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Review: the role of omega 3 fatty acids in intestinal inflammation

Jonathan E. Teitelbaum[a], W. Allan Walker[b,*]

[a]*MCP Hahnemann School of Medicine, Pediatric Gastroenterology and Nutrition, Monmouth Medical Center, Long Branch, NJ, USA*
[b]*Harvard Medical School, Division of Nutrition, Combined Program in Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital and The Children's Hospital, Boston, MA, USA*

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Abstract

The role of polyunsaturated fatty acids (PUFAs) in inflammatory lesions of the intestines is the subject of increasing research. This review begins with a background discussion of the source, elongation, and desaturation of PUFAs, as well as the role they have played in the human diet through evolution. The available data and hypotheses as to how manipulation of PUFAs might effect the various components of the immune system are then provided. Possible mechanisms by which PUFAs result in immunomodulation include alterations in eicosanoid synthesis, membrane fluidity, signal transduction, intraluminal bacteria, and gene expression. Attention is then turned to the known effects that these polyunsaturated fatty acids have on the various individual components of the immune system including lymphocytes, neutrophils, and antigen presenting cells, as well as the immunoregulatory process of apoptosis. Finally, laboratory data on the role of PUFAs in necrotizing enterocolitis, and to a greater extent inflammatory bowel disease, first as demonstrated in animal models of the disease, and second in human studies are then summarized.

Keywords: Omega 3 fatty acids; Fish oil; Anti-inflammatory; Inflammatory bowel disease

1. Introduction

"We are what we eat." This adage represents the force behind research efforts in diet manipulation as preventative measures and treatments of various disease states. Diet has long been known to effect the gastrointestinal tract, indeed Hypocrites had noticed the correlation of milk ingestion, in milk protein allergic patients, and illness. More recently researchers and clinicians have focused on the role of an elemental diet in the treatment of inflammatory bowel disease (IBD).

In the 1950s, investigators studied the effects of corn oil and fish oil in humans, demonstrating a reduction of serum cholesterol in patients with atherosclerosis [1]. The 1980s were a decade of increased interest and research into the effects of polyunsaturated fatty acids in general, and ω3 fatty acids in particular. Today we know that fatty acids are essential for normal growth and development, and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, autoimmune disorders, cancer, and other inflammatory states [2].

The role of polyunsaturated fatty acids in inflammatory lesions of the intestines is the subject of increasing research in animal models, tissue cultures, and humans since the intestinal tract represents the largest immune organ of the human body. Inflammatory bowel disease (IBD) is perhaps the archetypal form of inflammation of the gastrointestinal system. Classically IBD is divided into two entities, Crohn's disease and ulcerative colitis, although a considerable amount of overlap exists between these two forms.

Crohn's disease is a chronic, transmural, inflammatory process that may affect any segment of the gastrointestinal tract from mouth to anus, usually in a discontinuous fashion. The etiology of Crohn's disease remains unknown although evidence points to inappropriate activation of the immune system, perhaps due to an underlying immunoregulatory disorder, as the major cause of nonspecific tissue damage. Activation of T-cells is central to the inflammatory process and results in an expanded mucosal T-cell population, increased cytotoxic T-cell function, and increased expression and production of T-cell cytokines. Attention has focused the T_H1 cells, interleukin (IL)-1, IL-2, and IL-8 and tumor necrosis factor (TNF). These cytokines in turn have multiple effects on other immune cells including increased B-cell production of IgG, increased expression of MHC class II antigens by epithelial cells, and the recruitment of neutrophils and monocytes into the bowel mucosa. Amplification

* Corresponding author. Tel.: +1-617-355-6006; fax: +1-617-264-7253.

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of the inflammatory response occurs with the release of arachidonic acid metabolites, most notably leukotriene B4, and oxygen free radicals from these recruited cells [3].

Ulcerative colitis is a chronic relapsing inflammatory disease of the colonic and rectal mucosa. Unlike Crohn's disease, the inflammation of ulcerative colitis is limited primarily to the mucosa, and involvement is continuous extending from the rectum proximally with varying degrees of ulceration, hemorrhage, edema, and regenerating epithelium. Here too immune dysregulation is likely an etiologic factor with focus on the various cytokines and inflammatory mediators, including IL-1, IL-6, TNF, and interferon-gamma [4].

This review discusses the source, elongation, and desaturation of polyunsaturated fatty acids, and the role they have played in the human diet through evolution. The available data and hypotheses as to how manipulation of polyunsaturated fatty acids might effect the various components of the immune system are then provided. Attention is then turned to the known effects that these polyunsaturated fatty acids have on the various individual components of the immune system including lymphocytes, neutrophils, and antigen presenting cells, as well as the immunoregulatory process of apoptosis. Finally, these concepts are then applied to IBD specifically, first as demonstrated in animal models of the disease, and second in human studies.

2. Source, elongation, and desaturation of polyunsaturated fatty acids

Fatty acids are synthesized de novo from acetyl coenzyme A. There is little need for the synthesis of saturated fatty acids as the western diet normally supplies adequate amounts. However, cell membranes require unsaturated fatty acids to maintain their structure, fluidity, and function. Therefore mechanisms for the introduction of double bonds (i.e. desaturation) have evolved.

Unsaturated fatty acids consist of monounsaturates (MUFA) and polyunsaturates (PUFA). PUFAs are further divided between two classes, ω3 and ω6. It is the location of the first double bond, counting from the methyl end of the fatty acid molecule that distinguishes these classes. The end product of fatty acid synthetase is palmitic acid, a 16-carbon structure which is subsequently elongated to stearic acid. This 18-carbon saturated fatty acid becomes the backbone for unsaturated fatty acid synthesis. The introduction of a single double bond between carbon atoms nine and ten is catalyzed by delta-9-desaturase. This enzyme, universally present in both plants and animals, converts stearic acid to oleic acid. Plants, unlike animals, can insert additional double bonds into oleic acid between the existing double bond at the 9-position and the methyl terminus of the carbon chain. Thus, a delta-12-desaturase converts oleic acid into linoleic acid, while a delta-15-desaturase converts linoleic acid into alpha-linolenic acid (ALA) [5].

