in patients with active UC and CD compared with controls, together with increased ω-6 PUFA levels in patients with inactive UC [63]. Siguel and Lerman found higher levels of saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA), but lower values of ω-3 PUFA and ω-6 PUFA in patients with various chronic intestinal disorders compared with control subjects [64]. Kuroki confirmed low serum levels of total ω-3 PUFA and total PUFA, but found a significantly increased ratio of ω-6 to ω-3 PUFA in patients with CD when compared to controls, and a significant correlation of ω-3 PUFA and ω-6: ω-3 ratio with the CD activity index [65].

In addition, lower levels of ω-3 PUFA in the plasma phospholipids and in the adipose tissue were found in patients with both active and inactive CD as compared with healthy controls, suggesting a tight relationship between fatty acid profile, disease activity and serum antioxidant concentrations [66].

Similarly, UC and CD patients had significantly lower lipid intake with no significant differences in the levels of single ω-3 PUFA, but high levels of ω-6 LA and lower ω-6: ω-3 PUFA ratio were found in the erythrocyte membrane phospholipids and in the peripheral blood mononuclear cells (PBMC) of UC and CD patients compared with controls [67,68].

Patients with inactive UC as compared with patients with inactive CD had significantly higher plasma phospholipids with EPA, DHA and gamma-linolenic acid (GLA) compared with controls, but values of the principal ω-3 and ω-6 PUFA, AA and DHA were significantly higher in patients with UC but not in patients with CD, thus not supporting the concept of EPA or DHA deficiency in patients with either UC or CD [69].

Additionally, compared with 45 age- and BMI-matched healthy controls, patients with quiescent IBD showed similar levels of vitamin E and PUFA, but decreased plasma levels of carotenoids and vitamin C, increased levels of saturated FA and monounsaturated FA. No difference was found between patients with active disease and those with inactive disease compared with controls, suggesting that the FA

profile and antioxidant status are disturbed independently from disease activity and nutritional status [70].

As far as young patients are concerned, Levy found significantly lower levels of ω-6 LA in children with CD compared with healthy controls [71]. Trebble showed lower LA and ALA levels in children with active than in those with inactive CD, but the values of patients were not compared with those of healthy children [72]. To complicate the matter, Socha reported significantly lower levels of LA and AA in children with IBD compared with healthy controls, while values of ALA were higher in patients than in controls [73].

Taken as a whole, the findings concerning the effects of PUFA in adults and children affected by IBD are contradictory. Concerning the pathogenetic pathways, recent evidence suggests that ω-3 PUFA intake may positively affect the permeability of TJs of intestinal epithelial cells [74] and decrease the oxidative stress in patients with UC [75], competing with ω-6 PUFA and inhibiting the production of AA metabolites, such as leukotriene B4 and prostaglandin E2 [27].

4.4. The Effects of PUFA on Inflammatory Mediators and Tissue Damage

In two pilot clinical trials, UC patients receiving fish oil had reduced serum and *in vitro* neutrophil chemotactic activity and neutrophil LTB4 levels compared with controls [76, 77]. Then, Hillier demonstrated a readily incorporation of EPA and DHA in IBD affected bowel mucosa (7 fold and 1.5 fold increase, respectively), in patients with active UC or CD, who underwent mucosal tissue biopsy during colonoscopy before and after three weeks of orally and duodenal ω-3 PUFA supplementation. As expected, AA values fell throughout the study with significant reduction at 12 weeks, while mucosal PGE2, TXB2, and 6-keto prostaglandin F1 alpha synthesis were suppressed, and this reached significance at 3 and 12 weeks for PGE2 and at 12 weeks for TXB2 [78].

In UC patients participating in a double-blind crossover comparison with placebo, fish oil supplementation reduced the rectal dialysates content of LTx, improved the histologic lesions, even though there was no sigmoidoscopic improvement and also the clinical benefit was modest leading to a small reduction of steroid dose [79]. However, dietary fish oil supplementation resulted in reduced rectal LTB4 concentration and clinical improvement in active UC patients enrolled in a double-blind, placebo-controlled crossover study [80]. Similarly, the administration of intravenous EPA for 2 weeks increased the amount of LTB5 produced by polymorphonuclear leucocytes and the LTB5/LTB4 ratio in patients with CD [81].

A significant reduction in serum IL-2 and soluble IL-2 receptor levels has been reported in two placebo-controlled studies using EPA+ DHA supplementation (5.6 g /day) in patients with IBD, with the earliest response after 26 weeks in patients with UC. This change was accompanied by a significant reduction in serum LTB4 concentration, NK cell activity and sigmoidoscopic and histological scores, as well as decreased disease activity [82].

