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Nutritional AMD treatment phase I (NAT-1): feasibility of oral DHA supplementation in age-related macular degeneration

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PURPOSE. *To create a pilot study in order to evaluate the feasibility of a prospective case-control study of oral supplementation with fish oil (docosahexaenoic acid [DHA]; eicosapentaenoic acid [EPA]) in a population with age-related macular degeneration (AMD).*
METHODS. *A homogeneous group of 38 patients with drusenoid pigment epithelial detachment in one eye (PED) without choroidal new vessels (CNV) was selected. A complete ophthalmologic examination, and a complete profile of fatty acids in serum (S) and in red blood cell membranes (RBCM), were recorded at day 0 and month 6. In group 1, 22 patients were orally supplemented with EPA (720 mg/day) and DHA (480 mg/day) during 6 months. In group 2, 16 patients were followed as controls. Nutritional recommendations on fish consumption were given to both groups.*
RESULTS. *In group 1, after 6 months supplementation we observed a significant blood enrichment in EPA (EPA-S: 2.20 vs 0.79, p<0.0001 and EPA-RBCM: 2.24 vs 0.85, p<0.0001) and in DHA (DHA-S: 2.47 vs 1.56, p<0.0001 and DHA-RBCM: 6.47 vs 4.67, p<0.0001). No change was observed in group 2 despite nutritional recommendations. In this short follow-up, no evolution to CNV was noted in either of the two groups. Neither side effects nor dropouts were observed in either of the groups.*
CONCLUSIONS. *This study supports the feasibility of a long-term double-masked prospective case-control study in an AMD population in order to evaluate a potential benefit from oral supplementation with DHA. (Eur J Ophthalmol 2009; 19: 100-6)*

KEY WORDS. *Age-related macular degeneration, Apoptosis, Dietary supplementation, Docosahexaenoic acid, Omega-3, Polyunsaturated fatty acid*

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INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in absence of appropriate treatment in the United States, Europe, and other developed countries. Approximately 30% of individuals 75 years or older have the mild or intermediate forms of the disease (age-related maculopathy [ARM]) (1), which include retinal pigment epithelial changes and drusen. A smaller percentage of individuals (6–8%) in this age group have the advanced form of AMD, which includes geographic atrophy (dry AMD) and exudative choroidal neovascularization (wet AMD) (1).
The pathogenesis of AMD remains unclear. It is most likely a multifactorial disorder, with both environmental and polygenic components (1). To date, other than family his-

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tory of AMD, increasing age, ethnicity, and cigarette smoking, only a few risk factors have been consistently shown to be related to the onset of this disease (1-7). Nutrition is also associated with AMD (8-11). The AREDS study demonstrated that vitamin and mineral supplements reduces the risk for progression to the advanced forms of AMD and subsequent visual loss (12). Dietary sources of xanthophyll pigments also appear to play a protective role, especially lutein and zeaxanthin (13-15).

Over the last decade, several lines of evidence have raised the possibility that higher intake of some fatty acids and elevated cholesterol blood levels are related with an increased risk of AMD (16). In contrast, several epidemiologic studies suggest that omega-3 polyunsaturated fatty acids (PUFAs) could have a protective role, particularly for exudative AMD (17-21). The retina has a high concentration of omega-3 PUFAs that are important in maintaining fluidity of photoreceptor membranes, retinal integrity, and function (21, 22). Furthermore, these types of fatty acids have been shown to have protective effects (antiapoptotic and antiangiogenic functions) (23, 24). Long chain omega-3 PUFAs include eicosapentaenoic acid (EPA, C20:5n-3) and docosahexanoic acid (DHA, C22:6n-3), both found primarily in oily cold-water fish such as tuna, salmon, and mackerel. DHA is the major polyunsaturated fatty acid within rod outer segments and plays a crucial role in the retinal lipid balance.

Our aim was to evaluate the feasibility of a prospective case-control study of oral supplementation with fish oil (EPA and DHA) in an AMD population. For this purpose, we performed a pilot study in selected series of patients with AMD, phenotypically homogeneous.

