N-3 Polyunsaturated Fatty Acids and Autoimmune-Mediated Glomerulonephritis

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Abstract

Consumption of n-3 polyunsaturated fatty acids (PUFAs) found in fish oil suppresses inflammatory processes making these fatty acids attractive candidates for both the prevention and amelioration of several organ-specific and systemic autoimmune diseases. Both pre-clinical and clinical studies have been conducted to determine whether fish oils containing the n-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can be used in the prevention and treatment of immunoglobulin A nephropathy (IgAN) and lupus nephritis. In a toxin-induced mouse model that mimics the early stages of IgAN, n-3 PUFA consumption suppresses aberrant interleukin (IL)-6-driven IgA production and mesangial IgA immune complex deposition by impairing phosphorylation of upstream kinases and activation of transcription factors essential for IL-6 gene transcription. n-3 PUFAs can also suppress production of anti-double-stranded DNA IgG antibodies and the resultant development of lupus nephritis in the NZBW F1 mouse and related models. These effects have been linked in part to impaired expression of proinflammatory cytokines and adhesion molecules as well as increases in antioxidant enzymes in kidney and immune organs. Several recent clinical trials have provided compelling evidence that n-3 PUFA supplementation could be useful in treatment of human IgAN and lupus nephritis, although some other studies suggest such supplementation might be without benefit. Future investigations employing genomics/proteomics and novel genetically altered mice should provide further insight into how n-3 PUFAs modulate these diseases as well help to identify clinically relevant biomarkers. The latter could be employed in future well-designed, long-term clinical studies that will resolve current controversies on n-3 PUFA efficacy in autoimmune-mediated glomerulonephritis.

1. Introduction

Inflammation is the normal host response to infection or injury that mediates immune elimination of pathogens and tissue repair [1]. Inflammatory processes include increased production of cytokines, chemokines, nitric oxide and eicosanoids by the innate immune system in conjunction with altered leukocyte homing, all of which can greatly impact acquired immunity. Aberrant inflammatory responses not only evoke acute injury, as exemplified by endotoxic shock, but contribute significantly to chronic autoimmune diseases. The capacity of dietary n-3 polyunsaturated fatty acids (PUFAs) found in fish oil to suppress inflammation-
associated processes has made them attractive candidates for both the prevention and amelioration of a variety of organ-specific and systemic diseases [2,3]. This review specifically discusses pre-clinical and clinical studies of the efficacy of n-3 PUFAs in prevention and treatment of autoimmune-mediated kidney diseases.

2. N-3 PUFAs, inflammation and immune response

Since mammals require but cannot synthesize fatty acids with double bonds distal to the ninth carbon atom, long chain PUFAs are essential to their diet [4]. Linoleic acid (18:2n-6) is a major PUFA found in oils derived from plant seeds such as corn or safflower. Linoleic acid can be elongated and desaturated to yield arachidonic acid (20:4n-6; AA). The action of Δ15-desaturase in plants converts linoleic acid to α-linolenic acid (18:3n-3) which can be elongated to eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). These latter conversions to EPA and DHA occur slowly in mammals but are carried out readily by marine algae. Transfer of EPA and DHA from these algae through the food chain to fish makes fish oil the primary source of highly unsaturated n-3 PUFAs in the human diet as well as dietary supplements [5]. A CDC-NHIS survey determined that 11.7 % of U.S. adults (26 million individuals) consume n-3 PUFA supplements [6].

The capacity of n-3 PUFAs to modulate immune function and suppress inflammatory responses has been reviewed extensively [1,3,7,8]. n-3 PUFAs suppress proinflammatory cytokine production, lymphocyte proliferation, cytotoxic T cell activity, natural killer cell activity, macrophage-mediated cytotoxicity, neutrophil monocyte chemotaxis, MHCII expression and antigen presentation. Evidence that these cellular effects indeed impact immune function in vivo is reflected in n-3 PUFA attenuation of mediator production, leukocyte homing, delayed-type hypersensitivity, allograft rejection and acute inflammatory responses in experimental animals in which human inflammation and autoimmune diseases are modeled. n-3 PUFAs appear to mediate these pleiotropic effects via both eicosanoid-dependent and eicosanoid-independent pathways.