More recently, Trebble has shown that 2.7 g EPA+ DHA/day for 24 weeks reduces the ex-vivo production of IFN-γ and PGE2, but not TNF-α, by stimulated mononuclear cells from patients with CD [68]. Wellen found a lower production of key inflammatory mediators, PGE2 and IFN-γ by blood mononuclear cells in CD patients after dietary fish oil supplementation [83]. In patients with active CD, Nielsen showed that 3 g/day ω-3 PUFA as adjuvant therapy to corticosteroids reduced IL-1ß and IL-4 concentration compared to 7.8 g/day ω-6 fatty acids, while no significant changes were found in the concentrations of several other cytokines [84].

Finally, among 9 CD and 10 UC patients with IBD-related joint pain, those who self-administered seal oil through a nasoduodenal feeding tube 3 times daily for 10 days obtained a normalization of the ω-6: ω-3 fatty acids and AA: EPA ratios in blood and rectal mucosa with improved health related quality of life, as they experienced reduced duration of morning stiffness, number of tender joints intensity of pain and the doctor's scoring of rheumatic disease activity [85].

4.5. Randomized Controlled Clinical Studies

Initial evidence about the potential benefits of fish oil supplementation in human IBD comes from uncontrolled trials: in 6 active UC patients, supplementation with 3-4g/day EPA for 12 weeks resulted in improvement of symptoms, hystology and LTB4 production [86]; in 10 UC refractory to medication, 2.7 g/day EPA and 1.8 g/day DHA for 8 weeks improved the disease activity [87]; a diet rich in fish oil for two years promoted the maintenance of more prolonged remission in CD patients [88].

Controversial results emerged from a study on 29 CD and 10 UC patients enrolled in a 7 months placebo-controlled cross-over trial with 1 month wash-out where supplementation with 1.8 g of EPA daily improved the disease activity score in UC patients, but not in CD patients [89]. Consistently, Lorenz-Meyer did not find any difference in the relapse rate or in the length of remission over one year among 204 CD patients randomized to receive gelatine ω-3 PUFA capsules (3.3g/day of EPA and 1.8 gr/day of DHA) (n = 70), placebo (n = 65), or carbohydrate-reduced diet (84 g/day) (n = 69) [90].

In a one-year, double-blind, placebo-controlled study Belluzzi showed a reduced rate of relapse in 78 patients with CD in remission after supplementation with an enterically coated timed release fish oil preparation for 1 year (2.7 g of ω-3 fatty acids with special coating protecting the capsules against gastric acidity for at least 30 minutes). There was a significant difference in the proportion of patients who relapsed over 12 months (28% in the fish oil group versus 69% in the placebo group) and in the proportion of patients who remained in remission at 12 months (59% in the fish oil group versus 26% in the placebo group) [91]. Of note, the patients included in the study had been in clinical remission for less than 24 months before the study, and had a high risk of relapse based on laboratory evidence of inflammation.

Consistently, a benefit of ω-3 PUFA therapy [1.8g/day of EPA and 0.9 g/day of DHA] for maintenance of remission

was observed in patients with CD underwent ileal resection compared to controls during one year of follow-up [92]. Less patients from the intervention group had endoscopic/ radiographic and clinical relapse compared to the placebo group suggesting that enteric coated, timed release fish oil is effective for maintenance of remission after ileal resection in CD. In 62 patients with active CD randomised to three groups (a. a polymeric diet containing 35 g of lipids per 1000 kcal with high in oleate (79%) and low in linoleate (6.5%), b. an identical enteral diet except for the type of fat which was high in linoleate (45%) and low in oleate (28%), c. oral prednisone (1 mg/kg/day)) for 4 weeks, the rates of remission were 27%, 63% and 79%, respectively [93].

In 77 CD patients with evidence of raised laboratory markers of inflammation, high-dose fish oil (2.7 g EPA + DHA/day) plus antioxidants or placebo for 24 weeks had no significant effects on nutritional status, clinical and biochemical markers of disease activity, while treatment was associated with a lower production of IFN-γ and PGE2 by PBMC [94]. A reduced relapse rate was found in a paediatric group (treated with time dependent 5-ASA (50 mg/kg/d)+ω-3 PUFA in gastro-resistant capsules with 1.2 g/day of EPA and 0.6 g/day of DHA, as triglycerides) compared with a control group assuming time dependent 5-ASA (50 mg/kg/ d)+olive oil [95].