METHODS

The study was designed as a comparative study, not placebo-controlled, not double-blind, in an AMD population. Patients aged ≥55 years and <85 years with drusenoid pigment epithelial detachment (PED) without choroidal new vessels (CNV) were prospectively selected between October 1999 and April 2000 at the Retinal Department of Creteil University Hospital, in order to evaluate an homogenous subgroup of AMD. Given that AMD is a very heterogeneous disorder, in order to be able to compare the treated and the nontreated groups we had to select a homogenous population. Therefore, we decided to select a population where a lipidic imbalance could be involved (drusenoid PED) because of the lipidic component of confluent drusen. This study was performed in agreement with Declaration of Helsinki and French legislation, and was approved by our local ethics committee and institutional review board. In this 6-month trial, patients were randomly assigned to treatment (group 1, orally supplemented with fish oil, EPA 720 mg/day, and DHA 480 mg/day) or followed as controls (group 2, not orally supplemented). All patients from both series received a 60-minute interview with a nutritionist. Recommendations were provided in order to increase fish consumption in both groups at baseline.

A complete ophthalmologic examination including best-corrected visual acuity (BCVA) measured at 4 m with standard Early Treatment Diabetic Retinopathy Study (ETDRS) chart, fundus examination and red-free frame of the posterior pole, digital fluorescein angiography (FA), and optical coherence tomography (OCT 1 Humphrey Zeiss, San Leandro, CA) was performed in all patients at day 0 (D0) and month 6 (M6). OCT was performed to quantify the maximal elevation of drusenoid PED, using the tools of OCT software version 1 (two computer software-controlled cursors – calliper – that were manually placed on the superficial and deep boundaries). A complete profile of fatty acids in serum (S) and in red blood cell membranes (RBCM) performed by gas chromatography was recorded at day 0 and month 6. The ApoE ε4 allele frequency was also analyzed in our population and compared with our previously described control age-matched population (n=168) (25). Statistical calculations were performed using Epinfo 3.3 software package (CDC, Atlanta, GA). The Mann-Whitney/Wilcoxon two-sample test was used to compare ophthalmologic findings as well as the profile of fatty acids in group 1 and group 2. The chosen level of statistical significance was $p<0.05$.

RESULTS

Thirty-eight consecutive patients (72.74±6.25 years, 28 female, 10 male) were enrolled in this prospective 6-month pilot trial. In group 1, 22 patients were orally supplemented with fish oil (EPA: 720 mg/day and DHA, 480 mg/day) during 6 months. In group 2, 16 patients were followed without supplementation during 6 months as controls. Patients with milder disease in one eye, and more severe disease in the other eye, were equally included in group 1 and group 2. Neither side effects nor

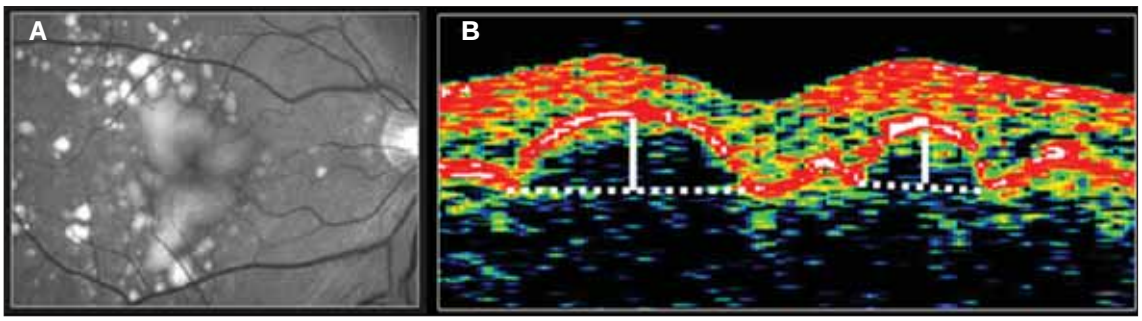

Fig. 1 - *Red-free frame* **(A)** *showing drusenoid pigment epithelium detachment (PED) in the macular area. Evaluation by optical coherence tomography at the mean elevation of the drusenoid PED* **(B)**.

dropouts were observed in either of both groups. No statistical difference for mean BCVA could be observed at M6 vs D0 in group 1 or in group 2 (mean BCVA D0 and M6 was 20/32 in group 1 and in group 2). During this short period of time, no progression was observed to central geographic atrophy. FA revealed no progression to CNV in any of the two groups within the 6-month period. On the interpretable OCT examinations (at least one 5 mm horizontal scan through the fixation point for each patient), the mean elevation of the drusenoid PED was lower in group 1 vs group 2 at M6 (148.42 µm vs 184.29 µm), but no significant statistical difference could be observed within 6 months (139.17 µm vs 140.34 µm, D0) ($p>0.05$) (Fig. 1). This result could probably be biased by the low resolution and absence of a standardized analysis software of OCT 1 (difficult quantification of elevation of the drusenoid PED).

