Role of Omega-3 Fatty Acids for Eye Health

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Introduction

Over the last few decades, there is an increasing interest in the role of omega-3 polyunsaturated fatty acids (PUFAs) and chronic inflammation. Numerous evidence exist from pre-clinical and clinical studies which prove the effectiveness of omega-3 PUFAs against heart disease, cancer, diabetes, neurological and autoimmune diseases [1]. This chapter will mainly focus on the role of omega-3 PUFAs in maintaining or improving the vision of different eye pathologies (either investigated in vivo or in a clinical setting), including age-related macular degeneration, macular dystrophies and severe dry eyes. In particular, emphasis will be given on the anti-inflammatory effect of omega-3 PUFAs. Also, some observational results from our patients are presented and future directions regarding how to benefit from the omega-3 PUFAs are briefly discussed.

Polyunsaturated Fatty Acids

Currently, several studies have been focusing on the therapeutic role of omega-3 PUFAs, which are considered anti-inflammatory molecules. The resolution of inflammation is an active process primarily driven by a new family of mediators, termed resolvins, derived from the omega-3 PUFAs, eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3) [2]. These PUFAs are highly concentrated in the brain and retina and have an important role in the neuronal development and damage repair [3]. DHA is abundantly expressed in the photoreceptors, and vital retinal functions depend on its existence [4].

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Among the major mediators of the inflammatory response is the generation of pro-inflammatory eicosanoids generated from the omega-6 PUFA, arachidonic acid (AA, C20:4ω-6). These include pro-inflammatory prostaglandins (e.g. PGE_2) and leukotrienes (e.g. LTB_4), which can act as mediators for leucocyte chemotaxis and inflammatory cytokine production. The balance between the pro- and anti-inflammatory molecules plays a key role in the disease progression and the resolution of an inflammatory response.

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly. It is estimated that 200 million people suffer worldwide and the number of these individuals is going to be increased up to 50 % by 2040 [5, 6]. The main symptom of early AMD is blurring of central vision which results in difficulty in reading and recognising faces. There are two different types of AMD: the dry form which is the most common one and occurs in 9 in 10 cases, and the wet form (choroid neovascularisation, CNV) which occurs in about 1 in 10 cases. In dry AMD, the retinal pigment epithelium (RPE) cells of the macula, which are crucial for the function of the rods and cones, will gradually degenerate. In wet AMD, in addition to the RPE cells' degeneration, newly formed blood vessels grow from the choroid, break through Bruch's membrane and migrate into the macula part of the retina. These vessels are immature and leak fluid within the retina resulting in scarring of the macula and damage of the rods and cones [7, 8].

Pathogenesis of Age-Related Macular Degeneration

Currently, there is no definite cause for the pathogenesis of AMD, but several different aetiologies. Ageing is one of the most common contributing factors of AMD, due to the

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accumulation of oxidised lipoproteins and free radicals in the retina and choroid. This in turn results in oxidative stress and a decrease in the number of RPE cells and photoreceptors [9, 10]. In addition to oxidative stress, natural age advancement also leads to immunosenescence which is the gradual deterioration of the immune system, specifically the T-cell population [11, 12].

Genetic predisposition, as with multiple pathologies, plays a role in the development of AMD; although there are several genetic associations, the most studied ones are some polymorphic links, in particular related to inflammatory genes, such as the *complement factor H* (*CFH*) and some complement components (e.g. *C3* and *C2*) [13, 14]. The *CFH* gene which controls the activation of the complement system through the alternative pathway was found to be associated with an increased risk of developing AMD [15, 16]. Environmental factors, including smoking, sunlight exposure, high-fat diet, obesity and diabetes, are all associated with the development and progression of AMD [17].