In two randomized, double-blind multicentric studies (Epanova Program in Crohn's Study 1 (EPIC-1) and EPIC-2) including 760 patients with CD Activity Index score of less than 150, there was no significant difference in the rate of relapse at 1 year in patients who received ω-3 PUFA or placebo without any other treatment allowed [96].

In 38 patients with IBD-related joint pain (21 CD and 17 UC), Brunborg found no significant difference between the two intervention groups (short-term oral administration of seal oil and cod liver oil) or between CD and UC patients concerning improvement in IBD activity, plasma LTB4 concentration, serum ω-6: ω-3 PUFA, and AA: EPA ratios and joint pain parameters [97].

Hawthorne found that fish oil supplementation produced a modest corticosteroid sparing effect in patients with active UC, but no benefit in the maintenance therapy. A similar rate of relapse was found among 96 adults in remission or recovery from relapsed UC (different stage of activity), treated with 5-ASA and corticosteroids and randomized to receive supplementation with triglyceride concentrate of ω-3 PUFA (5g/day of EPA and 1.2 g/day of DHA) or with olive oil for 1-year. UC patients randomized to fish oil experienced a measurable, but only limited, clinical benefit: in particular, patients on fish oil who entered the trial being in relapse had a significant reduction in corticosteroid requirement in CD patients; patients on fish oil who were in remission at the trial entry or during the trial had no significant difference in the rate of relapse [98]. In a multicenter, randomized, double-blind, placebo-controlled, crossover trail with 4-month treatment periods separated by a 1-month washout conducted in 24 patients with active UC, dietary fish oil supplementation (18 capsules daily with EPA3.24 g and DHA 2.16 g) resulted in a decrease in rectal dialysate levels of LTB4, improvement in acute and total histologic indexes as well as significant weight gain. No changes occurred in any variable during the placebo period. In addition, during the fish oil supplementation period, the mean prednisone dose decreased from 12.9 mg/day to 6.1 mg/day while it rose from 10.4 mg/dayto 12.9 mg/day during the placebo diet period [79]. On the contrary, among a small sample of patients with UC participating in double-blind, placebo-controlled, crossover trial, fish oil dietary supplementation (4.2 g/day of fish oil) resulted in clinical improvement and anti-inflammatory drugs reduction [80]. Patients with UC in remission or in low activity phase who were enrolled in a 2-year, double blind placebo controlled trial and randomized to 6 daily capsules containing 5.1 g/day of total ω-3 PUFA or placebo, experienced a similar relapse rate over a 2 year period [99]. Additionally, no benefit of 3.2g/day EPA and 2.1 g/day DHA versus olive oil was found for 1-year maintenance of remission in 40 adults with UC receiving 3.6 g/day mesalazine in three divided doses [100].

In 10 patients with mild to moderate UC randomized to sulphasalazine (2 g/day) or fish oil (3.2 g EPA + 2.16 g DHA/day) for 2 months in a crossover design, the former caused a lower disease activity (as detected by a significant decrease in platelet count, erythrocyte sedimentation rate, C-reactive protein, and total fecal nitrogen excretion). Neither treatment changed colonic histologic score from study entry; however, fish oil, but not sulphasalazine, significantly decreased the sigmoidoscopic score [101]. In a small case-control study performed in patients with UC, treated with sulfasalazine plus fish oil ω-3 PUFA or placebo for 2 months separated by 2 months, (when they only received sulfasalazin), had overall lower levels of oxidative stress markers, but no improvement in most laboratory indicators, sigmoidoscopic and histologic scores [75].

Based on evidence of potential anti-inflammatory proprieties, a supplement with ω-6 GLA (1.62 g/day) was added to EPA (0.27 g/day) and DHA (0.045 g/day) and administered to UC patients for 12 months. However, no difference in relapse rate or sigmoidoscopic score was observed in UC patients randomized to placebo or a preparation including GLA [102].

In addition, several reviews assessed the effects of ω-3 PUFA on clinical, sigmoidoscopic or histologic scores, rates of remission or relapse, or requirements for steroids and other immunosuppressive agents in both CD or UC patients. The authors concluded ummarized that ω-3 PUFA do not show any statistically significant beneficial effects on clinical, endoscopic or histologic scores, remission or relapse rates in patients with IBD. A marginal benefit of ω-3 therapy is supported for IBD patients: few studies have shown a significant improvement in clinical activity and a steroid-sparing effect, while others have shown a trend towards improvement. Although ω-3 PUFA are safe, especially as enteric coated capsules, the existing data do not support routine treatment of IBD patients [34, 103-109].