The ApoE ε4 allele frequency was 0.09 in our population (n=38) vs 0.15 in control age-matched population (n=168). Cholesterol, triglycerides, and apoB showed no significant change at M6 in group 1 (6.14±0.78, 0.86±0.41, 1.09±0.19, respectively) and in group 2 (6.19±1.18, 1.05±0.72, 1.09±0.18, respectively) vs D0 (6.14±0.93, 0.91±0.34, 1.12±0.20, respectively in group 1; 6.33±0.99, 1.08±0.66, 1.09±0.17, respectively in group 2), except for mild decrease of triglycerides in group 1.

At day 0, EPA and DHA concentrations in % were similar and normal for age in group 1 and group 2 (EPA-S: 0.79 vs 0.83, EPA-RBCM: 0.85 vs 0.75; DHA-S: 1.56 vs 1.52 and DHA-RBCM: 4.67 vs 4.61, respectively) (Figs. 2 and 3). In group 1, after 6 months omega-3 fatty acid supplementation, we observed a significant blood enrichment in EPA (EPA-S: 2.20 vs 0.79; 179%, $p<0.0001$, and EPA-RBCM: 2.24 vs 0.85; 164%, $p<0.0001$) (Fig. 2) and in DHA (DHA-S: 2.47 vs 1.56; 58%, $p<0.0001$, and DHA-RBCM: 6.47 vs 4.67; 39%, $p<0.0001$) (Fig. 3). No change was ob-

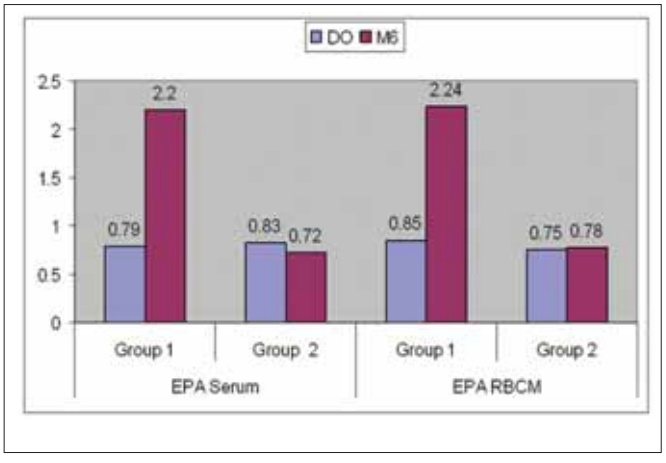

Fig. 2 - *Eicosapentaenoic acid values in treated and nontreated groups. EPA = eicosapentaenoic acid; RBCM = red blood cell membranes; D0 = day 0; M6 = month 6; group 1 = orally supplemented; group 2 = controls. Significant blood enrichment in EPA, in group 1, after 6 months omega-3 fatty acid supplementation ($p<0.0001$).*

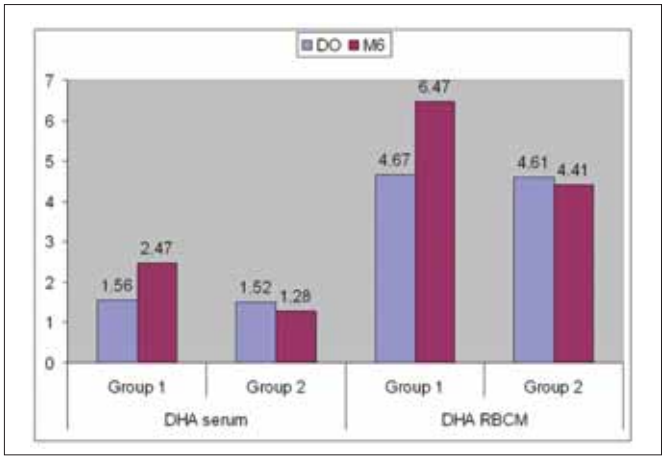

Fig. 3 - *Docosahexanoic acid values in treated and nontreated groups. DHA = docosahexanoic acid; RBCM = red blood cell membranes; D0 = day 0; M6 = month 6; group 1 = orally supplemented; group 2 = controls. Significant blood enrichment in DHA, in group 1, after 6 months omega-3 fatty acid supplementation ($p<0.0001$).*