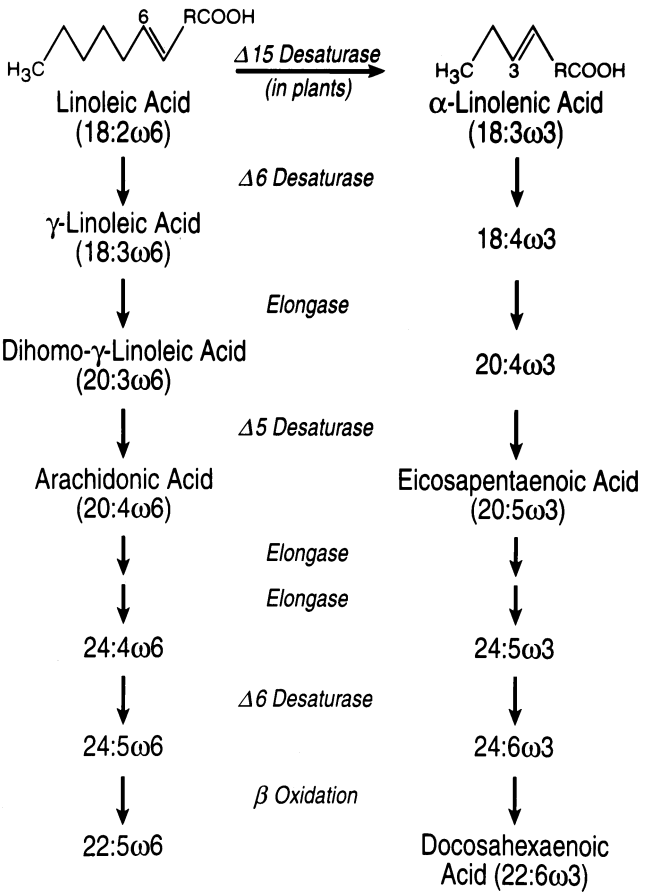

Fig. 1. Pathway for the synthesis of polyunsaturated fatty acids (PUFAs).

Using the pathway outlined in Fig. 1, animal's cells can convert dietary ALA into eicosapentaenoic acid (EPA). A similar series of reactions converts linoleic acid, via gamma-linoleic acid and dihomo-gamma-linoleic acid, to arachidonic acid (AA). The conversion of EPA into docosahexaenoic acid (DHA) involves four steps: two elongations, a delta-6-desaturation and one round of beta-oxidation [6]. It is still uncertain if the delta-6-desaturase is the same as that involved in the desaturation of linoleic acid and ALA. However, it is known that delta-6-desaturase preferentially acts on ω3 fatty acids over ω6 [7–9]. Furthermore, there is some evidence to suggest that delta-6-desaturase activity decreases with age [7], while premature infants appear to be limited in their ability to make EPA and DHA from linolenic acid [10]. The beta-oxidation step involves transfers of intermediates between the endoplasmic reticulum and peroxisomes, utilizing endoplasmic reticulum-associated processes for the final esterification reactions [11].

Omega-6 fatty acids are represented by linoleic acid while ω3 fatty acids are represented by alpha-linolenic acid. These fatty acids are essential fatty acids because mammals cannot synthesize them de novo and must obtain them in their diet. Linoleic acid is plentiful in nature and is found in the seeds of most plants except coconut, cocoa, and palm, and thus is the most predominant PUFA in the western diet.

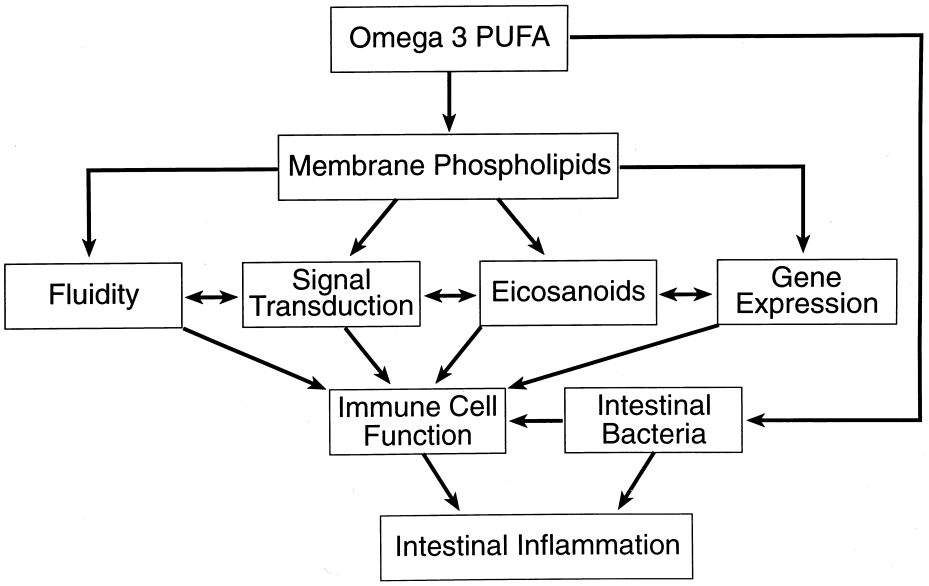

Fig. 2. Mechanism by which ω3 PUFAs might exert immunomodulatory and anti-inflammatory effects.

Many marine plants, especially the unicellular algae in phytoplankton, carry out chain elongation and further desaturation of ALA to yield EPA and DHA. It is the formation of these long chain ω3 PUFAs by marine algae and their transfer through the food chain to fish that accounts for the abundance of the ω3 PUFAs in some marine fish oils.