4.6. Conclusions on the Effects of PUFA on IBD

Although some studies showed a beneficial effect of fish derivatives PUFA on chronic inflammatory diseases, the available evidence concerning the use of ω-3 PUFA in the treatment of UC and CD are conflicting and insufficient to draw conclusions about the effectiveness of ω-3 PUFA alone

or in combination with standard treatment for IBD [34, 103-109].

A number of causes have been cited to explain the conflicting results from the trials, but the main reason is that the studies were both clinically and statistically heterogeneous. The main discrepancies among the study findings could reside in patients' heterogeneity , in terms of disease activity and treatments, as well as different study designs. In particular, the use of various formulations and dosages of ω-3 PUFA as well as of placebo, and the different compliance of patients to treatment or placebo hampered a direct comparison of trials. For instance, increased compliance was associated with new formulations of fish oils that have less unpleasant side effects, such as diarrhea and symptoms of the upper gastrointestinal tract, and therefore offer a potentially useful therapeutic modality for the management of IBD. Larger and better designed studies are necessary in order to make a definite recommendation.

5. RHEUMATOID ARTHRITIS

Rheumatoid arthritis [RA] is a chronic inflammatory disease characterised by joint inflammation. In the early stage, RA manifests with swelling, pain, functional impairment, morning stiffness, osteoporosis and muscle wasting, while in the late stage presents with destruction of bone and cartilage resulting in joint deformity and immobility. Joints are affected symmetrically and small joints are involved earlier than large, i.e. axial, joints. RA is also associated with the presence of rheumatoid factor [RF], typical RA erosions on radiological examination of hands and feet, systemic disturbance, i.e. fatigue, stiffness, anaemia and weight loss, and extra-articular features, i.e. rheumatoid nodules.

The aetiology of RA is unknown, but genetic factors are known to be involved and the locus has been identified. Whether genetic factors play a role in the susceptibility to RA rather than in the severity of RA remains controversial. It has been suggested that genetic susceptibility explains about 40% of the risk of developing RA [110]. Environmental and non-genetic constitutional factors may interact with genetic ones, resulting in additive effects and accounting for the majoeity of risks.

Putative agents have been proposed, the most likely being an infectious agents or physical damage which may trigger a persistent immune response that causes irreversible damage to tendons and joints by sustaining the infiltration of activated macrophages, T lymphocytes and plasma cells into the synovium and the proliferation of synovial cells. There is still little understanding as to why inflammation persists in such a condition [111], as well as to why high intake of fish, olive oil, and cooked vegetables confer a protective effect against the development of RA [112]. The section below aims to summarize the current evidence and the biological plausibility of the potential antiflammatory and therapeutic effects of ω-3 PUFA fatty acids in RA.

5.1. *In Vitro* and Animal Studies

The impact of dietary fatty acids on RA has been investigated in a number of in-vitro and animal studies. Inbred strains of mice developing spontaneous autoimmune disease have been originally used as models to study the effects of dietary intervention. In mice that are strongly predisposed genetically to systemic inflammatory diseases, a high ω-3 PUFA diet has been shown to have a marked preventive effect, but a weaker therapeutic effect on established diseases [113]. Mice fed with fish oil delay experienced a delay onset (mean 34 day versus 25 day), a reduced incidence (69% versus 93%) and severity (mean peak severity score 6.7 versus 9.8) of type II collagen-induced arthritis compared with those fed with vegetable oil [114]. However, not all animal studies of dietary ω-3 PUFA supplementation demonstrated beneficial effects: type II collagen-induced arthritis worsened in animals fed fish oil compared with those fed beef tallow [115].

The effects of ω-3 PUFA from fish oil on antigen presentation, T-cell reactivity and inflammatory lipid and peptide mediator production suggest that in animal models these fatty acids might have a role in decreasing the risk of development and the disease severity of RA. Most of the studies investigated purified EPA and DHA, but those investigating the effects of a mixture of EPA and DHA, the 2 major ω-3 PUFA constituents of fish oil, found the mixture more effective than either fatty acid [116]. In another study both EPA and DHA were found to suppress streptococcal cell wall-induced arthritis in rats, with EPA being more effective [117].

If EPA has been shown to inhibit the proliferation of human synovial lymphocytes [118], the most widely reported effects of EPA and DHA are the inhibition of T lymphocytes proliferation and IL-2 production [119-121]. However, EPA and DHA also inhibit the cytokine-induced upregulation of adhesion molecules on the surface of cultured endothelial cells, decrease binding of leucocytes to endothelial cells [122], and reduce the production of IL-1β and TNF-α by human monocytes and of IL-6 by rat macrophages [112].