Para-Inflammation in Age-Related Macular Degeneration

Furthermore, a tissue adaptive response, recently described as para-inflammation, where the innate immune system mount a low-grade inflammatory response in order to restore tissue homoeostasis, has been implicated in the pathogenesis of AMD [18, 19]. In particular, chronic inflammatory infiltrates, such as macrophages, lymphocytes and mast cells, have been detected in the choroid of AMD eye donors [20, 21]. Inflammatory-related proteins, including C-reactive protein (CRP) [22–25], interleukin-6 (IL-6) [25, 26] and tumour necrosis factor-α (TNF-α) [25, 27], have been associated with AMD; however, the results from different groups are inconsistent. The fact that systemic inflammatory markers are not strongly related to AMD might suggest that local low-grade inflammation is more likely to be involved in the pathogenesis of AMD. In addition, resident retinal microglia were found to be activated in the outer nuclear layer, in regions of ongoing photoreceptor cell death, in patients with AMD, retinitis pigmentosa and late-onset retinal degeneration [28]. The microglia cell activation is an indication of an immune response to ocular injury or inflammation, as well as retinal degeneration. During normal ageing and also in pathological conditions, such as AMD, there is an observed accumulation of microglia in the subretinal space, localised in the areas of RPE cell death. Apart from microglia activation, complement activation is also involved in ageing and in both forms of AMD. It is suggested that the damage of RPE cells and photoreceptors in AMD may, at least in part, be caused directly by complement activation at the retinal/choroidal interface [29, 30]. Evidence demonstrated that a local complement regulatory system exists in the eye, by the detection of complement components, such as the C3, membrane cofactor protein (MCP), decay-acceleration factor (DAF), membrane inhibitor of reactive lysis (CD59) and cell surface regulator of complement (Crry) [31, 32].

Treatment Options for Age-Related Macular Degeneration

With regards to the wet AMD, treatment options are based on anti-VEGF (vascular endothelial growth factor) therapies which aim to attenuate angiogenesis and vascular permeability. However, this type of targeted therapy does not lead to complete vascular or disease regression [33]. To assess the long-term outcomes, Rofagha et al. investigated the effect of intensive ranibizumab therapy in exudative AMD patients 7–8 years after initiation of the treatment. One-third of the patients demonstrated good visual outcomes 7 years post-treatment, whereas another third had poor outcomes. Almost half of the eyes were stable, whereas one-third declined by 15 letters or more and 37 % ended blind despite numerous injections [34]. These results may indicate that even a long-term therapeutic regime does not reduce the risk for substantial visual decline.

In contrast, for the dry form of AMD, there are no current guidelines for the first-line treatment, although several anti-oxidants, vitamins and zinc may reduce its progression according to the Age-related Eye Disease Study (AREDS). This study was a major clinical trial sponsored by the National Eye Institute which was designed to evaluate the effect of high doses of vitamin C (500 mg), vitamin E (400 international units), β-carotene (15 mg), zinc (80 mg) and copper (2 mg) on the progression of AMD and cataract [35].

Following the AREDS, an additional study was performed, the AREDS2, which was a multi-centre five-year randomised trial, designed to examine the effects of oral supplementation of macular xanthophylls (10 mg lutein and 2 mg zeaxanthin) and/or omega-3 PUFAs (650 mg EPA and 350 mg DHA) on the progression to advanced AMD. Overall, there was no additional benefit from adding the omega-3 PUFAs or a mixture of lutein and zeaxanthin to the formulation. Although the addition of omega-3 to the AREDS formulation was not proven beneficial, it is believed that higher doses of EPA and DHA may have a desirable effect.

To further examine the association of omega-3 dietary intake (from fish sources) with incidents of late-stage AMD (both neovascular and geographic atrophy), SanGiovanni et al. estimated nutrient and food intake from a validated food frequency questionnaire in AREDS participants. The data obtained indicated that people who were consuming the

highest levels of EPA and EPA + DHA had a 50 % reduced likelihood in disease progression (from bilateral drusen to central geographic atrophy) [36]. This shows a clear correlation between the dietary lipid intake and the development of AMD into a more severe clinical presentation.

Therefore, the inconclusive results from the clinical studies led to further investigations in order to examine the possible mechanisms of action of the omega-3 PUFAs and to assess any positive outcomes with regards to the disease progression.

Current Research in Age-Related Macular Degeneration

Numerous in vitro studies demonstrated that treatment of endothelial cells with omega-3 PUFAs effectively inhibited pro-inflammatory responses through modulation of nuclear factor-κB (NF-κB), TNF-α and interleukin-1β (IL-1β)-induced cell adhesion molecule (CAM) expression [37–40].