Eicosanoids are oxygenated and biologically active metabolites that include prostaglandins (PGs) and leukotrienes (LTs) synthesized by the cyclooxygenase (COX) and 5-lipooxygenase pathway, respectively. Immune cells produce eicosanoids a process which is in part influenced by the PUFA composition of the cell membrane [2,3]. The eicosanoid PGE2, a COX metabolite of AA, can be proinflammatory and modulate cytokine production. The 4-series LTs, lipooxygenase metabolites of AA, have chemotactic properties, promote inflammation and upregulate proinflammatory cytokine production. EPA and DHA competitively inhibit oxygenation of AA by COX and 5-lipooxygenase. EPA is also able to serve as substrate for both COX-2 and 5-lipooxygenase. n-3 PUFAs thus dramatically alter eicosanoid profiles by (1) decreasing membrane AA levels, (2) inhibiting generation of proinflammatory eicosanoids (2 series PGs and 4-series LTs), (3) promoting production of EPA metabolites (3 series PGs and 5 series LTs) and (4) suppressing COX-2 and 5-lipooxygenase expression. Recent elegant studies have demonstrated that n-3 PUFAs convert to a novel series of lipid mediators termed resolvins and protectins that can elicit protective and beneficial effects [9,10]. These mediators attenuate inflammation in a number of models, with the lipid autacoid resolvin E1 being extremely potent [11,12].

There is increasing recognition that eicosanoid-independent mechanisms play a critical role in n-3 PUFA suppression of inflammatory gene expression. Proposed mechanisms include (1) alteration of transcription factor activity or abundance as has been described for PPAR, LXR, NNF-4, NF-κB, AP-1 and CREB [4,13,14]; (2) interference with activity of critical second messenger-regulated kinases such as PKA, PKC, CaMKII, AKT and mitogen-activated protein kinases [15–17]; (3) changes in membrane lipid/lipid raft composition that alter G-protein...
receptor or tyrosine-kinase linked receptor signaling [18–23] and (4) interference with membrane receptors such as the TLR family [24,25].

Taken together, these anti-inflammatory and immunomodulating activities have led to the evaluation and application of n-3 PUFAs for prevention and treatment of inflammatory and autoimmune diseases. Of particular interest here are those studies that have focused on the kidney.

3. Chronic kidney disease and autoimmune-mediated glomerulonephridies

The kidney’s primary function is to remove excess metabolic waste products and water from the blood [26,27]. Damage to the glomeruli, the basic filtration units of the kidney, impairs this critical function. Glomerular damage can result from a kidney-specific disease or be a reflection of a broader systemic disease. Partial loss of kidney function is referred to as renal failure whereas total and permanent loss of kidney function is termed end-stage renal disease (ESRD). Both glomerular inflammation and tissue scarring, classified as glomerulonephritis and glomerulosclerosis, respectively, can contribute to ESRD. Kidney disease symptoms include proteinuria, haematuria, reduced glomerular filtration rate (GFR), low blood protein (hypoproteinemia) and oedema. Damage to the glomeruli can evoke varying degrees of renal failure. In chronic kidney disease (CKD), irreversible loss of kidney function typically occurs over a protracted period of time (>10 years).

Manifestations of CKD can range from relatively mild renal insufficiency to ESRD requiring dialysis or transplantation. Nearly 16% of persons above 20 years old in the U.S. have CKD with over 300,000 individuals undergoing dialysis each year [28,29]. CKD can specifically result from autoimmune diseases, infection, high blood pressure and diabetes, and vasculitis. Given that inflammation plays a significant role in autoimmune glomerulonephritides, it is not surprising that n-3 PUFAs have been extensively evaluated relative to their preventative and therapeutic properties in these diseases. Two of the most important causes of human autoimmune glomerulonephritis are immunoglobulin A nephropathy (IgAN) and systemic lupus erythematosus (SLE) and are therefore the focus of this review.