Human neutrophils, human monocytes and mouse macrophages cultured with EPA or DHA present reduced synthesis of superoxide, low cytokine-induced cell-surface expression of HLA-DR and HLA-DP complexes and low cytokine-induced cell-surface expression of major histocompatibility complex II, respectively [122]. In accordance, human monocytes cultured with EPA or DHA have reduced ability to present antigen to autologous lymphocytes [123]. Additionally, bovine chondrocytes cultured with ALA showed a marked decrease in the cytokine-mediated induction of the COX-2 expression, TNF-α and IL-1α genes [127]. Of note, ALA (but not palmitic, oleic or linoleic acids), inhibited the cytokine-mediated upregulation of aggrecanase activity and aggrecanase gene expression [124]. Aggrecanases degrade cartilage proteoglycan and their expression in cartilage is upregulated in response to the pro-inflammatory cytokines TNF-α and IL-1α. EPA and DHA exhibited the same effects as ALA [124]. However, it is not clear whether the reported actions of ω-3 PUFA are due to direct effects of the fatty acids themselves on COX-2, cytokine and aggrecanase gene expression, or whether they are eicosanoid-mediated effects. Whatever the mechanism, it appears that ω-3 PUFA can act within cells to decrease

actions that lead to joint chronic inflammation and destruction.

5.2. Ecological Studies

Ecological studies conducted in Greenland Eskimos suggested potential anti-inflammatory effects of essential fatty acids based on the low prevalence of RA associated with high intake of ω-3 PUFA from seafood [58]. In the Japanese population, an inverse relationship has been confirmed between high diet fish consumption and low incidence of RA: people with consumption of a diet rich in fish have a relatively high frequency of HLA-DRw15 (which bears the RA susceptibility pattern), but paradoxically displays a low prevalence of RA [125]. Some authors argued that the protective effect of dietary ω-3 PUFA looks even more likely in Japanese population when it is considered the high prevalence of the genetic polymorphism that confers to this population high susceptibility to RA [125,126].

The relationship between the intake of plant and marine oils, in particular olive oil consumption, and the risk of developing RA has been investigated in three case-control studies in Greece. Two hospital-based case-control studies carried out by the same group have demonstrated a strong negative association between greater olive oil consumption and the risk of RA [127,128]. The first study by Linos (1991) found that persons belonging to the top quintile of olive oil consumption versus those of the bottom one had statistically significant reduced risk of developing RA [127]. However, only four cases and eight controls consumed olive oil at the highest level of intake. A weak protective effect was also observed when fish consumption was evaluated as an independent risk factor. An interesting finding was the protective effect of adhering to the dietary restrictions of Greek Orthodox Lent, which emulate a typical Mediterranean diet, high in fruit, vegetables, cereals, olive oil and fish [129].

The second study by Linos (1999) confirmed that higher intakes of olive oil reduce the risk of developing RA, (highest quartile of intake v. lowest quartile of intake), but greater fish consumption was no longer protective [128]. In the third study, past fish consumption in 'usual' diet during the 5 years before the onset of symptoms was measured in 324 incident RA cases and 1,243 controls, all women, using self administered semi-quantitative food-frequency questionnaire. A reduced risk of RA was found in women consuming more than two servings of broiled or baked fish per week versus those with less than one serving, and the association was stronger in sieropositive RA cases [130]. Unlike the results from the Greek studies, no association was found with olive oil intake. In conclusion, a clear protective effect is not supported from these studies [127,128,130].

5.3. The Relation Between PUFA Intake and Inflammatory Markers

Based on the preclinical evidence of an anti-inflammatory action of PUFA, i.e. reduced synthesis of cytokines, eicosanoids and other inflammatory stimulating factors, on the synovial cells of RA patients and animal models [131-134], several authors investigated whether dietary PUFA may reduce the inflammatory markers and the activity of cartilage degradative enzymes in patients with RA. Whether there are conflicting results concerning the effects of ω-3 PUFA on cytokine production in ex-vivo studies [135], some beneficial effects of dietary fish oil supplementation, in particular EPA and DHA, have been demonstrated on inflammatory cytokines in healthy humans [136] and patients with RA [137-139].

