Oral Docosahexaenoic Acid in the Prevention of Exudative Age-Related Macular Degeneration

The Nutritional AMD Treatment 2 Study

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Objective: To evaluate the efficacy of docosahexaenoic acid (DHA)–enriched oral supplementation in preventing exudative age-related macular degeneration (AMD).

Design: The Nutritional AMD Treatment 2 study was a randomized, placebo-controlled, double-blind, parallel, comparative study.

Participants: Two hundred sixty-three patients 55 years of age or older and younger than 85 years with early lesions of age-related maculopathy and visual acuity better than 0.4 logarithm of minimum angle of resolution units in the study eye and neovascular AMD in the fellow eye.

Methods: Patients were assigned randomly to receive either 840 mg/day DHA and 270 mg/day eicosapentaenoic acid (EPA) from fish oil capsules or the placebo (olive oil capsules) for 3 years.

Main Outcome Measures: The primary outcome measure was time to occurrence of choroidal neovascularization (CNV) in the study eye. Secondary outcome measures in the study eye were: incidence of CNV developing in patients, changes in visual acuity, occurrence and progression of drusen, and changes in EPA plus DHA level in red blood cell membrane (RBCM).

Results: Time to occurrence and incidence of CNV in the study eye were not significantly different between the DHA group (19.5±10.9 months and 28.4%, respectively) and the placebo group (18.7±10.6 months and 25.6%, respectively). In the DHA group, EPA plus DHA levels increased significantly in RBCM (+70%; $P<0.001$), suggesting that DHA easily penetrated cells, but this occurred unexpectedly also in the placebo group (+9%; $P = 0.007$). In the DHA-allocated group, patients steadily achieving the highest tertile of EPA plus DHA levels in RBCM had significantly lower risk (−68%; $P = 0.047$; hazard ratio, 0.32; 95% confidence interval, 0.10–0.99) of CNV developing over 3 years. No marked changes from baseline in best-corrected visual acuity, drusen progression, or geographic atrophy in the study eye were observed throughout the study in either group.

Conclusions: In patients with unilateral exudative AMD, 3 years of oral DHA-enriched supplementation had the same effect on CNV incidence in the second eye as did the placebo. However, RBCM fatty acid measurements revealed that CNV incidence was significantly reduced in DHA-supplemented patients showing a steadily high EPA plus DHA index over 3 years.

Financial Disclosure(s): Proprietary or commercial disclosure may be found after the references. *Ophthalmology 2013;xx:xxx © 2013 by the American Academy of Ophthalmology.*

**Group members listed online in Appendix 1 (available at http://aaojournal.org).*

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in industrialized countries; its incidence increases with life expectancy.[1] Age-related macular degeneration is a multifactorial disease, with environmental risk factors such as smoking, dietary habits, or obesity interacting with genetic determinants.[2–4] Targeting lipid metabolism in AMD may prevent the development of exudative AMD. There is consistent evidence from a decade of epidemiologic observation in several populations that a high intake of docosahexaenoic acid (DHA; C22:6, n-3 [22 carbon chains and 6 double bonds, the first double bond is located at the third carbon from the omega end]), a long-chain omega-3 polyunsaturated fatty acid (PUFA) present in oily fish, is associated with a reduced risk of neovascular AMD.[2,5–14] Docosahexaenoic acid is an essential fatty acid partially converted into eicosapentaenoic acid (EPA; C20:5, n-3) after intestinal absorption that exerts numerous biological effects on blood vessels and tissues through signal transduction, gene regulation, and membrane structural and functional remodeling.[15] In the retina, DHA increases mi-

ISSN 0161-6420/13/$–see front matter
http://dx.doi.org/10.1016/j.ophtha.2013.01.005

tochondrial activity and has antioxidative, anti-inflammatory, antiapoptotic, and antiangiogenic effects.[16,17] Docosahexaenoic acid is a major lipid constituent (>50%) of photoreceptor membranes, where it plays a crucial role in maintaining their structural and functional integrity.[16] An imbalance in retinal lipids leads to photoreceptor degradation and the accumulation of lipid and lipoprotein debris in the retinal pigment epithelium layer.[16] Because the continuous renewal of retinal membranes requires a constant supply of omega-3 fatty acids by retinal pigment epithelium cells, diets rich in DHA may improve retinal function and may delay the development of exudative AMD.[2,5,16]