served in group 2 (EPA-S: 0.72 vs 0.83 and EPA-RBCM: 0.78 vs 0.75; DHA-S: 1.28 vs 1.52 and DHA-RBCM: 4.41 vs 4.61) ($p>0.05$) (Figs. 2 and 3). Moreover, despite recommendations we provided in order to increase fish consumption in both groups at baseline, EPA (EPA-S and EPA-RBCM) and DHA (DHA-S and DHA-RBCM) concentrations after 6 months were statistically higher in group 1 compared to group 2 ($p<0.001$).

DISCUSSION

To date, etiology of AMD remains unclear. AMD appears to be a multifactorial disease. Several environmental risk factors have been evidenced, including smoking, elevated serum cholesterol levels, and systemic hypertension (1). However, a number of studies suggest that genetic factors contribute to its development (26).

Four recent independent epidemiologic studies have shown a significant relationship between high intake of DHA and a decreased risk of neovascular AMD (19-22). In addition, two recently published studies have evaluated the role of omega 3 fatty acids in animal models of retinal and choroidal neovascularization (23, 24). Connor et al (23) showed that increasing omega-3 PUFA tissue levels by dietary or genetic means decreased the avascular area of the retina by increasing vessel regrowth after injury, thereby reducing the hypoxic stimulus for neovascularization. The bioactive omega-3 PUFA-derived mediators neuroprotectinD1, resolvinD1, and resolvinE1 also potently protected against neovascularization. The protective effect of omega-3 PUFAs and their bioactive metabolites was mediated, in part, through suppression of tumor necrosis factor-alpha. This inflammatory cytokine was found in a subset of microglia that was closely associated with retinal vessels. These findings indicate that increasing the sources of omega-3 PUFA or their bioactive products reduces pathologic angiogenesis. Koto et al (24) demonstrated that an EPA-rich diet results in significant suppression of CNV and CNV-related inflammatory molecules in vivo and in vitro. The authors showed how, in vivo, the CNV volume in EPA-fed animals was significantly suppressed compared with that in control mice. Moreover, they found in vitro that the mRNA expression and protein levels of intercellular adhesion molecule (ICAM)-1, monocyte chemotactic protein (MCP)-1, vascular endothelial growth factor (VEGF), and interleukin (IL)-6 after CNV induction were significantly reduced in EPA-supplemented mice, and that EPA-fed mice exhibited significantly higher levels of EPA in the serum and the RPE-choroid than control animals.

From a safety point of view, the only known side effects of omega 3 fatty acids are digestive intolerance and a modification of biochemical parameters related to platelet aggregation in humans consuming very high quantities of omega 3 fatty acids (higher than 4000 mg/day). The US Food and Drug Administration (FDA) performed a comprehensive evaluation of more than 2600 articles on docosahexaenoic and eicosapentaenoic acids (DHA and EPA), the two long chain polyunsaturated omega-3 fatty acids found in fish oil. The FDA concluded that dietary intakes of up to 3 g daily of DHA and EPA were generally recognized as safe (Substances affirmed as generally recognized as safe: menhaden oil. Fed Regist June 5, 1997; 62: 30751-7). The safety profile of PUFA has been a strong argument to conduct a study with a nutritional supplement in elderly subjects who are often polymedicated and more prone to adverse side effects with classic lipid-lowering drugs (27).

The protective effect of DHA for AMD may be related to different mechanisms (20, 28, 29). According to Seddon et al (16), the antithrombotic and hypolipidemic effects of the long chain omega 3 fatty acids on the cardiovascular system could also exert a beneficial effect on the vasculature of the choroids. Furthermore, DHA is known to have specific retinal effects that are likely to play a preventive role in AMD, such as increased mitochondrial activity, increased RPE acid lipase activity, anti-oxidative, antiproliferative, and antiapoptotic effects (30).