An ex-vivo decreased TNF-α and IL-1ß synthesis by monocytes and lipopolysaccharide (LPS)-induced IL-1 production by monocytes was found in RA patients supplemented with EPA+DHA (5.9 and 2.9 gr/d), while no effect was obtained on concanavallin A (ConA)-induced IL-2 production and ConA- or phytohemagglutinin (PHA)-induced lymphocyte proliferation [138]. In active RA patients, a decreased soluble TNF-α receptor and C-reactive protein levels were observed after supplementation with EPA+ DHA (3.4gr/d) and low ω-6 PUFA for 18 weeks, while the reduction in serum IL-6 and TNF-α became significant at 24 weeks [133]. Similarly, a reduction of the IL-1ß serum concentrations was obtained in RA patients supplemented with EPA+DHA (3.2 and 7.1 g/d) [138,139]. However, serum IL-6, IL-8 and IL-2 concentrations were unchanged following high dose of EPA+ DHA (7.1g/d) [138], and no effect on serum TNF-α concentrations was found after increasing fish oil consumption (EPA+ DHA 3.2 gr/day[139], 3.4 gr/day[133], 4.2 gr/day[141], 7.1 gr/day [134]).

Some biochemical markers relevant to inflammatory symptoms and CV risk were recently evaluated in early RA patients on disease-modifying antirheumatic drugs (DMARD) treatment and fish oil (4-4.5 g of EPA plus DHA) over a 3-year observation period. In patients who were compliant with the therapy and whose plasma EPA level was > 5% of total plasma phospholipid fatty acids, the index of AA availability for eicosanoid synthesis was 30% lower in platelets and 40% lower in PBMC of the fish oil compared to the no fish oil group. Correspondingly, there was a mean 35% decrease in platelet TXB2 production and 41% decrease in lipopolysaccharide stimulated PBMC synthesis of PGE2 in the fish oil compared to the no fish oil group. Favourable differences in plasma triglycerides, HDL cholesterol, and total cholesterol/HDL ratio were also seen in the fish oil group at 3 years but not the no fish oil group [134].

Overall, the studies addressing the anti-inflammatory proprieties of fish oil use in RA patients provide some biologic plausibility for the benefits of dietary habits on inflammatory markers. However, the clinical response to fish-oil supplements was not investigated in the majority of the studies, while in others it was modest and somehow not consistent with changes in inflammatory markers, suggesting that disease-specific clinical outcomes might be a more sensitive marker of anti-inflammatory effects of EPA+DHA than inflammatory cytokines [3].

5.4. Randomized Clinical Studies

Since 1985 several double-blind, placebo-controlled studies investigated the effects of dietary fish oil supplementation on the clinical outcomes of patients affected by RA. The main disease-specific outcomes were global clinical assessment, symptoms like duration of morning stiffness,

number of tender or swollen joints, joint pain, joint damage and disease activity as well as the requirement of corticosteroid or anti-inflammatory drugs.

As far as global assessment is concerned, RA patients randomized to diet high in PUFA (EPA 1.8 g/day) and low in SFA experienced an improvement with less morning stiffness and lower number of tender joints after 12 weeks of treatment, and a worsening of disease activity, pain and number of tender joints after 3 months of discontinuation compared with controls who had low PUFA/SFA dietary ratio [142]. Similar improvements were reported in active RA patients on fish oil-supplementation with and without naproxen [143], in active RA patients on 12-months supplementation with ω-3 PUFA (2.6 g/day) who also reduced their concomitant antirheumatic medications [144]; and in definitive RA patients on EPA (90 mg/d) which also ameliorated biological parameters of inflammation (LTB4, TXB2 and PG metabolites) [134], compared to those on placebo.

RA patients receiving fish oil supplementation (10 g/day) had an improvement in the global arthritic activity at 3 months and a small NSAID-sparing effect at 3 and 6 months compared with controls. Pain, duration of morning stiffness, functional capacity and biochemical markers of inflammation remained substantially unchanged [145]. No effect of fish oil supplementation was reported from other authors [132, 137,138].

As far as joint pain in RA patients is concerned, a significant improvement was reported in RA patient with ω-3 PUFA compared to baseline [132, 143] as well as to compared with the placebo group [144, 146]. Active RA patients (matched for age, sex, disease severity and use of DMARD), receiving different doses of fish oil supplementation (low dose group: 27 mg/kg EPA+18 mg/kg DHA; high dose group: 54 mg/kg EPA+36 mg/kg DHA versus oleic group: 6.8 gm of oleic acid), experienced similar improvement in the number of swollen joints compared with oleic acid, even if the number of tender joints decreased at week 24 in the low-dose group and at week 18 and 24 weeks in the high-dose group. Compared with baseline, only 8 and 21 of the 45 clinical measures significanlty improved in the low-dose and high-dose fish oil groups, respectively, while 5 over 45 in the oleic acid group [137]. In addition, after 3 months discontinuation, some patients returned to baseline conditions, but the majority (80%) of RA patients on NSAIDs and those in the oleic acid group became even worse [137]. Treatment with evening primrose oil (EPO) or EPO+fish oil for 12 months was associated with significant subjective clinical improvment and NSAID reduction compared to placebo, but after 3 months of placebo phase all the EPO patients and 80% of the EPO/fish oil patients had either returned to baseline or become worse [147]. A significant improvement of joint pain intensity and articular index for pain joints, physical performance and functioning have been demonstrated after 12 and 24 weeks of supplementation with both ω-3 PUFA and MUFA [148]. Other studies did not report any beneficial effects of PUFA supplementation on pain relief independent of the dose and the duration of PUFA exposure, the concomitant assumption of NSAIDs or antioxidant supplementation [137, 149-152].