In inherited retinitis pigmentosa, DHA supplementation slightly slowed retinal degeneration, without systemic or retinal side effects.[18,19] The Nutritional AMD Treatment 1 pilot study found that DHA supplementation (480 mg/day) was well tolerated in a homogeneous group of AMD patients without choroidal neovascularization (CNV).[20] Moreover, DHA levels increased significantly in serum and red blood cell membranes (RBCMs), despite a reduced capacity to incorporate long-chain PUFA in cellular membranes in the elderly.[21]

The Food and Drug Administration considers that a daily intake of up to 3 g of fish oil containing DHA and EPA is safe.[22] Because large cardiovascular trials using long-chain PUFA supplementation in patients of similar age to those affected with AMD have proven effective in reducing cardiovascular morbidity and mortality, without causing any increase in comorbidities or generating drug interactions or major adverse events,[23] the authors hypothesized that long-term oral DHA at similar doses may be a safe and efficient approach for preventing exudative AMD. The aim of the Nutritional AMD Treatment 2 (NAT2) study was to evaluate the efficacy of oral PUFA supplementation enriched in DHA on the progression of AMD in a 3-year prospective, single-center, double-blind, randomized, placebo-controlled trial.

Patients and Methods

Study Participants

Patients were enrolled prospectively from December 2003 until October 2005 (last visit of last patient included, October 2008) in a single centre at the Department of Ophthalmology, Hôpital Intercommunal de Creteil, Creteil, France. Eligible patients were affected by early age-related maculopathy (any drusen or reticular pseudodrusen with or without pigmentary changes) in the study eye and neovascular AMD in the fellow eye (the study eye was not affected by CNV at entry). Analysis of drusen for recruitment was performed using the semiology for phenotyping AMD grid, validated for interobserver and intraobserver agreement.[24] Inclusion criteria were as follows: (1) age 55 years or older and younger than 85 years, (2) signed informed consent, (3) visual acuity better than +0.4 logarithm of minimum angle of resolution units in the study eye, and (4) patients likely to attend follow-up visits during the study period. The main exclusion criteria were: (1) CNV in both eyes or no CNV in either eye, (2) wide central subfoveal atrophy of the study eye, (3) progressive ocular diseases (severe glaucoma or other severe retinopathy), (4) major corneal or lens opacities precluding retinal evaluation, (5) serious systemic disease (cancer, stroke, etc.) preventing long-term participation, (6) known allergy to the substances used in the study (fish oil, fluorescein, indocyanine green), (7) anticoagulant therapy (prohibited medication) or bleeding tendency, (8) current or recent treatment (<6 months) with nutritional supplements (oral supplement containing long-chain omega-3 fatty acids or α-tocopherol acetate), (9) any concomitant nutritional supplement, (10) participation in a clinical trial within the previous 30 days, (11) history of drug use or excessive use of medication, (12) patients likely to be lost to follow-up or unlikely to comply with the study protocol, (13) monocular patients for reasons other than AMD, and (14) patients not covered by the French National Health system or wards of the court.

The study was reviewed and approved by the relevant institutional review board (Comité de Protection des Personnes, Paris-Ile de France 5, Paris, France). It was conducted in compliance with local regulations and was approved by the national advisory commission on databases computing personal information (Commission Nationale Informatique et Libertés). It complied with International Conference on Harmonization on Good Clinical Practice guidelines and the tenets of the Declaration of Helsinki (1975, revised in 2000).

Study Design

The NAT2 was a double-blind, prospective, randomized, parallel, comparative trial in patients with neovascular AMD in 1 eye receiving oral DHA or placebo over 3 years, for which the assessment of time to occurrence of CNV in the study eye was the primary efficacy end point. The overall study design is presented in Figure 1.