Dry Age-Related Macular Degeneration

Furthermore, several animal studies focused on the dietary supplementation of omega-3 PUFAs in murine models for macular degeneration. In particular, Tuo et al. reported the therapeutic effect of a high omega-3 diet in the *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ double knockout model, which develops focal retinal lesions with certain features of AMD [41]. The high omega-3 diet included 1.9 wt % of each EPA and DHA, 0.66 wt % α-linolenic acid (α-LNA) and 0.4 wt % docosapentaenoic acid (DPA). The omega-6/omega-3 ratio was 2.9, where the omega-6 source was from the linoleic acid (LA) only. Specifically, animals that ingested a high omega-3 diet for up to 8 months of age showed progression of retinal lesions compared with the low omega-3 diet group. This effect was suggested to be through a reduction in the AA metabolism, as demonstrated by the decreased pro-inflammatory derivatives (PGE_2 and LTB_4). High levels of dietary omega-3 PUFAs may result in the incorporation of EPA into cell membrane phospholipids at the expense of AA, leading to less substrate available for eicosanoid synthesis [42]. In contrast to PGE_2, higher serum levels of PGD_2 (an anti-inflammatory mediator [43]) were observed in the high omega-3 PUFAs group, indicating a protective effect against inflammation. In addition, there was lower ocular *TNF-α* and *IL-6* transcript levels in the high omega-3 group, suggesting that reactive mediators of omega-3 PUFAs may also regulate differential gene expression [41].

Following treatment with the AREDS2 formulation, Ramkumar et al. reported its effect on the *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$model [44]. This formulation included high doses of omega-3 PUFAs (54.9 mmol EPA/kg diet and 25.2 mmol DHA/kg diet), 17.6 mmol lutein/kg diet and 1.76 mmol zeaxanthin/kg diet. After 3 months of treatment, the animals fed with the high omega-3 PUFAs diet showed significant lesion regression, following fundoscopic examination and a great reduction in the ocular A2E concentration (a fluorophore found in lipofuscin and RPE phagolysosomes). Morphological changes were noted, where the outer nuclear layer thickness was greater in the high omega-3-treated group than that in the control one. The retinal expression of pro-inflammatory mediators, including *TNF-α*, *Cyclooxygenase-2* (*Cox-2*), *IL-1β*, *VEGF* and *inducible nitric oxide synthase* (*iNos*), was much lower in the high omega-3-treated group compared with the control. The AA concentration in the retina was found to be lower and the EPA higher in the high omega-3 group compared to the control, whereas the retina AA/EPA ratio was estimated to be 2.26. However, the serum concentration of PGE_2 which is a metabolite of AA did not significantly differ between the two groups [44]. This fact may indicate that the concentration of fatty acids (and their metabolites) in the serum and retina is not directly correlated.

Observational Studies in Age-Related Macular Degeneration

In addition to the preclinical studies, an open-label pilot study performed by Georgiou et al. investigated the therapeutic effect of controlled doses of high omega-3 PUFAs in patients with dry AMD. The supplement formulation included 3.4 g EPA and 1.6 g DHA, where patients followed this treatment on a daily basis for 6 months. Significant improvement in visual acuity was observed in all patients with dry AMD, and by 4.5 months of treatment, all patients had gained a minimum of 1 line of vision consisting of 5 letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart and a third of patients gained 3 lines consisting of 15 letters [45].

Further observational studies took place with 24 eyes of twelve patients with dry AMD, both sexes and mean age of 75, where they were supplemented with 15 ml of omega-3 formulation divided into two daily doses. The omega-3 concentrates consisted of purified ethyl esters rich in EPA (400 mg) and DHA (200 mg) per gram for the liquid formulation. The dosage used in this pilot study provided approximately 5 g EPA and 2.5 g DHA per day. Patients were asked to continue with their current diet as normal and not to increase their consumption of food containing essential fatty acids.

Follow-up was performed at 1.5, 3, 4.5 and 6 months. Visual acuity was examined at each follow-up using the ETDRS chart, and the blood AA/EPA ratio was measured prior and during treatment using a gas chromatographic