4. n-3 PUFA and IgAN

4.1 Disease characteristics

IgAN is an autoimmune disease which has as its diagnostic hallmark diffuse mesangial deposition of IgA in kidney glomerulus frequently accompanied by haematuria. It has been estimated that IgAN affects almost 1% of the population and yet the diagnosis is often missed [30]. IgAN is extremely common worldwide accounting for up to 50% of glomerulonephropathies in Japan, 20 to 35% in Europe and 5 to 10% in North America [31, 32]. It has been estimated that IgAN is the most common glomerulonephritis and cause of ESRD in young adult Caucasians in the U.S. [33]. Nearly 150,000 people in the U.S. are diagnosed with IgAN with 4000 new cases occurring each year [34]. Approximately 25% IgAN patients progress to renal failure within 25 years [30].

The fundamental abnormality in IgAN lies within the IgA system and not the kidney since IgA deposition in IgAN patients recurs after renal transplantation [35]. An overly robust IgA response to mucosal infections and dietary antigens in terms of quantity, size (primarily polymeric), glycosylation status and immune complex formation is believed to contribute to IgAN [36–39]. Cases of IgA nephropathy vary with regard to clinical presentations, clinical and histopathologic risk factors for progressive renal disease, and time course [40]. While no consensus exists on how to best treat human IgAN, approaches include angiotensin-converting enzyme blockade, corticosteroids, cyclosporine, anticoagulants, antiplatelet drugs, phenytoin...
and tonsillectomy [40,41]. Epidemiologic studies suggest that negative association exists between tissue levels of n-3 PUFAs and IgAN [42] whereas a positive association exists for n-6 PUFAs [43] suggesting possible benefits to dietary supplementation with n-3 PUFAs. Both animal models and clinical studies have been used to evaluate the effectiveness of n-3 PUFAs in direct and adjuvant treatments for IgAN.

4.2. Animal studies

Experimental animal models developed to elucidate pathogenic mechanisms of IgAN include toxin-induced induction of polyclonal IgA response, mucosal immunization, injection of IgA-IC, viral infection and use of mice genetically prone to mesangial IgA deposition [44–48]. The toxin-induced IgA and mucosal immunization models in rodents have been used to study how n-3 PUFAs impact the early and late stages of IgAN, respectively.

Mice fed the trichotheccene mycotoxin deoxynivalenol (DON) develop early characteristic features of human IgAN including persistent elevation of serum polymeric IgA and IgA-IC and mesangial IgA deposition [49–59]. Relative to aberrant upregulation of the mucosal IgA response, it is known that several cytokines modulate B cell activation, class-switching, proliferation and terminal differentiation to IgA-producing plasma cells [60,61]. Relative to mucosal IgA immunity, IL-6 appears to be particularly critical based on production of this cytokine in the gut by macrophages, T-cells and other cells as well as because of its effects on IgA-committed B-cells in vitro [62]. IL-6 might similarly contribute to the induction of immunopathologic sequelae associated with human IgAN [62–68]. DON-induced IgA hyperproduction involves upregulated cytokine gene expression of which IL-6 appears to be most important in promoting polyclonal expansion of IgA secreting cells based on studies of (1) kinetics and magnitude of the IL-6 response relative to other cytokines [69,70], (2) ex vivo cell reconstitution [59], (3) antibody neutralization [58] and (4) IL-6 deficient mice [71].

Regulation of IL-6 gene expression involves multiple signal transduction pathways that impact transactivation, mRNA stability and translational efficiency. EMSA and point mutation analyses have shown that cAMP response element binding (CREB) protein, activating protein-1 (AP-1), CCAAT/enhancer binding protein β (C/EBPβ) and nuclear factor κB (NF-κB) all participate in IL-6 transcriptional upregulation [72–74]. DON induces binding of these factors both in vitro and in vivo models [75–78] as well as enhancing stability of mRNA for IL-6 and other cytokine genes [76,78–82].