Intervention

After baseline examination, eligible patients were randomized in a 1:1 ratio to receive either 3 daily fish oil capsules, each containing 280 mg DHA, 90 mg EPA, and 2 mg vitamin E (Reti-Nat, provided by Bausch & Lomb, Montpellier, France), or placebo

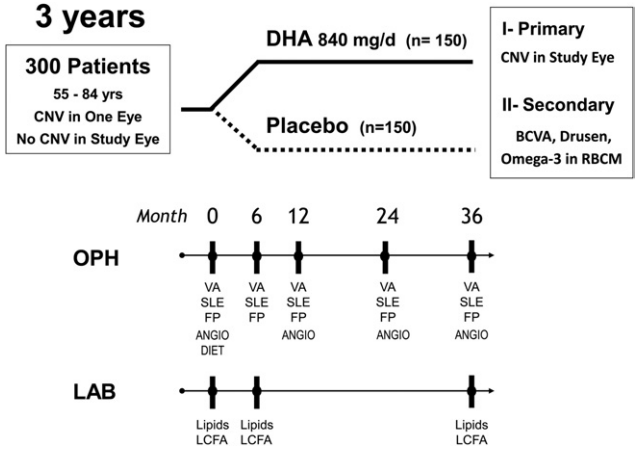


Figure 1. Diagram showing overall Nutritional AMD Treatment 2 Study design. AMD = age-related macular degeneration; ANGIO = fluorescein angiography, with or without indocyanine green; BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; DHA = docosahexaenoic acid; DIET = dietary habits assessed by food frequency questionnaire; FP = fundus photography; LAB = laboratory investigations; LCFA = long-chain fatty acids (eicosapentaenoic acid and DHA) in serum and red blood cell membranes (RBCM); lipids = plasma lipid profile (total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol); OPH = ophthalmologic investigations; SLE = slit-lamp examination; VA = visual acuity testing.

(602 mg olive oil). Based on the Nutritional AMD Treatment 1 study, the aim was to provide a dosage of between 600 and 1000 mg/day. The effective DHA dose thus was 840 mg/day, to be taken for 3 years on an outpatient basis. The capsules had the same appearance, the same size, and the same weight (602 mg) in both DHA and placebo groups. No masking flavor was added to the capsules, which were otherwise odorless. Participants were provided with 1 year's supply of capsules, with the medication dispensed during visits at baseline, year 1, and year 2. Treatment compliance was assessed during visits from unused capsules and serum PUFA levels. Prohibited medication or use of any other drugs was checked at each visit and recorded in the case report form.

Randomization and Blinding

QL-Ranclin software (Qualilab, Olivet, France) was used to generate the randomization list before enrollment. The patients and the study personnel both were blinded to the treatment assignment.

Examination Schedule

Patients were examined at baseline (visit 1), 6 months (visit 2), 1 year (visit 3), 2 years (visit 4), and 3 years (visit 5). The visit schedule allowed a ±15-day delay for visit 2 and a ±30-day delay for other visits. At baseline, clinical and ophthalmologic examinations of potentially eligible patients were checked against inclusion and exclusion criteria. Recorded data included demographic information, relevant ocular and medical history, and concomitant treatment. The following examinations were performed at each visit: (1) best-corrected visual acuity, (2) slit-lamp examination, (3) fundus photography, and (4) fluorescein angiography. Fundus photography and fluorescein angiography were not performed at baseline if they had been performed already within the previous 30 days with the same procedure. In addition, a food frequency questionnaire was completed by telephone with participants at baseline visit (see Appendix 2 for details, available at http://aaojournal.org).[25] Venous blood (15 ml, 12-hour fasting) was collected at the baseline, 6-month, and 3-year visits to monitor short-term and long-term changes in plasma lipoproteins, serum, and RBCM EPA-plus-DHA content.

