Omega-3 Fatty Acids and Age-Related Macular Degeneration

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Key Words

Macular degeneration · Epidemiology · Clinical trial · Fatty acids

Abstract

Against a background of considerable epidemiological and other evidence implicating omega-3 fatty acids in the prevention of age-related macular degeneration (AMD), the negative results of the Age-Related Disease Study 2 (AREDS2) were unexpected. The possibility that the design, setting, intake or subjects of AREDS2 may not have permitted the prophylactic potential of omega-3 to be adequately demonstrated is considered. Epidemiological studies had indicated potential preventative effects of omega-3, and an earlier randomised prospective study (NAT2) showed that patients who achieved high red blood cell membrane EPA/DHA (eicosapentaenoic acid/docosahexaenoic acid) levels were significantly protected against AMD compared with those with permanently low EPA/DHA levels. Various methodological differences between these studies are considered. NAT2 included a true placebo group, whereas control subjects in

AREDS2 received a nutritional formula already found to be effective in AREDS1, but no placebo for DHA/EPA supplementation. Differences in the handling of non-compliant subjects and the formulation of the test formulations are considered. Given these considerations, and other lines of evidence from laboratory and clinical studies, closing the chapter on omega-3 in AMD prevention may be premature.

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Introduction

The results of the recently published and keenly anticipated Age-Related Disease Study 2 (AREDS2) [1] were noted with great interest by many researchers in the field and to ophthalmologist clinicians interested in the prophylaxis of age-related macular degeneration (AMD). The results of this double-blind randomised clinical study did not support the prevailing view based on several lines of evidence from laboratory, epidemiological studies and other clinical trials that suggested omega-3 fatty acid intake might have a protective role in AMD progression.

AREDS2 is one of the foremost studies into nutrition and macular degeneration and the discrepancy between its results and those of the foregoing research may warrant a more detailed assessment. One interpretation of the AREDS2 results is that omega-3 supplementation does not protect against AMD progression. However, an alternative analysis suggests that the design, setting, intake or subjects of the AREDS2 study may not have permitted the prophylactic potential of omega-3 to be demonstrated. The objective here is to briefly examine the epidemiological and clinical evidence for and against the prevention of AMD by omega-3.

Epidemiological Studies

Although there have been few interventional studies, there have been a large number of epidemiological studies examining the effects of diets rich in omega-3 on the progression of AMD. They are mostly prospective cohort studies in which either patients at risk of AMD (drusen, fellow eye affected) or a general population sample are followed for a number of years and incident AMD is recorded. Nutritional intake of omega-3 or another dietary variable (such as fish consumption) is estimated from a food frequency questionnaire, usually at baseline. In the majority of studies, relative risk (RR) or odds ratio (OR) is calculated by comparing the highest and lowest quartile or quintile of the dietary variable measured.

These studies are summarised in table 1, from which it is clear that across a variety of different methodologies, populations and geographical sites there is a high degree of consistency with regard to a preventative effect of omega-3 in AMD. Of the studies estimating fish consumption, all show higher fish or oily fish consumption to be associated with a lower risk of AMD [2–7]. Other studies estimate omega-3 fatty acids or docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) intake from the food questionnaires and present similar results in the context of AMD [3–5, 8–14]. On the other hand, diets rich in total fat, saturated fat, animal fat, vegetable fat and trans-fat seem to be associated with an increased risk of AMD [15–19]. A single study measured plasma omega-3 levels, rather than using food frequency questionnaires to estimate intake, and confirmed relevant protective effects [20].

The study by Reynolds et al. [14] demonstrates that subjects homozygous for the ARMS2/HTRA1 genotype that confers high AMD risk were susceptible to protection from incident geographic atrophy by increased DHA intake [hazard ratio (HR) highest vs. lowest quintile 0.4,

$p = 0.002$], but that subjects who were homozygous for the non-risk-conferring ARMS2/HTRA1 genotype were not protected (HR 1.0, $p = 0.9$). Similarly, Ho et al. [12] identified significant interactions between the genetic variants CFH Y402H and LOC387715 A69S and EPA/DHA intake, suggesting that genetic predispositions to the development of AMD can be countered by dietary intake of omega-3.

The results of the epidemiological studies appear overall to be consistent, but there is the possibility of bias in that populations who eat greater quantities of fish may differ in other ways from those that do not, although many potential confounders (such as age, gender, education, body mass index, physical activity, smoking and genetics) were taken into account in these studies.