Consumption of menhaden fish oil or DHA- and EPA-enriched fish oils attenuates DON-induced increases in serum IgA, IgA immune complexes (IC) and IgA deposition in the kidney in DON-fed mice whereas high α-linolenic acid-containing flaxseed oil has no effect [83–85]. DHA-enriched fish oil consumption did not inhibit specific IgA or IgG responses to reovirus strain Type 1 Lang, (T1/L), a model mucosal pathogen, in the intestinal or systemic compartments [86]. Both splenic IL-6 mRNA and heteronuclear nuclear RNA (hnRNA), an indicator of IL-6 transcription, are significantly reduced in spleens of DON-fed mice consuming fish oil or DHA [84,87]. Furthermore, in macrophages from mice fed DHA or EPA, induction of IL-6 hnRNA expression, activation of CREB and AP-1, CREB/ATF1 phosphorylation and in vitro and intranuclear binding of the CREB/ATF transcription factor family are suppressed whereas DON enhancement of IL-6 mRNA half-life is unaffected [14,88]. Thresholds for these ameliorative effects were 1% and 5% diet for DHA and EPA, respectively, suggesting DHA to be more potent than EPA. Collectively, these data suggest that n-3 PUFA consumption suppress DON-induced transcriptional activation of IL-6 gene in the macrophage by interfering with phosphorylation and subsequent binding of CREB/ATF1 to the IL-6 promoter, whereas post-transcriptional mechanisms are unaffected.
There are at least five possible kinase pathways for CREB phosphorylation [89,90]. DON does not appear to markedly affect classical pathways for CREB phosphorylation involving adenylate cyclase/PKA, or Ca MKII, whereas pathways involving the kinases MSK1, RSK1 and AKT1 are affected by DON [91]. MSK1 is widely distributed in mammalian cells and can be activated by growth factors, cell-damaging stimuli and proinflammatory cytokines through both the ERK and p38 pathway while RSK1 (p90 RSK) is only activated through the ERK pathway. AKT1, also known as PKB [92], functions as a critical controller of cell homeostasis mediates of phosphorylation of several proteins including CREB. DHA might thus act by phosphorylation, and/or modified activity of MSK1, RSK1 or AKT.

Shi et al. [17] determined how consumption of fish oil enriched with DHA suppresses DON-induced CREB phosphorylation in peritoneal macrophages ex vivo. DON-induced IL-6 expression in naïve macrophages maximally at 3 h. Pharmacologic inhibition of the CREB kinases, AKT, MSK1 and RSK1 as well as knockdown of the transcription factor CREB downregulated DON-induced IL-6 expression. Inhibition of double-stranded RNA-activated protein kinase (PKR) suppressed not only IL-6 expression but also phosphorylation of CREB and its upstream kinases, AKT1, MSK1 and RSK1. PKR, CREB kinases and CREB phosphorylation were suppressed in peritoneal macrophages isolated DHA-fed mice. The effects of DHA could not be explained by increased activity of protein phosphatase 1 and 2A since both of these were suppressed in mice consuming the DHA diet. While cells cultured directly with DHA expressed less IL-6 compared to cells cultured with AA, neither fatty acid treatment affected DON-induced protein phosphorylation. Both DHA and AA similarly inhibited cell-free protein kinase activity. Thus DON-induced IL-6 expression is CREB-mediated and PKR-dependent and requisite kinase activities for these pathways are suppressed in macrophages from mice fed DHA for an extended period.

While the DON-induced IgAN mode replicates early aberrant IgA hyperproduction and mesangial deposition, it does not recapitulate the latter stages of the disease involving mesangial proliferation and glomerular inflammation within the two to three months in which DON is fed. There has been at least one alternative model employing in which IgAN was induced in male Sprague-Dawley rats by oral and i.v. immunization with bovine γ-globulin for 8 wk as evidenced by haematuria, proteinuria, and IgA deposition in the mesangium [93]. Four diets were compared that included standard rodent chow containing (1) α-tocopherol, (2) fish oil stripped of α-tocopherol preservative, (3) corn oil supplemented with α-tocopherol or (4) fish oil supplemented with α-tocopherol. Fish oil with α-tocopherol attenuated renal injury in this model, while fish oil without α-tocopherol did not. It was concluded that α-tocopherol was more critical than the fish oil to mitigating injury and promoting repair in experimental model for the later stages of IgAN. Clearly, further studies with n-3 PUFAs are warranted that employ additional rodent models that mimic the latter stages of human IgAN with progressive kidney damage.