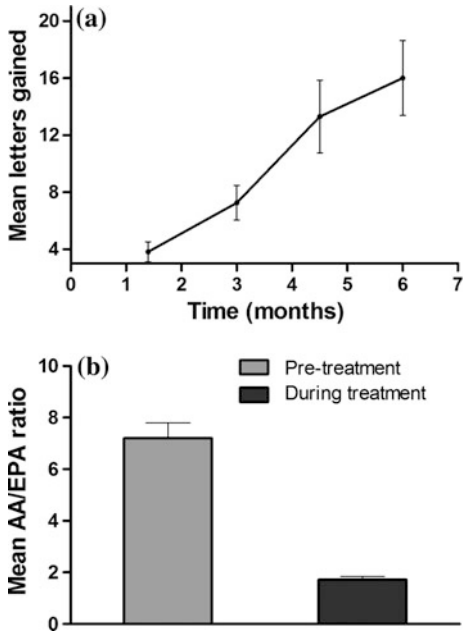

Fig. 20.1 The effect of omega-3 supplementation on **a** visual acuity in patients with dry AMD according to the letters gained and **b** the AA/EPA ratio pre-treatment and during treatment

technique. The optimum AA/EPA ratio is believed to be between 1 and 2 for maximum anti-inflammatory effects. The mean initial visual acuity of patients was 6/18 + 2 (36 %). The mean gain of letters at 1.5, 3, 4.5 and 6 months was 3.8 ± 0.7, 7.3 ± 1.2, 13.3 ± 2.3 and 16.0 ± 2.6, respectively (Fig. 20.1a). The mean AA/EPA ratio prior to treatment was estimated to be 7.2 ± 0.6, whereas during the period of treatment was reduced to 1.7 ± 0.1 (Fig. 20.1b). No side effects were reported by any of the patients treated in the study. The closer the AA/EPA to 1, the more pronounced the effect was.

Wet Age-Related Macular Degeneration

Additional animal studies have been performed using models of wet AMD and other ocular pathologies, including retinal angiogenesis or diabetic retinopathy. Yanai et al. demonstrated that metabolites derived from the omega-3 PUFAs, which are generated through the cytochrome P450 (CYP450), are potent inhibitors of intraocular neovascular disease, such as wet AMD [46]. It is known that the omega-6 PUFAs, including AA, generate CYP-metabolites, the epoxyeicosatrienoic acids (EETs), which are associated with the VEGF-activated signalling cascade, leading to angiogenesis [47]. On the other hand, the CYP-generated metabolites of EPA and DHA, namely 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP), respectively, have shown anti-angiogenic properties [48]. Therefore, Yanai et al. investigated the dietary enrichment with omega-3 PUFAs in a mouse model of laser-induced CNV and demonstrated suppression of CNV (possibly through increased expression of peroxisome proliferator-activated receptor-γ, PPAR-γ), vascular leakage, immune cell recruitment and adhesion molecules (E-selectin and intracellular adhesion molecule-1, Icam-1) to the lesion site. In addition, VEGF expression was suppressed in the retina and choroid of the mice fed with the high omega-3 diet. A significant increase in the serum levels of anti-inflammatory eicosanoids was observed in the high omega-3 group, which was mediated through the CYP-metabolites, 17,18-EEQ and 19,20-EDP [46].

Similar studies by Connor et al. were previously performed, evaluating the therapeutic effects of omega-3 PUFAs on hypoxia-induced pathological neovascularisation in a mouse model of oxygen-induced retinopathy [49]. The results suggested that by increasing the level of omega-3 PUFAs either by dietary or genetic means (using *Fat-1* transgenic model which converts omega-6 to omega-3 PUFAs), there was a reduced hypoxic stimulus for neovascularisation. This effect was mediated through the bioactive metabolites neuroprotectin D1, resolvin D1 and resolvin E1, through reduction in the TNF-α expression which was found to be present in a subset of microglia within the retinal vessels [49].

Furthermore, in a clinical setting, Renzende et al. examined the effect of omega-3 PUFAs supplementation (1052 mg fish oil, 600 mg EPA and DHA) on the levels of vitreous VEGFA, in patients with wet AMD who were receiving intravitreal anti-VEGF therapy. Interestingly, the group of patients that was supplemented with omega-3 showed lower levels of VEGFA in the vitreous but similar levels in the plasma compared to the other groups [50]. This indicates that omega-3 PUFAs could also be useful in minimising progression of wet AMD.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) refers to an inherited, genetically heterogeneous condition which can result from mutations in several different genes (>45 known genes), including the rhodopsin and cyclic guanosine monophosphate phosphodiesterase (cGMP) β-subunit genes [51–53]. RP is affecting approximately 1 in 3500 people pan-ethnically and is a major cause of blindness in adults [54]. What drives the disease progression is the dysregulation and degeneration of the photoreceptors through apoptotic signals, initiating from the rods followed by the cones at a later stage. The main characteristics of RP are night blindness, retinal pigmentary deposit [55] and gradual loss of peripheral vision. As the degeneration of the photoreceptors progress, the vision loss will be increased leading to eventual blindness.