Clinical Studies

There is a clear need then for the results of good-quality interventional studies. However, such studies face a number of challenges. The number of subjects required is high in order to achieve sufficient statistical power and the studies need to be long-term in order for effects to be realised on what is a slowly but relentlessly evolving illness. Fortunately, the results of such studies have recently become available.

Although there have been earlier interventional studies, some used less rigorous methodologies or multiple supplements making it impossible to differentiate the effects of omega-3 from other components [21, 22]. There have been, however, two well-conducted randomised, double-blind controlled trials of omega-3 in the prevention of AMD, although both studies raise some questions.

AREDS2

AREDS2 is a large, multicentre, randomised, double-masked, placebo-controlled study in which 4,203 subjects at risk of progression to advanced AMD (having either bilateral large drusen or unilateral large drusen in one eye and advanced AMD in the other eye) were included. The study objective was to identify a reduction in AMD risk of 25% or more (the degree of risk reduction observed in the original AREDS study [23]). Given that all participants in AREDS2 received the AREDS formulation as background treatment, to achieve a further 25% risk reduction could be considered ambitious. The protocol distributed the 4,203 enrolled subjects between a total of 20

Table 1. Brief summary of epidemiological studies

Study, year	n	Results
Beaver Dam Study Mares-Perlman et al. [15], 1995	1,968	Intake of saturated fat and cholesterol in the highest vs. lowest quintile had 80 and 60% increased odds for early AMD
Smith et al. [2], 2000	3,654	Fish consumed once weekly or more, OR 0.5
Cho et al. [16], 2001	567	Highest vs. lowest quartile RR: Total fat intake: 1.54 (95% CI 1.17–2.01, $p = 0.008$) Linoleic acid: 1.49 (95% CI 1.15–1.94, $p = 0.0009$) DHA: 0.70 (95% CI 0.52–0.93, $p = 0.05$) Fish consumed >4 times per week vs. <3 times per month: 0.65 (95% CI 0.46–0.91, $p = 0.009$)
Heuberger et al. [17], 2001	7,883	Highest vs. lowest quartile: Total fat OR 1.4 (95% CI 0.9–2.2, $p = 0.10$) Similar results for saturated fat, polyunsaturated fat and monounsaturated fat
Seddon et al. [18], 2001	349 subjects with advanced neo-vascular AMD 504 controls without AMD, but with other ocular diseases	Highest vs. lowest quartile OR: Vegetable fat consumption: 2.22 (95% CI 1.32–3.74, $p = 0.007$) Monounsaturated: 1.71 ($p = 0.03$) Polyunsaturated: 1.86 ($p = 0.03$) Higher intake of linoleic acid associated with increased risk ($p = 0.02$) Omega-3 fatty acids associated with a lower risk in those consuming diets low in linoleic acid Neither omega-3 fatty acids nor fish intake were related to risk for AMD among people with high levels of linoleic acid intake ($p = 0.05$) Higher intake of fish reduced AMD risk when diet was low in linoleic acid
Seddon et al. [19], 2003	261	Highest vs. lowest quartile RR: Total fat intake: 2.90 (95% CI 1.15–7.32, $p = 0.01$) Animal fat intake: 2.29 (95% CI 0.91–5.72, n.s.) Vegetable fat intake: 3.82 (95% CI 1.58–9.28, $p = 0.003$) Saturated fat intake: 2.09 ($p = 0.08$) Monounsaturated: 2.21 ($p = 0.04$) Polyunsaturated: 2.28 ($p = 0.04$) Trans-unsaturated: 2.39 ($p = 0.008$) Higher fish intake associated with a lower risk of AMD progression among subjects with lower linoleic acid intake Processed baked goods increased rate of AMD progression approximately 2-fold; nuts were protective
US Twin Study Seddon et al. [4], 2006	681	Dietary omega-3 fatty intake inversely associated with AMD (OR 0.55, 95% CI 0.32–0.95) comparing the highest vs. lowest quartile
Blue Mountains Eye Study Chua et al. [3], 2006	3,654	High consumption of omega-3 fatty acids associated with reduced risk for incident early AMD (OR = 0.41, 95% CI 0.22–0.75) High fish consumption (≥ 3 per week) associated with reduced risk for incident late AMD (OR = 0.25, 95% CI 0.06–1.00)
POLANUT Delcourt et al. [5], 2007	2,584	Total OR 4.74, $p = 0.007$ Saturated OR 2.70, $p = 0.04$ Monounsaturated OR 3.50, $p = 0.03$ Total PUFA not significantly associated with ARM Total and white fish intake not significantly associated with ARM Fatty fish intake (>once/month vs. <once/month) OR 0.42, $p = 0.01$
AREDS Report 23 SanGiovanni et al. [8], 2008	2,132	Progression from bilateral drusen to central geographic atrophy among people who reported the highest levels of EPA (OR 0.44, 95% CI 0.23–0.87) and EPA + DHA (OR 0.45, 95% CI 0.23–0.90) consumption
AREDS Report 30 SanGiovanni et al. [9], 2009	1,837	Participants who reported the highest omega 3 LCPUFA intake (median 0.11% of total energy intake) were 30% less likely than their peers to develop central geographic atrophy and neovascular AMD; the respective ORs were 0.65 (95% CI 0.45–0.92, $p = 0.02$) and 0.68 (95% CI 0.49–0.94, $p = 0.02$)