Linoleic acid and linolenic acid, as well as their long-chain derivatives, are important components of animal and plant cell membranes. In mammals and birds the ω3 fatty acids are distributed selectively among lipid classes. Linolenic acid is found in triglycerides, cholesterol esters and in very small amounts in phospholipids. EPA is found in cholesterol esters, triglycerides and phospholipids. DHA is found mostly in phospholipids and is one of the most abundant components of the brain's structural lipids.

3. Evolutionary and epidemiological aspects of PUFAs

Throughout the evolution of the *Homo sapiens* species the ratio of food consumption between ω6 and ω3 PUFAs was approximately one [12–14], however it is estimated that this ratio is currently closer to 10–11:1 [15] or even 20–25:1 [16]. The increase in consumption of ω6 fatty acids during the last 100 years can be attributed to the development of technology in the late 1800s that marked the beginning of the modern vegetable-oil industry, and to modern agriculture practices. Inventions such as the screw press, and steam vacuum deodorization allowed for the industrial production of vegetable oils for cooking. Subsequently the partial selective hydrogenation of soybean oil reduced the linolenic acid content of the oil while leaving a high concentration of linoleic acid [2]. Modern agricultural practices have further decreased the amount of ω3 fatty acids in animal carcasses. Domestic beef contains very small or undetectable amounts of linolenic acid because cattle are fed grain rich in ω6 fatty acids and poor in ω3 fatty acids [17]. Indeed wild deer that forage on ferns and mosses contain more ω3 fatty acids in their meat. Furthermore, modern aquaculture produces fish that contains less ω3 fatty acid than do fish grown naturally in the oceans, rivers, and lakes [18].

4. Mechanisms by which PUFAs result in immunomodulation (Fig. 2)

Due to the documented role PUFAs have in various disease states, researchers have attempted to discover the mechanism by which they exert their effects. The following sections delineate the available laboratory data substantiating the most accepted hypotheses surrounding the immunomodulatory effects of PUFAs.

Modulation of eicosanoid synthesis. The eicosanoids are a family of 20 carbon oxygenated derivatives of dihomo-gamma-linolenic, arachadonic, and eicosapentaenoic acids. Eicosanoids include prostaglandins (PGs) and thromboxanes (TXs) which are termed prostanoids, as well as leukotrienes (LTs) lipoxins (LXs), hydroperoxyeicosatetraenoic acids (HPETEs), and hydroxyeicosatetraenoic acids (HETEs). In most conditions, the principal precursor for these compounds is AA. Indeed the eicosanoids produced from AA have more potent biologic functions than those from dihomo-gamma-linolenic acid or EPA. The precursor PUFA is released from the membrane phosphatidylcholine by phospholipase A2 or phosphatidylinositol-4,5-bisphosphate (PIP2) by the actions of phospholipase C (PC) and a diacylglycerol (DAG) lipase [5].

Among immunocompetent cells macrophages appear to

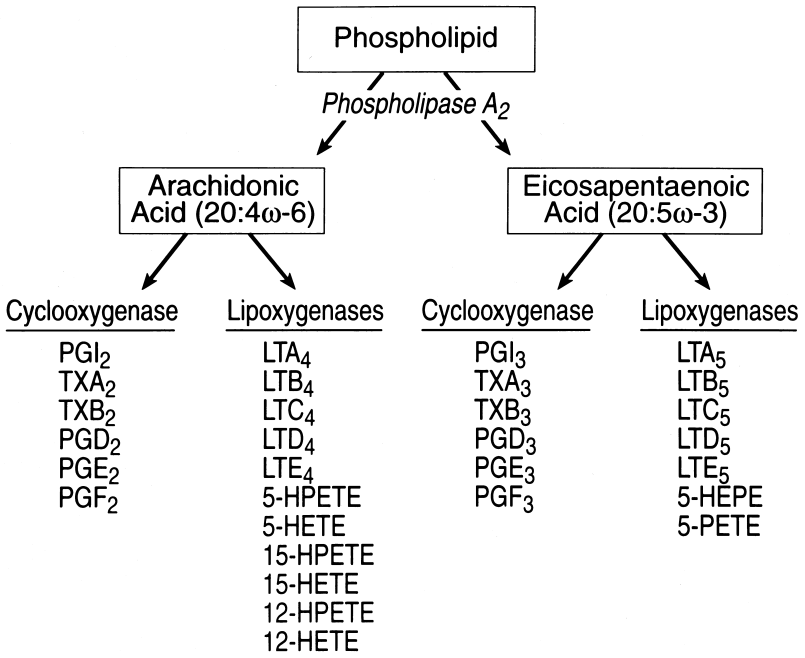

Fig. 3. Eicosanoid synthesis from arachidonic acid and EPA (reproduced with permission from reference 5).

be the principal if not the only source of eicosanoids. However, their production is affected via interaction with lymphocytes. Eicosanoid synthesis begins with the cyclooxygenase pathway which yields the PGs and TXs or with the 5-, 12-, or 15-lipoxygenases which yield LTs, HPETES, HETEs, and LXs. AA is the precursor of the two series of prostenoids (PGA2, PGE2, PGI2 (prostacyclin), PGF2a, and TXA2) and the 4 series of leukotrienes (LTB4). EPA gives rise to the 3-series PG and TXs, and the 5-series LT, these are typically less active then their corresponding products from AA (Fig. 3). These compounds have a short half-life and act in the immediate proximity to the cell from which they are produced. Their production, initiated by specific stimuli, can in turn modify the response to the stimulus [5].