Inflammation plays an important role in the pathogenesis of RP as demonstrated by Newsome et al. who found inflammatory cells infiltrate into the vitreous cavity of RP patients [56]. In addition, Yoshida et al. investigated the inflammatory response in the aqueous humour and vitreous fluid of RP patients by examining different pro-inflammatory cytokines and chemokines performed by ELISA analysis. There was a significant increase of MCP-1 and IL-8 in the aqueous humour, whereas in vitreous fluid, there was an increase in a variety of cytokines/chemokines, including IL-1α, IL-1β and MCP-1 [57].

Current Research in Retinitis Pigmentosa

At present, there is no available treatment which targets the regression of RP; thus, numerous studies are ongoing. The necessity of omega-3 fatty acids for proper retina functioning was demonstrated by Bush et al., where a reduced capacity for photoabsorption by rhodopsin could play a role in lowering retinal sensitivity to light in omega-3 PUFAs-deficient rats [58]. Omega-3 PUFAs and some anti-oxidants or vitamins have been used in several laboratory and clinical studies in order to examine their effect on RP progression, aiming to reduce inflammation.

The nature of the inflammatory response in the rd10 model of RP was evaluated by Yoshida et al., who found an increased expression of pro-inflammatory cytokines/chemokines in the retina, activated microglia and photoreceptor apoptosis. However, treatment of animals with an anti-oxidant, N-acetylcysteine (NAC), prevented the photoreceptor cell death and reduced the inflammatory response [59].

In addition, the effect of DHA was examined in a mouse model of inherited retinal degeneration ($Rs1h^{-/Y}$). Supplementation of animals with DHA demonstrated enhancement of the photoreceptors' survival, transformation of the activated microglia to a quiescent phenotype and reduction in the pro-inflammatory gene expression [60]. This indicated that the retinal DHA levels could control the activity of microglia and perhaps the extent of retinal degeneration.

A randomised, controlled, double-masked trial was performed in order to determine the effect of DHA in patients with RP receiving vitamin A treatment. Patients were given either 1200 mg/day of DHA or control capsules over a 4-year period, in addition to vitamin A. The end results showed that supplementation of DHA over a 4-year interval did not slow the course of disease in RP patients [61]. Similar disappointing results were obtained by a different clinical trial using DHA supplementation (400 mg/day) with X-linked RP patients [62]. In contrast, Berson et al. analysed questionnaires completed by patients with RP who were receiving vitamin A for 4–6 years. The difference in visual acuity was compared between those with high ($\geq$0.20 g/day) versus low (<0.2 g/day) omega-3 intake. The study concluded that patients receiving vitamin A and who consumed a diet rich in omega-3 fatty acids had slower decline in visual acuity than those who consumed a low omega-3 diet [63].

An overview of the clinical findings was presented by Hodge et al. which analysed 6 different studies involved investigation of the intake of omega-3 fatty acids. The review suggests that the data obtained from those studies did not provide a conclusive result as to whether or not the intake of omega-3 fatty acids could slow the progression of RP [64].

Stargardt Disease

The most common form of autosomal recessive macular dystrophy is Stargardt disease which affects the RPE and photoreceptor layer and is usually associated with mutations in the *ABCA4* gene (ATP-binding cassette 4) [65], which leads to accumulation of lipofuscin [66]. Stargardt disease is characterised by a juvenile onset (first two decades), a rapidly progressive course and a poor visual outcome. Loss in central vision is observed caused by a progressive central atrophy.

Another type of macular dystrophy is the autosomal dominant Stargardt-like macular dystrophy type 3 (STGD3), which is caused by 3 sets of mutations in the *ELOVL4* gene (elongation of very long chain fatty acids protein 4) [67, 68]. The ELOVL4 protein is involved in fatty acids' elongation which is necessary for the generation of long chain PUFAs [69]. Mouse models and human clinical studies indicated that a deficiency of very long chain PUFAs in the retina is likely to be a key factor in the macular pathology seen in STGD3 [70, 71].