Table 1 (continued)

Study, year	n	Results
Chong et al. [10], 2009	6,734	Trans-fat: OR 1.76 (95% CI 0.92–3.37, $p = 0.02$) Omega-3: OR 0.85 (95% CI 0.71–1.02, $p = 0.03$; early AMD) Olive oil intake: OR 0.48 (95% CI 0.22–1.04, $p = 0.03$; late AMD) No significant associations with AMD were observed for intakes of fish, total fat, butter or margarine
Blue Mountain Eye Study Tan et al. [6], 2009	3,654	Fish once per week: RR early AMD 0.68, 95% CI 0.48–0.98 (primarily in those with low linoleic acid consumption) Nuts once or twice per week: RR 0.65, 95% CI 0.47–0.91
Swenor et al. [7], 2010	2,520	AMD (choroidal neovascularisation or geographic atrophy) sufferers significantly less likely to consume fish/shellfish high in omega-3 fatty acids (OR 0.4, 95% CI 0.2–0.8)
Women's Health Study Christen et al. [11], 2011	39,876	Highest vs. lowest tertile for DHA intake vs. lowest tertile: RR 0.62, 95% CI 0.44 vs. 0.87 Highest vs. lowest tertile for EPA intake vs. lowest tertile: RR 0.66, 95% CI 0.48 vs. 0.92
Rotterdam Study Ho et al. [12], 2011	2,167	EPA/DHA risk reduction from 1.97 to 1.30
ALIENOR Merle et al. [13], 2011	666	High intake of long-chain omega-3 PUFA associated with a reduced risk for: Early AMD (OR 0.83, 95% CI 0.71–0.98, $p = 0.03$) Late neovascular AMD (OR 0.26, 95% CI 0.08–0.83, $p < 0.02$) Late atrophic AMD (OR 0.74, 95% CI 0.52–1.06, $p = 0.10$) Overall, high intakes of long-chain omega-3 PUFA were associated with a reduced risk for late AMD (OR 0.59, 95% CI 0.39–0.88, $p = 0.01$)
ALIENOR Merle et al. [20], 2013	963	High plasma total omega-3 PUFA associated with a reduced risk for late AMD: OR 0.62 for 1-SD increase (95% CI 0.44–0.88, $p = 0.008$) Similar for plasma: 18:3 omega-3: OR 0.62 (95% CI 0.43–0.88, $p = 0.008$) Omega-3 long-chain PUFA: OR 0.65 (95% CI 0.46–0.92, $p = 0.01$)
AREDS Reynolds et al. [14], 2013	2,531	Increased intake of DHA was significantly associated with reduced risk of progression to geographic atrophy Total omega-3 long chain polyunsaturated (DHA + EPA) fatty acid intake significantly associated with reduced risk of progression in model B (p trend = 0.02) Monounsaturated fat was associated with increased risk in model A (p trend = 0.05) DHA intake was significantly associated with reduced risk of incident geographic atrophy among those with the ARMS2/HTRA1 homozygous risk genotype (HR Q5 vs. Q1 0.4, $p = 0.002$, for interaction between gene and fat intake, $p = 0.05$) DHA was not associated with a reduced risk of geographic atrophy among those with the homozygous ARMS2/HTRA1 non-risk genotype (HR 1.0, $p = 0.90$)

experimental groups requiring 2 separate randomisations (fig. 1). Despite an extensive protocol, no real placebo group was included, the control group comprising subjects taking the AREDS1 formulation [500 mg vitamin C, 400 IU vitamin E, 15 mg beta carotene, 80 mg zinc (as zinc oxide), and 2 mg copper (as cupric oxide)] [24].