Outcome Measures: Main and Secondary

The primary outcome was the time to occurrence of CNV in the study eye. The secondary outcome efficacy measures were: (1) percentage of patients in whom CNV developed; (2) changes in visual acuity from baseline, measured in logarithm of minimum angle of resolution (logMAR) units, and the proportion of patients with a visual acuity decrease of 15 letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart; (3) drusen burden and progression, based on automatic detection of their number, size, and area on fundus photography[26]; and (4) changes in RBCM EPA-plus-DHA levels. Safety was assessed by determining ocular and systemic tolerance to study treatment and included slit-lamp examination and evaluation of lens opacity. Blood lipids were assessed to check patient compliance and for safety reasons. Systemic tolerance was estimated from fasting plasma lipoprotein profile, signs of intolerance related to fish oil consumption, and occurrence of systemic adverse events.

Measurement of Study Variables

Ophthalmology. Fluorescein angiography was performed to screen for the presence of CNV at each planned visit and in patients experiencing visual symptoms at any time during the study. In the latter case, patients immediately were asked to return for further examination. Fluorescein angiography was performed to detect the presence and to specify the location (extrafoveal, juxtafoveal, or subfoveal) and type (classic or occult) of CNV. Indocyanine green angiography was performed to confirm the diagnosis in patients with suspected CNV. All patients with CNV were treated with laser, photodynamic therapy, or anti-vascular endothelial growth factor (VEGF) intravitreal injections according to the decision of the investigators. Further details on angiographic and all other procedures are provided in Appendix 2 (available at http://aaojournal.org). Changes in visual acuity were measured in logMAR units and by standardized refraction and visual acuity protocol by experienced certified examiners. At each visit, the same examiner evaluated the best-corrected visual acuity before pupil dilation. Furthermore, the proportion of patients with a decrease of 15 letters or more was another secondary parameter in the NAT2 study. For this purpose, the number of letters counting on ETDRS charts were recorded. A decrease in the identification of more than 15 letters was considered significant.

Changes in drusen were determined by fundus photography through the dilated pupil at each visit using a Topcon 501A CCD camera (Topcon, Tokyo, Japan) to record images centered on the macula. Automated drusen segmentation was performed by geometric background leveling and threshold selection, an automated solution (Matlab 7.0; The Mathworks, Inc, Natick, MA) to analyze fundus photographs and autofluorescence images, as previously described[26] (see Supplementary Material, Appendix 2 for details, available at http://aaojournal.org). Lens opacity was evaluated through dilated pupils and was rated on the Lens Opacities Classification System II scale.[27]

Biology. Overnight fasting blood samples were delivered to a single clinical chemistry laboratory (Hôpital Saint Antoine, APHP, Paris) within 5 hours and processed immediately. A detailed description of laboratory methods is given in Appendix 2 (available at http://aaojournal.org). Briefly, plasma total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein cholesterol were measured by enzymatic colorimetric and electrophoretic methods as previously described.[28] Fatty acids from serum and RBCM were transmethylated by diazomethane and were analyzed by gas chromatography (see details Appendix 2, available at http://aaojournal.org). Results for EPA and DHA content were expressed as a percentage of the total fatty acid profile in serum and RBCM.

Sample Size Determination

The sample size calculation was performed for a survival analysis (log-rank test). The time to event (here, occurrence of CNV) was estimated on the basis of a 10% to 12% annual rate of CNV occurrence (33% over 3 years).[29] The sample size calculation took into account a regular recruitment and an inclusion period of 2 years. On the basis of previous studies, a 40% reduction in CNV occurrence over time was hypothesized.[6,8,11,30,31] The sample size was calculated assuming that the 3-year risk of developing CNV in the study eye was 19.8% and 33% for the DHA and placebo groups, respectively, with regular occurrence of CNV over time. With a 0.05 type I error, a 0.20 type II error (80% power), and an expected dropout rate of 10%, 298 patients were to be included in the study. Three hundred patients were recruited, expecting 150 patients in each study arm.