Current Research in Stargardt Disease

The effect of omega-3 supplementation is believed to play a role in the disease progression. In particular, Dornstauder et al. used the ELOVL4 transgenic model (which displays extensive age-related retina dysfunction and A2E accumulation), to study the effect of dietary DHA supplementation. The data obtained indicated that following DHA supplementation for several months (starting at different stages), there was preserved retina function at mid-degenerative stages and reduced A2E levels in the mouse models [72]. Furthermore, Querques et al. reported a study where 840 mg/day DHA was given to 20 late-onset Stargardt disease patients for six months. A complete ophthalmological examination was performed pre- and post-treatment. The evidence from the study demonstrated

that DHA influenced some functional parameters in those patients; however, there was not significant short-term benefit [73].

Additionally, some emerging therapeutic options for Stargardt disease are being considered, including drugs which modify the functional activity of ABCA4 protein [74], which slow down the visual cycle [75] or gene-targeted therapy [76, 77].

Cone Dystrophies

Cone dystrophies are associated with genetic mutations (e.g. guanylate cyclase activating protein 1, *GCAP1*) which result in functional abnormalities in the cone photoreceptors and sometimes in their post-receptoral pathways [78]. This could be inherited as an autosomal recessive, autosomal dominant or X-linked recessive trait. Within these types of dystrophies, there are subtypes including the stationary cone dystrophy and the progressive (cone-rod dystrophy) which have a different time of the disease presentation. All forms of cone dystrophies show reduced visual acuity and colour vision deficiency, along with dysfunction of the cone electrophysiology [79, 80]. Although these are inherited disorders, chronic inflammation is an important component that contributes to the disease progression. Shiose et al. demonstrated the involvement of Toll-like receptor 3 (TLR3) in a cone-rod dystrophy animal model lacking the ABCA4 and RDH8 proteins (which are critical for all-trans-retinal clearance in the retina). These findings suggested that endogenous products from degenerating retina can stimulate TLR3, causing apoptosis and retinal inflammation. In addition, the loss of TLR3 showed a protective effect from cone-rod dystrophy [81].

Observational Studies in Macular Dystrophies

The strong evidence from the literature regarding the positive outcomes from the supplementation of omega-3 PUFAs in retinopathies and following the promising results from the preliminary study with dry AMD patients [45] led us to investigate the effect of omega-3 in patients with macular dystrophies. Observational studies included a total of 40 eyes of 21 patients with macular dystrophies, 9 patients with Stargardt disease with mean age of 33.6 ± 6.1, 6 patients with RP with mean age of 54.2 ± 6.4 and 6 patients with cone dystrophies and mean age of 41.3 ± 4.4. Patients were supplemented twice daily with an omega-3 formulation, and follow-up was performed at 1.2, 3, 4.5 and 6 months. RP patients were supplemented with 5 g/day of EPA/DHA (ratio 2:1), and the Stargardt's and cone dystrophy patients were supplemented with 7.5 g/day of EPA/DHA (ratio 2:1). Visual acuity was examined at each follow-up using the ETDRS chart, and the blood AA/EPA ratio was monitored. The mean initial visual acuity of all patients was 6/18 (33 %). The overall mean gain of letters for all types of macular dystrophies at 1.2, 3, 4.5 and 6 months was 5.2 ± 0.9, 9.7 ± 1.1, 10.5 ± 0.9, 11.2 ± 0.9, respectively (Fig. 20.2a). The mean AA/EPA ratio pre-treatment in patients with macular dystrophies was 9.9 ± 0.6, while during treatment, the ratio was reduced to 1.4 ± 0.09 (Fig. 20.2b).

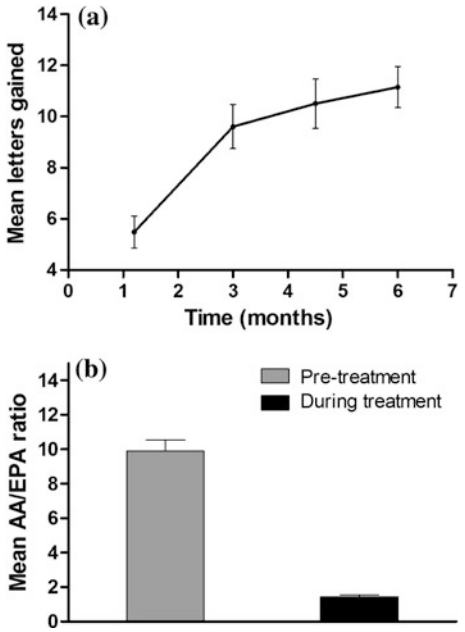

Fig. 20.2 The effect of omega-3 supplementation on **a** visual acuity in patients with macular dystrophies according to the letters gained and **b** the AA/EPA ratio pre-treatment and during treatment