During prostaglandin formation there is competition between the two different classes of PUFA. EPA and DHA compete with AA for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level [19]. When humans ingest fish or fish oil, the ω3 fatty acids EPA and DHA partially replace the ω6 fatty acids, especially AA, in cell membranes including the membranes of platelets, erythrocytes, neutrophils, monocytes and hepatocytes. As a result, ingestion of EPA and DHA leads to a decrease production of prostaglandin E2 metabolites (PGE2). In vivo, PGs are involved in modulating the intensity and duration of immune responses. PGE2's pro-inflammatory affects include fever, erythema, increased vascular permeability, vasodilation, and enhancement of pain and edema caused by bradykinin and histamine. In chronic inflammatory conditions one sees increased suppressor T cells activity and increased PGE2 production. PGs further play a role in the differentiation of T and B lymphocytes. PGE1 and 2 suppress T lymphocyte proliferation and cytotoxicity, as well as IL2 production, and NK activity. PGs also inhibit production of IL1 and TNF by macrophages [5].

Increased membrane EPA and DHA, with a corresponding decrease in AA, additionally leads to a decrease in thromboxane A2 (a potent platelet aggregator and vasoconstrictor), and a decrease in leukotriene B4 formation (an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence). Furthermore, one sees an increase in thromboxane A3 (a weak platelet aggregator and a weak vasoconstrictor), an increase in prostacyclin PGI3 (an active vasodilator and inhibitor of platelet aggregation), and an increase in leukotriene B5 (a weak inducer of inflammation and a weak chemotactic agent) [20–21]. Thus, ω3 PUFA replacement of AA in the phospholipid layer modulates the immune cell functions by eicosanoid mediated effects.

Alteration of membrane fluidity. Fatty acids also have important roles in membrane structure and can thus affect membrane protein function. According to the fluid mosaic model, biological membranes are dynamic and responsive structures. Within these membranes, domains exist allowing for highly specific lipid-protein and lipid-lipid interactions. Both membrane bound proteins and receptors (including adrenergic and insulin) are sensitive to their fatty acid environment. The basis of this is not fully understood, however suggestions include changes in membrane fluidity [22–23], and fatty acid dependent effects on the conformation of the protein complex [24].

Signal transduction. PUFA may have further immune modulatory effects via an effect on signal transduction. Many

lipids are directly involved in intracellular signaling pathways. For example, hydrolysis of phospholipids such as PIP and PC by phospholipases generates second messengers such as DAG. Other phospholipids have roles in the activation of stabilizing enzymes involved in intracellular signaling, such as phosphatidylserine which is required for protein kinase C activation. Furthermore, there is evidence that fatty acids can alter the intracellular free calcium concentration, often a key component in the intracellular signaling pathway. It has been suggested that this occurs via the fatty acid's ability to directly affect receptor operated calcium channels [25].

Effects on intraluminal bacteria. The role of intraluminal bacteria in the pathogenesis of IBD has been the focus of recent research. Such efforts are inspired by experimental models of IBD using interleukin knockout mice that are only symptomatic when removed from a germ-free environment, as well as the role of antibiotics in the treatment of IBD. The effect of EPA on colonic bacteria was investigated by Thompson and Spiller [26]. Their in vitro study demonstrated that EPA significantly inhibited the growth of an obligate anaerobic *Bacteroides* species, but not that of the facultative anaerobe *E. coli.* The mechanism of inhibition is unknown however it is hypothesized that disruption of the outer membrane is the site of action. Thus, changes in the bacterial milieu in patients supplemented with fish oil may have secondary effects on intestinal inflammation.

Changes in gene expression. It is now well documented that fatty acids effect the expression of genes involved in hepatic fatty acid and lipoprotein metabolism. There is speculation that this effect is analogous to that of steroid hormones, possessing intracellular receptors which directly influence transcription [27–28]. Alternatively, the effect may be mediated by their interaction with other mediators such as eicosanoids which in turn alter gene expression. Rosa et al. [29] suggest that PUFAs alter gene products responsible for protein and carbohydrate metabolism as a means of immune modulation. Lymphocytes have the capacity to utilize large quantities of glucose and glutamine and this utilization is essential for their proliferative capacity. An omega 3 PUFA-rich diet induces a reduction in glutaminase (GLUTase) and citrate synthase (CS) activities which were accompanied by a reduction in glucose and glutamine decarboxylation in lymphocytes. Furthermore there is impairment of glutamine utilization and an alteration of the flux of substrates through the Krebs cycle. These changes result in a decrease in lymphocyte proliferation.

5. Immunomodulatory and anti-inflammatory effects of PUFAs on specific components of the immune system

Proper functioning of the immune system relies on multiple interactions among various cell types, antigens, and inflammatory mediators. The complexity of this system makes it impossible for researchers to study it as a whole. Therefore, individual components are analyzed with the hope that in the end, the many small pieces of this elaborate puzzle can be put together. The inflammatory lesions seen in IBD are thought to be secondary to dysregulation of the immune system, and specifically T-helper cell populations. This results in a mixed cell infiltrate that includes lymphocytes, neutrophils, and eosinophils. This section outlines the known effects of PUFAs on various individual components of the intestinal immune system.

Lymphocytes. Studies analyzing the variations of T-cell function between patients with inflammatory bowel disease (IBD) and controls have lead to discrepant results. However, since T-cells play important roles in antigen presentation, macrophage activation, and release of inflammatory mediators researchers have investigated the potential effect of PUFAs on this cell type. A number of studies have shown that linolenic acid, EPA and DHA inhibit the proliferation of lymphocytes isolated from rodent lymph nodes, spleen and thymus as well as human peripheral blood [30–32]. One such study involved weaning rats fed for 6 weeks on high fat diets. These diets differed in the ratio of ω6 to ω3, as well as the absolute amount of PUFA. Data analysis revealed that the ex vivo proliferation of spleen lymphocytes from rats fed the low PUFA diets decreased as the ω6 to ω3 PUFA ratio of the diet decreased. The proliferation of spleen lymphocytes from those rats fed a high PUFA diet was less effected by the ratio of ω6 to ω3. This suggests that it is not only the amount of PUFA introduced in the diet but the ratio of ω6 to ω3 PUFAs that is modulatory.