### 4.3 Clinical studies

A number of clinical trials have demonstrated that n-3 PUFA consumption retards renal disease progression in patients with IgAN by reducing inflammation and glomerulosclerosis. An early Japanese randomized trial of 20 IgAN patients compared the effects of consuming EPA (1.6 g) and DHA (1.0 g) per day to an unsupplemented group and reported improved renal function [94]. Holman [95] compared plasma phospholipid fatty acids, nonesterified fatty acids, urine protein excretion, and GFR in 15 IgAN patients before and after supplementation with fish oil. Following supplementation with fish oil, increased DHA and EPA were found to accompany decreases in proteinuria and improved GFR. The authors proposed that n-3 PUFAs favorably influenced IgAN by a modulation of the pathologic actions of eicosanoids and other diverse actions on various mediators produced by an initial immune injury. A Polish trial followed...
renal reserve (% change of basal creatinine clearance) in IgAN patients consuming EPA (540 mg) and DHA (810 mg) daily over 12 months [96]. The therapy was associated with increased renal reserve and decreased proteinuria.

A definitive series of studies were conducted by the Mayo Nephrology Collaborative Group that support the contention that n-3 PUFA supplementation stabilizes renal function in IgAN patients. Daily regimens included EPA (1.8 g) and DHA (1.2 g) for 2 years [97] or 6.6 years [98] as well as low (EPA, 1.88g and DHA 1.47 g) and high (EPA, 3.76 g and DHA, 2.94 g) dosages [99]. Those individuals ingesting fish oil exhibited reduced tendency for plasma creatinine increase and slower decline in the GFR as well as less death, dialysis, or transplantation. The investigators further observed that low-dose and high-dose omega-3 fatty acids were similar in slowing the rate of renal function loss in high-risk IgAN patients.

The effects of very low dose n-3 PUFA supplementation on the progression of severe IgAN was also assessed in a randomized, prospective, controlled 4 year trial in which 14 patients received EPA (0.85 g) and DHA (0.57 g) daily and 14 patients were treated symptomatically and used as controls [100]. As observed in the Mayo studies, the fish oil-consuming patients showed reduced tendencies for plasma creatinine increase and GFR decline.

In contrast to the above studies, some investigators have concluded that n-3 PUFA supplementation to be without benefit in human IgAN. A two-year prospective Australian trial was conducted in which 37 IgAN patients with biopsy-proven mesangial IgAN were allocated to either two years of treatment with EPA at 10 g per day or no treatment [101]. EPA was not found to alter the course of established mesangial IgAN. Pettersson et al. [102] carried out a prospective, randomized, placebo-controlled six-month study of 32 adult IgAN patients in Sweden in which effects of daily consumption of 6 g of a high n-3 PUFA-containing fish oil product (55% eicosapentenoic and 30% docosahexenoic acid) were compared to daily consumption of 6 g of corn oil. Fish oil was not found to benefit the IgAN patients. Another randomized, placebo-controlled, double-blind trial evaluated the role of prednisone and n-3 PUFA on renal failure in 96 IgAN patients [103]. When groups receiving prednisone, 4 g/d n-3 PUFA (1.88 g eicosapentaenoic acid, 1.48 g docosahexenoic acid; O3FA group) or placebo for 2 yr were compared, neither treatment was demonstrated to slow renal disease progression.