The subjects in AREDS2 were well educated (more than 60% were educated to degree level or higher), predominantly white (more than 95%) and well nourished. It can also be inferred that they were health conscious; more than 40% were taking cholesterol-lowering drugs and less than 7% were smokers (compared with the US average of 19%) [25]. Moreover, more than 10% of sub-

jects in the control groups took nutritional supplements contravening the protocol guidelines. Indeed, a number of key baseline nutritional parameters, including serum lutein, zeaxanthin and DHA/EPA, were significantly better in the AREDS subjects than for the US population as a whole, which is unexpected in AMD subjects according to the epidemiological evidence exposed above [1].

In the present context, the results regarding dietary supplementation with omega-3 fatty acids are of interest. In total, 1,749 patients were randomised to DHA/EPA supplementation and 1,691 to reference treatment. There were 507 advanced AMD events in the DHA/EPA supplementation group and 493 in the reference treatment

Primary randomization (AREDS with lutein and zeaxanthin, DHA and EPA, or both)

Secondary randomization (AREDS with no beta carotene, with low-dose zinc, or both)

(For legend see next page.)

groups, yielding an HR of 0.97 (95% CI 0.82–1.16, $p = 0.7$), suggesting that DHA/EPA does not significantly protect against AMD.

NAT2

The only other prospective randomised study of the prophylactic effect of omega-3 on AMD was reported almost simultaneously with AREDS2 [26]. Although this double-blind study only randomised 263 patients with early signs of AMD in the study eye and neovascular AMD in the other eye, the study specifically focused on a potential prophylactic effect of omega-3, mainly DHA, and therefore only two groups were required, one of which was a true placebo group (olive oil capsules). No background treatment was allowed in this study. In common with AREDS2, around half of all subjects were taking cholesterol-lowering drugs and the proportion of smokers was also relatively low. The primary efficacy variable was defined as the time to occurrence of choroidal neovascularisation (CNV) and the study continued for 3 years. In contrast to AREDS2, subjects in NAT2 who reported taking supplements not permitted in the protocol were not included in the efficacy analysis. The time to occurrence of CNV in the study eye was not significantly different between the DHA group (19.5 ± 10.9 months) and the placebo group (18.7 ± 10.6 months). Similarly, the incidence of CNV was not significantly different between the two groups (28.4 vs. 25.6%, respectively).

Prima facie the results of NAT2 do not appear different to those of AREDS2. The authors observed poor compliance in both arms, demonstrated by red blood cell membrane (RBCM) analysis. In addition, increased omega-3 consumption was demonstrated in the placebo group by RBCM analysis (EPA levels rose by 24% after 3 years). These two deviations led to a degree of overlap between the groups in the serum and RBCM EPA and DHA

levels that, in combination with the degree of patient dropout inherent in studies in older patient populations, led to a lack of discrimination between the two groups.

However, this study included a predefined RBCM analysis for all participants which revealed that, in the treated group, patients who maintained consistently high RBCM EPA/DHA levels (a secondary efficacy parameter) were significantly protected against AMD compared with those with permanently low EPA/DHA levels (HR 0.32, 95% CI 0.10–0.99), a finding somewhat analogous to that observed in the epidemiological studies described earlier (fig. 2).

A subsequent analysis of the NAT2 data indicates yet more strongly the relationship between polyunsaturated fatty acids and AMD incidence. Patients from the NAT2 study with neovascular AMD in one eye and early lesions in the other eye were compared with 144 normal controls without AMD. Not only was dietary oily fish and seafood consumption significantly lower in patients with AMD than in controls, but serum red blood cell EPA and EPA + DHA were associated with a substantial and significantly lower risk of neovascular AMD (OR = 0.25, 95% CI 0.13–0.47, $p < 0.0001$, and OR = 0.52, 95% CI 0.29–0.94; $p = 0.03$, respectively) [27].

Discussion

Clearly there are complementarities between the results of AREDS2 and NAT2 that need to be analysed in order to form a view on the utility of omega-3 prophylaxis in AMD. Although superficially similar, the two studies have a number of important methodological differences. Whilst AREDS2 enrolled an impressive 4,203 subjects, a complex protocol distributed them among 20 separate treatment groups in two randomisations that evaluated the effects of lutein/zeaxanthin, beta carotene and a lower dose of zinc, as well as that of DHA/EPA. In contrast, the NAT2 was focused specifically on the effects of DHA on the progression of AMD.