Sasaki et al. [33] proposed that this effect on lymphocyte proliferation may be modulated via DHA's effect on surface markers involved in T cell proliferation. The primary signal for clonal expansion of antigen-specific T cells is mediated by the physical association of a T cell receptor (TCR)/CD3 complex and a coreceptor such as CD4 or CD8, with the MHC antigen-peptide complex expressed on antigen presenting cells. Furthermore, a costimulatory signal is needed simultaneously with the signal for full T cell activation. CD-28 a surface molecule involved in costimulation can prevent induction of clonal anergy. Sasaki et al demonstrated that in weaning male mice an increase in dietary DHA was associated with a decrease in surface expression of CD4 and CD8, and an increase in CD28 on splenic T cells.

The proliferation of lymphocytes and the regulation of the function of cytotoxic lymphocytes (CTLs), natural killer (NK) cells, B-cells and macrophages depend on the production of IL-1 and IL-2. ALA, EPA, and DHA all suppress the production of IL-2 by mitogen stimulated rat or human lymphocytes [34–37]. Endres et al. [38] studied nine healthy volunteers after consumption of 18 g of fish oil concentrate per day in addition to the western diet for six weeks. Peripheral blood mononuclear cells demonstrated a

suppression of IL-1 beta after the six weeks of supplementation, as well as ten weeks after the end of supplementation. The production of tumor necrosis factor responded in a similar fashion. This decrease was associated with a decrease on the ratio of AA to EPA in the membrane phospholipids of the mononuclear cells. Twenty weeks after the end of supplementation the production of IL-1 and TNF had returned to the pre-supplement level.

Neutrophils. An influx of neutrophils from the circulation into the mucosa and intestinal lumen has been associated with colitis, including Crohn's disease colitis [39–41]. However, the effects of PUFA on neutrophils has yielded contradictory results. While some authors have demonstrated that PUFAs, particularly AA, can stimulate superoxide production [42], induce degranulation [43], suppress cell chemotaxis [44], and alter cell adhesion to endothelial cells [45] others have found opposing or no effect [5,46]. The pro-inflammatory effects of ω6 PUFA were demonstrated by Robinson et al. [47] by revealing ω6 activates phospholipase A2 in neutrophils, a potentially critical role in stimulating the respiratory bust. Fisher et al. [48] studied the affects of daily cod liver oil on healthy human volunteers and found a corresponding decrease in superoxide production. Analysis of the volunteer's neutrophils and platelet fatty acids revealed that the appearance of EPA correlated with a decrease in AA.

Contradictory results were reported by Chawla et al. [49] who investigated the neutrophil response in a formyl-methionyl-leucyl-phenylalanine (fMLP) induced ileitis. fMLP is a potent chemotactic agent for neutrophils, causing infiltration of the mucosa and degranulation. The study compared ω3 vs. ω6 PUFA fed rats and found that although the ω3 fed rats had no alteration in ileal permeability as compared to controls, the ω6 group had a reduced permeability response. The ω6 group also had decreased mucosal infiltration of neutrophils and decreased chemotaxis. The authors thus suggest that fish oil is unlikely to have a significant anti-inflammatory role in Crohn's disease.

Antigen presenting cells. Cell-mediated immune responses are initiated when mononuclear phagocyte cell types process and subsequently express antigens on their surface membranes for recognition by appropriate T cells. A prerequisite for antigen presentation is the expression of MHC class II antigens (or human leukocyte adhesion in man), such as HLA-DR, HLA-DP and HLA–DQ [50]. Indeed the T-cell proliferative response to antigen is proportional to the number of MHC II on the surface of the antigen presenting cell [51]. The percentage of MHC II positive cells and their density on the cell surface can alter the degree of responsiveness of an individual [52]. Studies by Hughes et al. have demonstrated that the ω3 PUFA EPA inhibits the in vitro expression of HLA-DR on human monocytes in a dose-dependent manner. However, there is a significant increase in the expression of HLA-DR and -DP after incubation of monocytes with DHA. The combined effect of EPA and DHA given in a ratio of 3:2, the natural ratio observed in fish oil, yielded no significant effect on the expression of HLA-DR in vitro on unstimulated monocytes. IFN-gamma typically up-regulates the expression of MHC II. However, when IFN-gamma activated monocytes were incubated with fish oil the expression of MHC II antigens was inhibited. Furthermore, there was a reduction in the ability of these monocytes to present tetanus toxoid antigen to autologous lymphocytes [53]. Other animal data revealed that dietary ω3 PUFAs diminish the percentage of peritoneal exudate cells bearing MHC II antigens on their surface [54–56]. Hughes et al showed that supplementation of the human diet with ω3 polyunsaturated rich fish oil for 3 weeks resulted in a decreased level of MHC II expression on the surface of peripheral blood monocytes [57].

In humans, dendritic cells are the key to antigen presentation. Studies suggest that feeding rats a diet of 20% ω3 PUFA significantly diminishes the expression of antigens on dendritic cells and ex vivo antigen presentation by dendritic cells to sensitized spleen lymphocytes [58]. These observations suggest that diets rich in ω3 PUFA will result in diminished antigen presentation.

Apoptosis. PUFAs have been shown to have anti-carcinogenic effects, however the exact mechanism is unclear. Researchers investigating their effects on colon cancer hypothesized that the anti-neoplastic properties are achieved by modulation of epithelial cell turnover in the colonic crypt. Animal models of tumor genesis suggest that exposure to carcinogens results in an acute suppression of cell proliferation and high levels of apoptosis within the colonic crypt [59]. Following this acute response, there is a compensatory phase of cell growth. Those cells that experienced carcinogen-induced DNA damage but escape apoptosis subsequently become the origin of tumor growth. Latham et al. [60] studied the effects of fish oil on colonic crypt cell apoptosis and proliferation in rats after exposure to 1,2 dimethylhydrazine (DMH), a known carcinogen. The consumption of a diet rich in fish oil was associated with increased apoptotic cell death and suppression of proliferation. Histologic analysis of these same rats revealed fewer aberrant crypt foci, early markers of incipient neoplastic change. The authors hypothesized that the mechanism by which PUFAs achieve this altered balance between apoptosis and proliferation is through oxidative stress. Specifically, the DMH provided an acute oxidant stress, while the EPA and DHA from the fish oil further compromised the antioxidant status of the cell via increased lipid peroxidation. The cell's inability to compensate for this oxidative stress leads to selective apoptosis.