Hogg et al. (2006) questioned the discordant results among clinical trials for IgAN and lack of a unifying hypothesis by evaluating results of the North American IgA Nephropathy Study for a dosage-dependent effect of Omacor, a purified n-3 PUFA preparation, in IgAN patients. Correlations were observed between (1) phospholipid EPA/AA and DHA/AA and Omacor dosage (mg/kg bw), (2) phospholipid EPA/AA and DHA/AA levels and percentage change in urine protein/creatinine ratio after 21 to 24 mo of therapy and (3) Omacor dosage (mg/kg bw) and change in proteinuria after 21 to 24 mo. The authors concluded that Omacor’s effects on proteinuria in IgAN patients were dose dependent and were associated with changes in plasma phospholipid EPA and DHA levels. Donadio et al. [104] attempted to confirm these findings by reexamining archived data from earlier studies by the Mayo group in which two dosages of Omacor were given to 73 adult IgAN patients who were at high risk for developing progressive renal disease. Post hoc analysis of body weight and BMI, plasma n-3 PUFA status, and renal outcome failed to demonstrate that treatment efficacy of n-3 PUFA was dosage dependent on the basis of body size. Differences in outcomes between this and the study by Hogg and coworkers were suggested to reflect (1) different patient populations that were studied (adults vs. children and young adults, respectively); (2) different disease severity (severe vs. moderate, respectively); (3) limited statistical power because of small patient numbers; and (4) the possibility that the relationship does not exist.
Finally, Henoch-Schönlein purpura (HSP) is a vasculitic syndrome with palpable purpura and renal involvement with IgA deposits similar to IgAN. Dixit et al. [105] treated 5 children with biopsy-proven HSP with repeated episodes of haematuria and proteinuria with ACEI and fish oil (1 g orally twice daily) for an average of 49 weeks. The treatment was associated decreased protein excretion rate, reduced hypertension and increased GFR. While preliminary in nature, these findings suggested the need for randomized prospective trials in children with HSP to confirm these observations.

5. n-3 PUFA and Lupus Nephritis

5.1. Disease manifestations

Systemic lupus erythmatosus (SLE) affects 1 in 2500 Americans with most cases occurring in women of childbearing age (1:750) and in individuals of African and Asian descent (1:250) [106]. SLE etiology is complex with both genetic and environmental factors thought to contribute to its development. Defects in apoptotic cell clearance and aberrant uptake by macrophages appear to contribute to autoimmunity due to presentation of nuclear antigens to T and B cells [107]. Mortality from SLE is significantly correlated with the development of glomerulonephritis (~ 50% incidence rate) [108]. Binding of autoantibodies, most notably IgG anti-dsDNA, and immune complex deposition within the kidney recruits circulating and migrating leukocytes [109]. This infiltration results in an inflammatory response that can lead to potentially irreparable parenchymal damage. Disease severity is typically monitored by microscopic evaluation of IgG and C3 deposition, leukocyte infiltration, glomerular changes and proteinuria [110].

Conventional treatment options for lupus nephritis include cyclophosphamide, methylprednisone, azathioprine, corticosteroids, or mycophenolate mofetil [111]; however, potential side effects include bone loss, cataracts, and increased risks of infection and liver toxicity. Clinical trials have also been conducted on antibody therapies such as Tocilizumab (anti-IL6 receptor antibody), Rituximab (anti-CD20+ B cells) and Infliximab (anti-TNF) [112]. Contradictory findings from these studies demonstrate the complex nature of lupus nephritis with some patients being unresponsive and some exhibiting increased susceptibility to infection. Since as many as half of SLE patients appear to be unresponsive to conventional medicine, it is not surprising that they seek other therapy options, including nutritional supplementation [113]. The efficacy of using n-3 PUFAs to prevent and ameliorate lupus nephritis has been studied extensively in rodent models and human clinical studies.

5.2. Animal Studies

The New Zealand Black White (F1) mouse (NZB/NZW F1) spontaneously develops the characteristics of lupus nephritis between approximately 30 to 40 weeks of age resulting in a drastically shortened lifespan. The NZB parental strain contributes to B cell hyperactivity while the NZW strain contributes to MHC class II SLE [114]. Disease pathogenesis in these mice resembles that of human lupus nephritis in that they develop high titers of IgG, IgG anti-dsDNA autoantibodies, antichromatin and other antinuclear antibodies. These form immune complexes which deposit in the kidney and drive complement-mediated glomerular injury. Two other genetically susceptible mouse strains, MRL<sup>lpr</sup> and C57BL/6 x satin beige mice (BXSB) [115] have also been used to model lupus. MRL<sup>lpr</sup> mice have a fas gene mutation that allows autoreactive lymphocytes to escape thymic selection. MRL<sup>lpr</sup> mice also develop rheumatoid arthritis and central nervous disorders that do not reflect human SLE. BXSB mice exhibit greater disease proclivity in males and overexpression of TLR7/8 [116].