However, a recent meta-analysis on the effects of dietary ω-3 PUFA supplementation concludes that ω-3 PUFA supplementation improves pain outcomes after three months, particularly with respect to pain, duration of morning stiffness, number of painful and/or tender joints, and NSAID consumption [153].

Few double blind, placebo controlled trials investigated the effects of ω-3 PUFA on swollen joint count in RA patients with significant benefits in treated RA patients relative to placebo [132,154], and to baseline [137,143,145]. In particular, a reduction in the number of swollen joints was observed among subjects treated with fish oil who were on a modified lacto-vegetarian diet relative to a Western diet [141]. No changes in swollen joint count were observed in a fish oil-treated arm during and after 1-2 month discontinuation of the fish oil [136,138,147,148-152]. Some authors did not found any benefit of dietary ω-3 PUFA from fish consumption on joint damage when it was estimated based on the radiographic Larsen score [154]. Consistently, a meta-analyses concluded about a favourable but not significant effect of ω-3 fatty acids over placebo on swollen joints in RA patients [155].

The majority of the studies investigating the effects of fish oil on disease activity, by means of Erythrocyte Sedimentation Rate (ESR), did not report any significant improvement relative to placebo [132,145,148-152, 154]. As exception, Kremer found a significant ESR improvement in patients treated with a lower dose of fish oil at 24 and 36 weeks compared with baseline, while treated with a high dose of fish oil had it only at 24 weeks [137]. Magaro and Alpigiani found an improvement of ESR activity from baseline relative to placebo in children with jouvanile RA treated with cod liver oil [156,157]. No studies clearly specified about the sustainment of the effects of ω-3 PUFA on ESR or CRP in RA patients.

Some studies investigating the effects of ω-3 PUFA supplementation on the anti-inflammatory and/or immunosuppressive drug requirements of RA patients found a lower drug requirement in those treated with ω-3 PUFA compared with placebo [26,138,152], and a decreased longitudinal drug requirements compared with the baseline [141,145,147]. In an open label and patient-directed reduction of NSAID use, RA patients consuming fish oil could significantly lowered or discontinued their use of NSAID compared with a control group [145], while in an open label and physician-directed reduction of NSAID use, subjects on EPA and γ-ALA (240 mg+ 450 mg daily), could significantly reduce NSAID doses after 1 year [147]. Moreover, patients receiving fish oil could significantly reduce NSAID dose and better tolerate the discontinuation [152, 143]. Even if an additive effect of dietary supplementation with ω-3 PUFA adjunct to NSAID therapy is supported [3], the magnitude of the improvement does not seem to change among patients consuming different dosages of ω-3 PUFA (3-6 g/day) [158]. Particularly, patients consuming high-dose dietary supplements of fish oil while taking NSAID had a significant reduction in clinical outcomes and were better able to discontinue use of NSAID than a control population given corn oil [138]. No difference in NSAID requirement between treated and controls was

found only in one study [154], while an unsustained effect of ω-3 PUFA was demonstrated in other studies [147,153].

Unfortunately, no studies on NSAID requirement in RA patients taking fish oils used a composite score incorporating subjective and objective measures of disease activity [144,145,152]. Positive findings are from short (<6 months) double blind and placebo controlled studies, with small numbers of patients, based in one centre only, and which did not include a reduction of NSAID requirement as their primary end point [159]. Only recently, Gallagara investigated NSAID requirement as main outcome measure (with a specified protocol for the reduction of the NSAID dose), in a double-blind randomized clinical trial with 97 RA patients taking either 10g of cod liver oil (containing 2.2 g of ω-3 EFAs) or air-filled identical placebo capsules. The NSAID requirement was reduced by more than one-third in 39% of patients with RA that started ω-3 PUFA supplementation and almost two-thirds of patients who continued to take it, without worsening of disease activity [160].