In lymphocytes apoptosis plays a key role in maintaining the T cell repertoire and deletion of autoreactive T and B lymphocytes so as to decrease autoimmune responses. Avula et al. [61] studied the effects of PUFA on T cell apoptosis. They found that ω3 PUFA diets fed to mice

resulted in increased levels of serum and splenocyte lipid peroxisomes and a decrease in the antioxidant vitamin E, thus supporting the hypothesis of Latham et al. [60] mentioned above. Furthermore, they found that there was increased apoptosis, via gene products Fas-Ligand (Fas-L) and CD3, as well as necrosis. Fas-L is a type-2 membrane protein belonging to the tumor necrosis factor receptor family and is found predominantly on activated T cells. Fas-L mediates cell death by cross-linking of the Fas-L receptor. The Fas-L gene product was increased in greater numbers while the Bcl-2a ligand, an inhibitor of apoptosis found on the outer mitochondrial membrane, was decreased. These findings likely contribute to the decreased proliferation of ω3 PUFA fed splenocytes.

Intestinal structure and function. Microvillar membranes of intestinal epithelial cells are associated with a layer of carbohydrate-rich material. The biosynthesis of this glycoprotein is highly sensitive to nutritional and developmental variations [62]. Intestinal glycosyltransferase activities are involved in cell differentiation and postnatal maturation of the small intestine. A study by Alessandri et al. [63] demonstrated that PUFA deficiency decreased the intestinal glycosylation of brush border and goblet cell mucus as demonstrated by loss of binding of specific lectins. The animals supplemented with ω3 had increased glycosylation of intestinal mucosa and goblet cells, thus suggesting that PUFA can play a role in intestinal cell differentiation.

Tight junctions are the apical most structures in epithelial and endothelial cells and play a key role in the control of permeability. Jaing et al. [64] demonstrated that treatment of endothelial cells with gamma-linolenic acid increased the transendothelial cell resistance and reduced the pericellular permeability to large molecules. These effects were independent of cell viability. In addition occluden, a transmembrane protein integral in the formation of tight junctions, was up regulated by this fatty acid. EPA also resulted in occluden up-regulation, while AA and linoleic acid down regulated its expression. These findings suggest a role for PUFAs in treating inflamed mucosa and control of tumor extravasation.

6. The role of PUFAs in necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a disease of focal or diffuse ulceration and necrosis of the gastrointestinal tract that occurs almost exclusively in the neonatal period. It is the source of significant morbidity and mortality, specifically among premature neonates. The etiology remains obscure, however it is likely multifactorial and includes factors such as intestinal barrier immaturity, infectious agents, and intraluminal components such as formula.

Akisu et al. [65] investigated the protective effect of fish oil on a hypoxia-induced model of necrotizing enterocolitis (NEC). Mice fed a diet deficient in ω3 fatty acids had increased levels of platelet activating factor (PAF) and LTB4 and had histologic lesions consistent with ischemic injury. The lesions, although present, were milder in the ω3 supplemented group of mice. This study suggests that PAF and LTB4 play a role in mediating hypoxia induced intestinal necrosis.

A study by Carlson et al. [66] compared the outcome of preterm infants fed a traditional formula versus those fed a diet supplemented with egg phospholipids which contained increased amounts of esterified choline, AA, and DHA. The supplemented group developed significantly less stage II and III NEC compared to the control group, while having similar rates of other complications of prematurity such as bronchopulmonary dysplasia, septicemia, and retinopathy of prematurity. Although which component of the egg phospholipids, as well as the mechanism responsible for these changes are unclear, this study does suggest that fatty acid supplementation may have role in the treatment and or prevention of NEC in neonates.

7. PUFAs and inflammatory bowel disease (IBD)

The evidence for the influence of diet on the pathogenesis of IBD consists mostly of unreliable retrospective data. Studies have linked excessive sugar intake and deficient fresh fruit to Crohn's disease. Other studies have pointed to the ingestion of toothpaste as a possible source of granuloma formation given the abrasive phosphate salts [67]. However, population-based epidemiologic studies, do suggest that fatty acids may play a role in the pathogenesis of IBD. A study of the 1800 inhabitants of the Upernavik district of Greenland between 1950 and 1974 [68] documented the incidences of various disease states through hospital records. When compared to western populations the incidence of IBD, as well as myocardial infarction, diabetes mellitus, thyrotoxicosis, bronchial asthma, multiple sclerosis and psoriasis in this population was decreased. Assumptions were made that it was the increased ingestion of fish in this whaling and sealing population that was responsible for these differences. A similar study by Shoda et al. [69] examined the correlation between the incidence of Crohn's disease and dietary change in a Japanese population. Changes in diet and disease incidence were compared annually from 1966 to 1985. During this time period there was a shift from a predominantly fish based diet to a more western diet. Data analysis revealed that the increased incidence of Crohn's disease was strongly correlated with the increased dietary intake of total fat, animal fat, ω6 polyunsaturated fats, animal protein, and an increased ratio of ω6 to ω3 FA.

Still, the primary events that trigger IBD remain unknown. Many of the mediators that amplify the inflammatory response have been identified including platelet-activating factor, biogenic amines, kinins, complement-derived peptides, cytokines, chemotactic oligopeptides, and neu-

ropeptides. Eicosanoids have also been proposed to play an important role in the inflammatory response.