Early studies demonstrated that initiating the feeding of EPA-enriched menhaden oil to NZB/NZW mice at 5–6 weeks of age or 5 months of age resulted in markedly reduced severity and...
incidence of renal disease as well as an extended lifespan as compared to mice fed beef tallow [117,118]. Menhaden oil consumption over a defined period (6 weeks to 5 to 7 months) followed by beef tallow, however, offered no long term protection [119]. Increased lifespan and reduced renal disease in fish oil-fed NZB/NZW mice have been linked to reductions of anti-ds-DNA and circulating immune complexes [120]. The effects of feeding NZB/NZW mice DHA ethyl ester (DHA-E), EPA ethyl ester (EPA-E), refined fish oil and beef tallow prior to the development of overt renal disease at 22 weeks of age and continuing for 14 weeks were compared [121]. Significant renal protection was offered by consuming 10% fish oil, 10% EPA-E or 6% or 10% DHA-E as compared to beef tallow whereas 3 or 6% EPA-E or 3% DHA-E were less effective. Suppressed anti dsDNA antibody production in fish oil-fed NZB/NZW mice has been linked to altered T cell activity and increased CD8+ T cells (Wu et al., 2001 NOT CITED CORRECTLY).

Ameliorative effects of n-3 PUFAs in mice were similarly replicated in BXSB/MpJ and male MRL-1pr/1pr mice, as evidenced by decreased proteinuria and glomerular injury as well as increased lifespan following fish oil feeding [122]. Menhaden fish oil was more effective at reducing glomerulonephritis in MRL-1pr/1pr mice than MaxEPA containing the same amount of EPA [123]. The renal protective effects of fish oil in MRL-1pr/1pr mice have been linked to altered eicosanoid metabolism, specifically production of the 5-lipoxygenase metabolites by peritoneal macrophages and within the kidney [124,125]. Relatedly, fish oil consumption by MRL/lpr mice suppresses serum concentrations of PGE$_2$, TXB$_2$ and LTB$_4$ as well as of IL-6, IL-10, IL-12, TNF-α and some of these effects can be enhance by vitamin E [126].

The elegant studies of the Fernandes laboratory have related transcript and protein changes evoked by fish oil consumption in NZB/NZW mice to delayed onset and decreased severity of renal disease [127]. Fish oil consumption reduced IL-1β, TNF-α, TGFβ1, ICAM-1 and fibronectin 1 in NZB/NZW mouse kidneys but increased antioxidant enzymes [128–130] whereas increased TGFβ1 expression in spleens of fish oil-fed mice correlated with decreased expression of the oncogenes c-Myc and c-Ha-Ras [131]. Consistent with these findings, Jung et al. [132] reported that fish oil ingestion increased antioxidant enzyme activities and ROS in kidney. Fish oil consumption also increased levels of catalase, superoxide dismutase and glutathione peroxidase in livers of NZB/NZW mice which might potentially be beneficial when cyclophosphamide is used in treatment of lupus nephritis [133].

The beneficial effects of fish oil in NZB/NZW mice are remarkably augmented by caloric restriction [134–136]. The augmenting effect of energy restriction was linked to maintenance of a T cell phenotype consistent with young mice that included increased and decreased capacity for TH1 and TH2 responses, respectively (Jolly et al., 2001B). Muthuhumar et al. (2004) found that both fish oil intake and food restriction decreased costimulatory (CD80 and CD86) and adhesion (ICAM-1, PGP-1, LFA-1 and Mac1) molecule expression in peripheral blood mononuclear cells of NZB/NZW mice.