NAT2 included a true placebo group (olive oil), whilst the control group in AREDS2 received the nutritional supplement already found effective in AREDS1 (the control group was also randomised in the second randomisation), but no placebo for the DHA/EPA treatment. Patients in both studies appeared to be both well educated and health conscious, although the nutritional status of the AREDS2 group for DHA/EPA and some other parameters was significantly above the national average. In AREDS, 11% of the subjects reported taking DHA/EPA

Fig. 1. AREDS2 randomisation and patient paths. The original AREDS supplement comprised vitamin C (500 mg), vitamin E (400 IU), beta carotene (15 mg), zinc (80 mg, as zinc oxide) and copper (2 mg, as cupric oxide). ^a Patients could be excluded for more than one reason. ^b The participants assigned to the placebo group were also given the AREDS supplement either within or outside the secondary randomisation for the 4 variations of the AREDS supplements; thus, there is no true placebo group. ^c Smokers were not randomised to groups receiving beta carotene ($n = 181$, AREDS with no beta carotene; $n = 166$, AREDS with no beta carotene and with low-dose zinc).

Fig. 2. HRs for 3-year CNV incidence as a function of RBCM PUFA (area under the curve, AUC). HRs are for the higher versus lower quartile of EPA + DHA AUC for the 3 years of the NAT2 study [27]. PBO = Placebo.

supplements in violation of the protocol but remained in the study. In this context it is worthwhile noting that the serum level of DHA in the control group had increased by 14% by the end of the study. In NAT2, the protocol required that such non-compliant subjects were withdrawn from the study.

Such differences in methodology might account for some of the differences in the results between the two studies; the control group in AREDS2 received supplements already shown to be effective in the prevention of AMD progression and, moreover, more than 10% added DHA/EPA supplements to their diet. These two factors might be responsible for a ‘ceiling effect’ in AREDS2 that could make it more difficult to demonstrate a real effect of DHA/EPA. Such a hypothesis is supported by the finding in NAT2 that only those patients who had consistently raised DHA/EPA levels (mean 8.68% of total fatty acids in RBCM) had delayed progression of their disease. Such an analysis for the AREDS2 data is not currently available for the 244 patients whose DHA/EPA levels were measured at 3 years and, in any case, the AREDS2 investigators chose to measure serum DHA/EPA rather than RBCM DHA/EPA, the latter being a more reliable indicator of ongoing DHA/EPA status [28, 29].

There are further formulation differences between the two studies. Although the overall dose of omega-3 used was the same (1 g per day), the formulations used were different; in AREDS2 ethyl-esters of the fatty acids were administered with a DHA/EPA ratio of 1:2 compared with the triglyceride formulation with a DHA/EPA ratio of 3:1 in NAT2. Studies suggest that the bioavailability of the triglyceride formulation is significantly higher than that of the ethyl-ester fatty acid formulation [30]. Moreover, the differences in the proportion of DHA in the supplements may also be significant; DHA comprises around 50% of

the fatty acids in retinal photoreceptor membranes (compared with EPA that comprises merely 0.1%) and DHA has proven anti-oxidant, anti-inflammatory, anti-apoptotic, neuroprotective and anti-angiogenic properties [31]. Health claims regarding the role of DHA and EPA in the maintenance of vision have recently received approval from the European Food Safety Authority [32].

Epidemiological studies have, for more than a decade, pointed towards a beneficial effect of dietary omega-3 in the prophylaxis of AMD. Whilst it is tempting to accept the results of AREDS2 at face value, the contradictory results from NAT2 suggest that the situation could be somewhat more complex. NAT2 points to a situation where only those patients who achieve a consistent increase in DHA/EPA receive benefit from long-chain omega-3 supplementation (mainly DHA). The conditions of AREDS2 in which well-nourished and well-motivated patients, already well supplemented with the AREDS formulation, received a, perhaps, suboptimal formulation of DHA/EPA may not have been ideal to tease out any extra benefit from DHA/EPA.

AREDS and AREDS2 represent important steps towards elucidating the role of micronutrition in the prevention of AMD. However, given the findings in other interventional studies in this area and the limitations expressed above, we would urge caution in closing the chapter on omega-3, and instead suggest building on these findings with more detailed exploration of its role with different formulations and populations.

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