Severe Dry Eyes

Dry eye disease is a multifactorial disorder of the tears and ocular surface that represents one of the most prevalent ocular conditions, and it is a frequent reason that people seek eye care [82]. Dry eye disease is caused by disequilibrium of tear film components and results in symptoms of chronic ocular discomfort, functional visual disturbance that interferes with quality of life [83–85]. Around 3.2 million women in the USA aged 50 years and older have dry eye disease [86], and depending on the population, age and definition, the estimates of the prevalence of dry eyes in the general population range from <1 % to 30 % [83, 87].

Although there has been some progress in understanding the natural history of dry eye disease, current treatment

options for severe dry eye disease have limited efficacy and there are no means of prevention. Artificial tears are the standard of care for the treatment of dry eyes [88]. However, they can provide only temporary symptom control and they do not affect the causative factors of the inflammation that occurs in dry eyes. As a result, artificial tears may only be suited for milder dry eye disease [89]. In more severe disease, multiple treatments are often required, in particular one survey of treating ophthalmologists found that patients with severe dry eye disease usually need to use around five different treatment approaches to manage this condition [90], with the most frequently used treatment being artificial tears, followed by anti-inflammatory drugs and secretagogues [89].

Restasis® (0.05 % cyclosporine ophthalmic emulsion; Allergan) is the only pharmacologic treatment approved by the FDA to increase tear production, but its licensed indication does not extend to the treatment of the symptoms associated with dry eye disease. The onset of action is 24 weeks and many patients are intolerant due to side effects, particularly ocular burning sensation, which occurs in up to 17 % of treated patients [91].

Inflammation, particularly of the ocular surface and meibomian glands, is thought to be an important contributor in the pathogenesis of dry eye disease [92, 93]. Patients with dry eye disease have an increased concentration of inflammatory molecules (such as TNF-α, VEGF, IFN-γ, IL-1, IL-6, epidermal growth factor and fractalkine) in the tear film [93]. Inflammation can be relieved by topical steroids and anti-inflammatory drugs, but most are unsuitable for long-term use and their use may be limited by side effects [94]. There is a clear need for additional options to provide continuous relief of severe dry eye disease symptoms and that are acceptable to patients.

Several studies have suggested that omega-3 PUFAs (including α-LA, EPA and DHA) systemic and topical administration is effective as adjunctive therapy in dry eye disease [95–100]. Possible mechanisms suggested effects on tear secretion, prevention of oxidative damage and reduction in inflammation.

In a cross-sectional observational study, Milanović et al. found that the prevalence of dry eyes was substantially lower among women who had a higher intake of omega-3 PUFAs, compared with those with a lower intake, where the prevalence was higher [95]. In this study, intake of omega-3 PUFAs was estimated by a questionnaire of dietary intake. To date, most studies to investigate omega-3 supplements in dry eye disease have been limited in design and size, and the doses of omega-3 PUFAs have varied, making meaningful comparisons difficult. There is a need for additional trials using omega-3 PUFAs to provide the evidential basis for their use as an adjunct to current therapies for dry eye disease.

Observational Studies in Severe Dry Eyes

We hypothesised that high-dose omega-3 PUFAs may provide a viable alternative treatment for dry eye disease. Therefore, an observational study was performed in order to investigate this hypothesis, where patients with severe dry eyes who had not responded to other standard treatment were supplemented with 10 ml of omega-3 formulation (providing approximately 3.4 grams of EPA and 1.6 grams of DHA per day). The dosage was divided into two daily doses of 5 ml each. Sixty eyes of 30 patients with dry eyes were included in the study, 26 females and 4 males, with mean age of 58 ± 9.9 years. Patients were asked to continue with their current diet as normal, and not to increase their consumption of food containing essential fatty acids. Visual acuity using the ETDRS chart, tear break-up time and fluorescein staining score was determined prior to the initiation of the study and then at 1.5 and 3 months post-treatment.