5.3 Clinical studies

Early clinical trials suggested that n-3 PUFAs might have little value in treating lupus nephritis. A double blind crossover study on 34 SLE patients compared the effects of consuming MaxEPA and olive oil [137]. During the first 3 months, patients consuming Max EPA showed significant clinical and serologic improvement, however, differences were undetectable after 6 months, suggesting that MaxEPA’s effects might be short-lived. Another double-blind, randomized crossover trial was conducted in 26 SLE patients given 15 g/d of fish oil or olive oil for 1 year followed by a 10 week washout period and then 1 year of the reverse treatment [138]. GFR and serum creatinine were unaffected while a nonsignificant trend toward reduced 24 h proteinuria was observed. The authors concluded that this fish oil regimen is without effect in the SLE patients.
Other clinical investigations contradict the negative findings of the two aforementioned studies. In a prospective double blind crossover study, 27 SLE patients were given either 20 g of MaxEPA daily or 20 g of olive oil for 12 wk as part of a standardized isoenergetic low fat diet [139]. When outcome measures were assessed in the 17 compliant patients, 14 of those who received MaxEPA showed benefits when evaluated for generalized lupus disease criteria. A double blind, double placebo controlled factorial trial on 52 SLE patients revealed that those consuming 3 g MaxEPA per day over a 24 week period exhibited significant declines in their Systemic Lupus Activity Measure (SLAM-R) scores [140]. The authors interpreted these findings to suggest benefits of n-3 PUFAs in controlling effects of lupus.

Recently, Wright et al. [141] conducted a 24 week double-blind placebo-controlled parallel trial in which the effects of daily consumption of 3 g of n-3 PUFAs (1.2 g DHA plus 1.8 g EPA) for 24 weeks were compared to control patients consuming an olive oil placebo. In the fish oil group, there were significant improvements in the SLAM-R scores and the British Isles Lupus Assessment Group Index of disease activity for SLE as well in endothelial function. It was concluded that low dose n-3 PUFA supplementation has a therapeutic effect on disease activity and might further confer cardiovascular benefits to SLE patients.

6. Conclusions

Taken together, animal studies have demonstrated the potential for n-3 PUFAs to suppress onset (e.g. aberrant Ig production and glomerular deposition) and progression (e.g. inflammation, glomerular injury and proteinuria) of disease in several animal models of autoimmune glomerulonephritis. However, major gaps still exist in our understanding of the precise molecular mechanisms by n-3 PUFAs act and identities of their cellular targets. While several human clinical trials similarly suggest that n-3 PUFA supplementation could be useful in treatment of IgAN and lupus nephritis, sufficient controversy remains to preclude widespread recommendation of fish oil and its derivatives to patients with these diseases. Recent animal studies employing genomics, proteomics, genetically susceptible strains and targeted gene deletions have provided unique insights into mechanisms of autoimmune glomerulonephridies [46,142–145]. Such approaches should be amenable to new investigations that improve our understanding of the mode of action, efficacy and effective dose of n-3 PUFAs as well as identifying novel and clinically relevant biomarkers of the effect of n-3 PUFAs. New collaborative human trials on n-3 PUFAs and glomerulonephritis employing such biomarkers could be conducted to resolve the aforementioned controversies on human efficacy. These must have sufficiently large recruitments, maintain long-term follow-up and take dose-dependency of effects into account to ensure that questions about n-3 PUFAs be definitively answered.

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Abbreviations

- PUFAs: polyunsaturated fatty acids
- DHA: docosahexaenoic acid
- EPA: eicosapentaenoic acid
- IgAN: immunoglobulin A nephropathy
- PGs: prostaglandins
- LTs: leukotrienes

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COX  cyclooxygenase
ESRD  end-stage renal disease
CKD  chronic kidney disease
SLE  systemic lupus erythematosus
DON  deoxynivalenol
CREB  cAMP response element binding
C/EPBβ  CCAAT/enhancer binding protein β
NF-κB  nuclear factor κB
IC  immune complexes
hnRNA  heteronuclear nuclear RNA
PKR  RNA-activated protein kinase
AA  arachidonic acid
HSP  Henoch-Schonlein purpura
SLE  systemic lupus erythematosus
NZBW F1  New Zealand Black White (F1) mouse
BXSB  C57BL/6 x satin beige mice
DHA-E  DHA ethyl ester
EPA-E  EPA ethyl ester
SLAM-R  Systemic Lupus Activity Measure

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