One study, which assessed the effect of ω-3 PUFA on steroid requirements, demonstrated significant improvement relative to placebo [153]. No study assessed the effect of ω-3 PUFA on DMARD requirement. However, among RA patients on early DMARD treatment and consuming anti-inflammatory dose of fish oil (4-4.5 g of EPA plus DHA), the disease remission rate at 3 years was lower in the fish oil group than in the no fish oil group [134]. Patients who consumed high doses of fish oil were able to lower or discontinue their use of NSAID with a proportion of patients in remission at 3 years greater in the fish oil than the no fish oil group (72% vs 31%) [134].

The meta-analysis by Fortin, which included trials published between 1985 and 1992 and one unpublished trial, concluded that 'dietary fish oil supplementation for three months significantly reduced tender joint count and morning stiffness" [155]. The systematic review of trials published between 1985 and 2002 concluded that fish oil supplementation in RA patients has no clear effect on pain, swollen joint count, disease activity or patient's global assessment [161]. Limitations of the studies were mainly the absence of scores derived from subjective and objective measures of disease activity, and methods to assess how the supplements affected requirements for DMARD [162]. Finally, a recent meta-analysis of data from seventeen trials confirmed the efficacy of ω-3 PUFA supplementation in the management of joint pain [153].

5.5. Conclusion on the Effects of PUFA on RA

Although no studies examined the effect of ω-3 PUFA supplementation on the incidence of RA, several studies have reported at least some clinical improvement in the mangment of symptomatic established RA. Such clinical improvement includes amelioration of symptoms such as duration of morning stiffness, number of tender or swollen joints, joint pain, with tendency to decrease the requirement for corticosteroid or anti-inflammatory drugs. Dietary supplementation with ω-3 PUFA, particularly in the form of fish oil, alone or in combination with reduced ω-6 PUFA and adequate intake of MUFAs, may have beneficial effects on clinical outcomes of RA patients but it cannot still be regarded as an established treatment for RA [162-164]. In fact, some cautions in the interpretation of the findings of the majority of studies should be acknowledged. The available evidence on the effects of dietary fish-oil supplements are predominantly from white RA patients with different background dietary habits, in particular concerning fish consumption. Additionally, PUFA supplements have been studied in comparison with a variety of dietary interventions, including inert paraffin wax [142], corn oil [138,143], olive oil [137], and a specially prepared mixture of fatty acids designed to reproduce local dietary intake [147,149]. The combination of fish oil with an anti-inflammatory diet or supplements containing other lipid classes (i.e. MUFAs, GLA, other.), may have additive benefits on RA clinical outcomes and may also modify other risk factors, such as cardiovascular disease. Plant sources of ω-3 PUFA in the form of ALA, such as leafy green vegetables, flaxseed, rapeseed, and canola oils, have little evidence for efficacy in the treatment of RA as well as γ-linolelic acid in the form of evening primrose oil has shown mixed results [165].

5. CONCLUSIONS AND PERSPECTIVES

A large body of preclinical evidence supports the therapeutic use of dietary ω-3 PUFA supplementation, alone or in combination with standard drug treatments, in patients affected from autoimmune-mediated conditions, i.e. IBD and RA.

The efficacy of ω-3 PUFA, especially from fish oil, has consistently been demonstrated in chemically induced colitis in animal models. Several observational studies suggest a protective effect of high dietary intake of ω-3 PUFA on the levels of inflammatory-related markers at intestinal and systemic level. These findings have not been consistently replicated in available clinical trials, thus no strong clinical effect has emerged from their pooled analysis. Better designed and larger clinical trials are required to assess the effects and the therapeutic potential of ω-3 PUFA on IBDs. Of course, future studies should include large sample of patients with CD and UC, but they should report findings separately for these groups, as well as they should assess the effects of ω-3 fatty acids on clinical outcomes using standard validated instruments.

Both experimental animal models and randomized clinical trials showed somewaht stronger evidence for the clinical efficacy of ω-3 PUFA in RA patients. Althought not consistently, some beneficial effects of ω-3 PUFA supplementation have been demonstrated on different clinical outcomes independently of the age of RA patients, the dosage of supplement and the disease activity. However, evidence from pooled analysis of clinical trials is not strong enough to demonstrate that ω-3 PUFA supplementation is a treatment for RA. Also in this condition, large clinical trials are required. They should include people from different geographical areas or socio-economic levels, and with diverse ethnicity. They should evaluate the effect of specific ω-3 PUFA source and dosages taking into account the baseline PUFA intake of participanting patients.

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