DHA appears to play an important role in the normal development, morphology, and function of the retina (21, 31-33). In animals (rats, guinea pig), dietary deficiency in omega 3 fatty acids has been associated with altered function of the retina. Interestingly, impaired vision due to a long period of DHA deprivation remains reversible with a DHA enriched diet. More recently, studies in infants, premature or not, have confirmed the essential role of DHA for the functional development of the retina in humans (31). DHA represents the main component (>50%) of the lipids of the external membrane of the photoreceptors cells, suggesting a major role in the maintenance of the structure of these cells. Imbalance of the lipidic composition of the retina leads to the degradation of the photoreceptors, and accumulation of deposits of debris made of lipids and lipoproteins, localized at the level of retinal pigment epithelium cells. Since photoreceptor outer seg-

ments have a high DHA content and require a constant supply of these omega 3 fatty acids due to their continuous renewal, diets rich in DHA may improve retinal function and protect against the development of exudative AMD (16, 17).

Our purpose in this preliminary study called NAT-1 was to evaluate the feasibility of a prospective case-control study of oral supplementation with fish oil (EPA and DHA) in an homogeneous AMD population. Among 38 AMD patients without CNV, 22 patients were orally given DHA at the dose of 480 mg per day in combination with EPA for 6 months. Neither adverse effect nor dropout were observed. No statistical differences for mean BCVA were observed within the 6-month period in group 1 or in group 2 ($p>0.05$). During this short period of time, no progression was observed to central atrophy. FA revealed no progression to CNV in either of the two groups within the 6-month period. Indeed, one could object that such a small sample size of patients and short term of follow-up may be insufficient to detect any clinical result (fundus examination, BCVA, FA, and OCT) or adverse effect (also if the usual common effect that could have been observed, mainly diarrhea, usually occurs within the first weeks). Thus, no conclusion can be drawn from the ophthalmologic data in our series. On the other hand, a mild decrease of triglycerides (not statistically significant) was observed in group 1 during this short period of time. Interestingly, it was also observed that oral intake of 480 mg DHA significantly increased the levels in serum and red blood cell membranes, even in the elderly population.

At the time of NAT-1 patient recruitment, no epidemiologic study supported our hypothesis, excepting the one by Seddon et al (34).

It is notable that exudative AMD is less frequent in populations whose nutritional diet is rich in fish oil and that AMD has begun to appear in Iceland or Japan with the shift to Westernized diet (35-39). Moreover, to date eight studies report a beneficial effect of fish oil/DHA consumption for AMD (40). However, all these studies are non-interventional (based on nutritional questionnaire).The AREDS group also investigated the role of omega-3 in the occurrence of exudative AMD (19). They observed a significantly lower relative risk of neovascular AMD associated with higher consumption of omega-3. Higher fish consumption was also inversely associated with neovascular AMD. Conversely, dietary arachidonic acid was directly associated with neovascular AMD prevalence. Therefore, they concluded that a higher intake of omega-3 and fish was associated with decreased likelihood of having neovascular AMD. AREDS 2 is an ongoing interventional multicenter randomized trial designed to assess the effects of oral supplementation of high doses of macular xanthophylls (lutein and zeaxanthin) and/or omega-3 LCPUFAs (DHA and EPA) on progression to advanced AMD. This objective will be accomplished by collecting and assessing the data on approximately 4000 AREDS 2 participants aged 50 to 85 years, who at the time of enrollment have either 1) bilateral large drusen or 2) large drusen in one eye and advanced AMD (neovascular AMD or central geographic atrophy) in the fellow eye.

In our study, nutritional recommendation for increasing fish consumption did not show any effect on group 2 (not supplemented); EPA and DHA values did not substantially change from D0 to M6. These data would have been mainly due to the difficulty in changing habits in an elderly population. On the other hand, our AMD population did not show major troubles in absorption of omega-3, nor of incorporation to RBCM, which approximate incorporation to photoreceptors (20).

Of note, in our series, 480 mg DHA supplementation determined a mild increase in DHA-S and DHA-RBCM, compared to 720 mg EPA: this could be due either to the properties of DHA vs EPA, or to the different dosage. Thus, for the purposes of large study on DHA, we decided to increase the DHA dose, to over 700 mg/day.

This study shows that supplementation with EPA and DHA increases levels of these nutrients in the blood. There were no reported side effects within 6 months of supplementation. Results support the use of these nutrients in a double masked randomized trial in an AMD population. This clinical trial, NAT-2 study (which is ongoing), will evaluate a potential preventive effect by oral (three tablets daily) supplementation with DHA (840 mg daily) against the occurrence of new vessels.

The authors have no proprietary interest in the materials used in this study.

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