Eyes that had the greatest gain in visual acuities were those who started with the worst initial vision. The patients who had reduced vision (e.g. 6/15 or worse) had a significant ($p < 0.05$) improvement in vision (Fig. 20.3a). Prior to the omega-3 supplementation, the fluorescein staining was either moderate (17 %) or severe (83 %) in all patients, whereas at

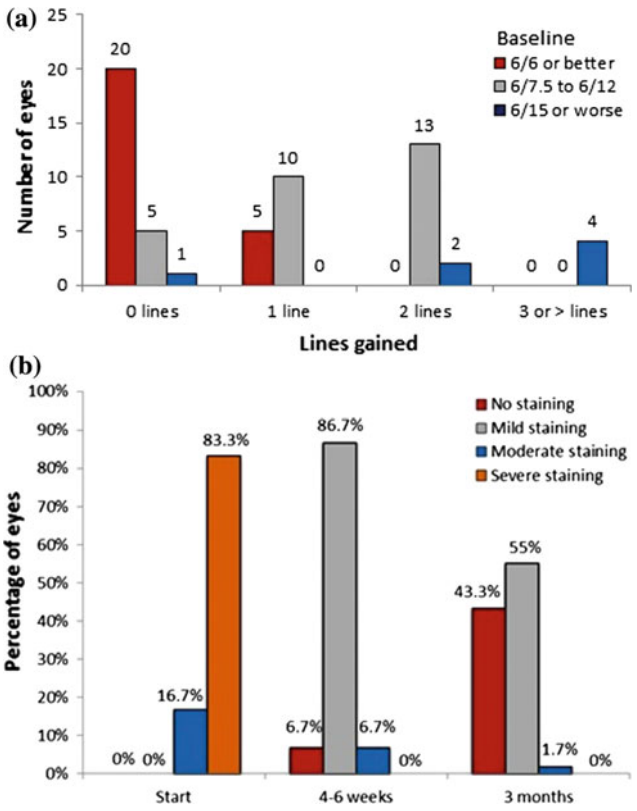

Fig. 20.3 **a** Number of ETDRS chart lines gained at 3 months in patients with severe dry eyes and **b** corneal fluorescein staining scores following supplementation with an omega-3 formulation

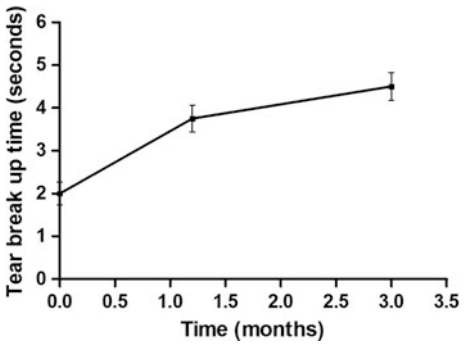

Fig. 20.4 Improvement in tear film break-up time in patients with severe dry eyes following supplementation with an omega-3 formulation for 3 months

the end of the study, no eye had severe staining, and only one eye had moderate staining, illustrating that staining scores improved significantly ($p < 0.001$, Fig. 20.3b). Tear break-up time increased significantly from a mean of 1.78–4.4 s at 3 months ($p < 0.001$, Fig. 20.4). No side effects were reported by any of the patients treated in the study.

In summary, evidence from the literature and from our observational studies suggest that supplementation with high doses of omega-3 PUFAs of patients with retinopathies and severe dry eyes results in disease regression by a substantial improvement in visual acuity. A point for consideration is the monitoring of the AA/EPA ratio, where this could not only provide the optimum therapeutic effect (between 1 and 2) but it could also prevent undesirable side effects by remaining in the safety range (≥ 1).

Conclusions

Chronic low-grade inflammation attacks the body's organs for years until enough damage occurs to produce a chronic disease. It is inflammation below the threshold of pain which is not initially noticeable until chronic organ damage occurs. As for the eyes, reduced vision is the first obvious symptom. Anti-inflammatory drugs, such as steroids and non-steroidal anti-inflammatory drugs (NSAIDS), could reduce chronic inflammation; although side effects such as gastric bleeding, immunosuppression, osteoporosis and heart failure do not make them ideal agents for long-term use. In addition, substantial long-term improvement in visual acuity has not been demonstrated with this class of drugs. Maintaining the AA/EPA ratio between 1 and 2 using omega-3 supplementation could provide an excellent therapeutic regimen for reducing inflammation in the retina and the optic nerve with no side effects. Further studies are ongoing in order to get a better optimisation of the anti-inflammatory effect of omega-3 PUFAs and to have an improved understanding on their mechanism of action and their long-term effects.

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