Fatty acid profiles in IBD. Researchers have analyzed fatty acid profiles of patients with IBD. Patients with inflammatory bowel disease often suffer from fat malabsorption and nutritional deficiencies. This is due to weight loss secondary to poor intake and catabolic stress of illness, as well as to loss of absorptive surface through disease or surgery in patients with Crohn's disease. One would suspect that these factors plus the increased demand for essential fatty acids (EFA) during tissue repair and membrane formation would lead to essential fatty acid deficiency (EFAD), abnormal fatty acid profiles, abnormal precursors of eicosanoids, and suboptimal cell function in these patients. Studies have shown decreased total serum PUFA, as well as deficiencies of both ω6 and ω3 in Crohn's patients [70–71]. Siguel and Lerman [72] examined plasma fatty acid patterns in 47 patients with chronic intestinal disorders (25 Crohn's disease, 11 ulcerative colitis, 7 short bowel syndrome, 4 celiac disease). Here too there were decreased concentrations of total PUFA. Furthermore, patients exhibited a shift in fatty acid metabolism similar to that previously shown to be associated with EFAD. Compared to controls, patients with chronic intestinal disorders had decreased PUFA levels, increased monounsaturated fatty acid levels, and higher ratios of ω9 to ω6. More than 25% had biochemical evidence of EFAD by at least one criterion. EFAD would contribute to the pathology of IBD as EFA and their derivatives are key membrane components and are essential for the formation of new intestinal cells, cells normally renewed every few days. EFA insufficiency may prevent formation of normal cells and therefore produce further pathology (malabsorption, absorption of large protein molecules causing systemic immune alterations, ulceration, etc).

These studies contradict the studies by Esteve et al who found an abnormal FA profile in patients with both active and inactive IBD [73–75]. These abnormalities included increased plasma concentrations of both ALA and DHA, with a stepwise decrease in all PUFA, especially ω6, as the disease became more severe. This implied an increase in PUFA biosynthesis in patients with IBD, which is counterbalanced by increased FA utilization during active disease. A study of 63 ulcerative colitis patients after colectomy [76] revealed this increase in ω3 PUFA in patients with IBD is not maintained long-term after colectomy. However, there did appear to be a persistent increase in saturated fatty acids for many years after surgery. This study further described the mucosal fatty acid pattern within the post-colectomy reservoirs or pouches created from the ileum to improve fecal continence. It was found that there is an increased percentage of ω6, and milder increase in ω3 PUFA with a decrease in their essential precursors. These changes were more marked in patients with inflammation within the reservoir or pouchitis. This pattern is essentially identical to that described in the colonic mucosa of patients with active ulcerative colitis [77–78], Crohn's disease [79], as well as experimental models of colitis. This reinforces the concept that this pattern may be shared by all forms of intestinal inflammation.

Animal models of IBD. Shoda et al [80] used trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats as an experimental model of Crohn's disease and observed the effects of diet manipulation. Those rats fed an elemental diet plus 2% ω3 PUFA rich perilla oil had significantly suppressed plasma LTB4 and ulcer index as compared to those with supplemental ω6. The ulcer index and LTB4 were significantly correlated, suggesting that the therapeutic effect of ω3 supplementation in reducing colic damage is mediated through a decrease in LTB4.

Emply et al. [81] studied the effects of a fish oil supplemented diet on the colonic and ileal morphology, histology, and *in vivo* fluid absorption of rats with 4% acetic acid induced mild colitis. The EPA enriched diet reversed net colonic fluid secretion towards increased absorption and prevented macroscopic and histologic injury compared to controls. The fish oil protective effect occurred in the presence of a 30 fold enhancement of PGE2 synthesis and 2 fold increase in LTB4. This protective effect was also demonstrated by Salas et al. [82] in their study in which rats were pretreated with ω3 or ω6 FA prior to the induction of granulomatous colitis by TNBS. The ω3 supplemented group had significantly less colonic damage macroscopically (as measured by presence of adhesions to surrounding tissues, strictures, ulcerations and wall thickness), and histologically (as demonstrated by ulceration, inflammation, depth of the lesions and fibrosis) by day 30 after induction of colitis, as compared to the ω6 supplemented group.

Human studies of PUFAs in IBD. Despite the multitude of laboratory based data in animal models of IBD and immune function suggesting a therapeutic role of PUFAs in the treatment of IBD, the human studies have yielded conflicting results (Table 1). In 1993 Grimminger et al. [83] supplied ω3 FA to a 36 year old female with steroid dependent ulcerative colitis both parenterally and enterally. During the nine day infusion of the fish-oil derived lipid emulsion disease activity rapidly declined and the steroids were quickly tapered. The patient was subsequently provided enteral fish oil capsules. At the end of two months severe colitis recurred. A reintroduction of the parenteral fish oil once again resulted in improvement in the disease score. During the periods of parenteral administration of the fish oil there was a several fold increase in total plasma EPA and DHA, however this was maintained only to a minor extent during enteral supplementation.

To study the role of fish oil in patients with active disease Lorenz et al. [84] studied 39 patients with chronic, active inflammatory bowel disease in a seven month, double-blind placebo controlled cross-over trial of dietary supplementation with fish oil. Biopsies from controls revealed higher

Table 1
Human studies of the effects of PUFA on IBD

Study	Year	Study design	Study population	Clinical results
Lorenz	1989	Double-blind placebo controlled cross-over	39 UC and CD, active	CD—no clinical effect UC—minimal effect
Stenson	1992	Double-blind placebo controlled cross-over	18 UC, active	Improved body weight, and decreased colitis
Aslan	1992	Double-blind placebo controlled cross-over	11 UC, mild/moderate	Decreased disease activity
Hawthorne	1992	Single-blind placebo controlled	53 UC, active 69 UC, inactive	Active UC; decreased steroid requirement Inactive UC: no effect
Grimminger	1993	Case report	1 UC, moderately active	Parenteral, not enteral, fish oil improved disease activity
Greenfield	1993	Placebo controlled	43 UC, stable	No symptomatic or histologic improvement
Belluzzi	1996	Double-blind placebo controlled	78 CD in remission	Improved remission rate (59%) vs. placebo (26%) at one year
Lorenz-Mayer	1996	Placebo controlled, muti-center	135 CD in remission	No effect on remission rate
Loeschke	1996	Double-blind placebo controlled	64 UC, remission	No symptomatic or histologic improvement, no lasting change in relapse rate
Alimallah	1998	Double-blind placebo controlled	9 UC, active	Improved gross and histologic colitis

UC-ulcerative colitis, CD-Crohn's disease.

levels of AA in areas of inflamed mucosa as compared to uninvolved mucosa. Dietary ω3 were preferentially incorporated into mucosa phospholipids at the expense of ω6 FA. The fish oil reduced the production of AA derived prostanoids, and macroscopic involvement of the bowel was moderately improved. However, in those patients with Crohn's disease clinical activity was unchanged, whereas the clinical disease activity fell in those patients with ulcerative colitis, although not to a significant degree. The authors thus dispute the role of fish oils in active Crohn's disease despite the reduced production of prostanoids. The role of fish oils in ulcerative colitis is less clear.

Stenson et al. [85] studied the effects of oral fish oil in patients with ulcerative colitis in a double-blind placebo controlled cross-over trial of 18 patients. They found that the fish oil supplementation resulted in a significant decrease in rectal dialysate levels of LTB4, as well as significant improvements in the histologic appearance of the colon. Furthermore, patients had a significant increase in body weight, average 1.74 kg, as compared to controls, who had no change in body weight. Aslan et al. [86] demonstrated the clinical improvement of 11 patients with mild to moderate ulcerative colitis with the ingestion of fish oil ω3 fatty acids. The clinical improvement was quantified using a disease activity index, and while the mean score declined 56% while on fish oil, it declined only 4% while on placebo. However, no associated reduction in mucosal LTB4 production could be demonstrated. Alimallah et al. [87–88] also demonstrated beneficial effects of fish oil in patients with procto-colitis. After six months of supplementation, the ω3 group had significant improvement in the sigmoidoscopic and histologic appearance of the distal colon. Furthermore, blood analysis revealed reduced NK cell activity and markedly decreased LTB4 serum levels. This decrease correlated with a decrease in IL-2.

These observations are in contrast to those of Hawthorne et al. [89] who revealed that despite the increased synthesis of LTB5 and suppression of LTB4 in ω3 supplemented ulcerative colitis patients there was only limited clinical benefit. Furthermore, disappointing results were found by Greenfield et al. [90] who studied 43 patients with stable ulcerative colitis supplemented with either oral EPA or super evening primrose (an oil rich in ω6 PUFAs). This study failed to demonstrate a difference in stool frequency, rectal bleeding, disease relapse, sigmoidoscopic appearance, or rectal histology as compared to controls.

Others hypothesized that the anti-inflammatory effects of fish oil, though not clearly effective in active disease, may be effective as a maintenance therapy and thus reduce the frequency of relapses in IBD. Belluzzi et al. [91] studied a fish oil preparation, previously shown to have few side effects [92], in a one year double-blind placebo controlled study of the maintenance of remission in 78 patients with Crohn's disease. After one year, the remission rate of the group receiving 2.7 g of daily ω3 fatty acids group was 59 percent, a significantly greater proportion when compared to the 26 percent remission rate of the placebo group. This study thus provided encouraging evidence that fish oil supplementation can be useful as a maintenance therapy in patients with IBD in order to maintain remission. However, a similar study by Lorenz-Meyer et al. [93] of 135 patients with Crohn's disease in remission did not show an effect on the extension of remission when compared to placebo. Specifically, 30% of patients on 5 g per day of highly concentrated ω3 fatty acids maintained remission at one year, which was identical to the 30% of patients on placebo who

remained in remission. Similarly, a study by Loeschke et al. [94] of 64 patients with ulcerative colitis in remission revealed no consistent difference in clinical, macroscopic, or histologic disease between patients supplemented with ω3 PUFAs and controls. Also, although the ω3 group showed improvement during months two and three, the cumulative relapse rate was statistically similar to the placebo group at two years.

Thus a review of the available in vivo trials in laboratory animals and humans with IBD yield conflicting results. The source of variability of between these studies is likely multifactorial and includes the type and dose of PUFA tested, as well as different patient populations. Further research and clinical studies will hopefully create a clearer picture of the role PUFAs have in the treatment and prevention of intestinal inflammation.

8. Summary

The use of ω3 fatty acids in the treatment and prevention of disease has been well documented in a number of disease states. The mechanism by which these changes occur is unknown. Current research, however, has allowed for the substantiation of many hypotheses including alteration of eicosanoid synthesis with resultant changes in inflammatory mediators, alteration of membrane fluidity, signal transduction, the bacterial milieu, and gene expression. The resultant immunomodulatory effects include alteration of lymphocyte proliferation, neutrophil function, antigen presentation, and induction of apoptosis. In addition, there are affects on intestinal structure and function including changes in the cellular glycoproteins and tight junctions.

Laboratory work on animal models of inflammatory bowel disease have yielded conflicting results, as have human trials. The differences in results of these studies are likely multifactorial but appear to be altered by the composition of the fatty acids that are supplied. This includes not only the absolute quantity, or daily dose, but also the relative proportions of linoleic, linolenic, EPA, and DHA provided. Furthermore, the patient's disease type, Crohn's versus ulcerative colitis, disease state, active versus in remission, severity, and location of disease all may impact on the effect that these supplements have on individual patients. Clearly the data demonstrates that ω3 fatty acids are not a "cure-all" for IBD, but their role as an adjuvant therapy has yet to be defined.

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