Statistical Analyses

The safety population included all randomized patients who received at least 1 unit of the study medication. The full analysis set

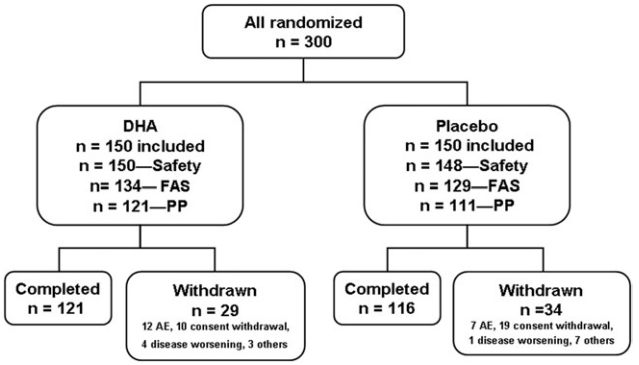


Figure 2. Diagram showing the populations of the Nutritional AMD Treatment 2 Study. The safety population included all subjects who were confirmed to have received the study treatment. Full analysis set (FAS) included all subjects in the safety set having at least 1 postbaseline value regarding occurrence of choroidal neovascularization. Per protocol (PP) population included all FAS subjects with no major protocol deviation. Among those withdrawn were 3 deaths in the docosahexaenoic acid (DHA) group and 6 deaths in the placebo group.

(FAS) was defined as all patients in the safety set with at least 1 postbaseline visit assessing whether CNV had occurred in the study eye. The per-protocol (PP) population included patients from the FAS without major deviation from the protocol that could jeopardize the primary outcome (Fig 2). Any temporary discontinuation of the treatment was considered to be a deviation from the study protocol. Discontinuation for more than 5 months was considered to be a major deviation from the study protocol. Participants who dropped out were taken in account in the survival analysis and occurrence of CNV and were counted at last angiography performed. Descriptive statistics were performed for baseline characteristics of the FAS. The incidence of CNV in the 2 treatment groups was compared using life-table methods. Treatments were compared using a Cox model adjusting for age, smoking status, and maculopathy stage at baseline. A secondary analysis of the difference between treatments used a second Cox model with 4 additional factors: gender, family history of AMD, baseline DHA level in the RBCM, and body mass index. Secondary analyses on a prespecified secondary efficacy criterion studied the risk of CNV occurrence according to DHA plus EPA levels. The values of the area under the receiver operating characteristic curve (AUC) of EPA plus DHA levels in serum and RBCM were calculated in patients from the FAS with data available at visits 1, 2, and 5 to assess the level of these fatty acids throughout the study. Hazard ratios for occurrence of CNV according to tertiles of AUC of serum or RBCM EPA plus DHA were compared using Cox modeling.

Secondary efficacy and safety qualitative data were compared for the 2 treatment groups using the chi-square or Fisher exact tests. Quantitative data were compared between visits and across treatment groups using the Student *t* test or Wilcoxon rank-sum test, depending on data normality. Adverse events were counted and described by visit. Where a nonnegligible number of adverse events was reported, the percentage of patients with 1 or more adverse events was compared across groups using the chi-square test. Serious adverse events were counted and described. Statistical analyses were carried out using SAS software version 8.2 for PC (SAS Institute, Inc, Cary, NC). The study was registered on the International Standard Randomized Controlled Trial Number Register and was allocated registration number ISRCTN98246501.

Results

Baseline Characteristics

Among the 300 randomized patients, 298 were included in the safety set (150 patients in the DHA group and 148 in the placebo group), and 263 had at least 1 postbaseline assessment of CNV and could be included in the FAS. Baseline characteristics are described in Tables 1 and 2. Patients were 73.5 years of age, on average. There were more women (64.6%) than men, with a mean body mass index of 25.68 kg/m^2. Mean age at AMD diagnosis was 70.7 years. Approximately 1 in 4 patients had a family history of AMD. The results of ophthalmic evaluation and description of AMD in the study eye were similar in both groups regarding visual acuity, the number and type of drusen, stage of maculopathy, and presence of atrophy. Approximately two-thirds of patients had cataracts. In the fellow eye, the mean ± standard deviation (SD) visual acuity was 1.040±0.487) logMAR in the DHA group and 1.044±0.507 logMAR in the placebo group, corresponding to the values of legal blindness (>1 logMAR). Most patients had been treated previously for AMD (91.0% in the DHA group and 86.0% in the placebo group) in the fellow eye (nonstudy eye). Photodynamic therapy (PDT; 61.5%) and laser photocoagulation (54.5%) were the most frequent treatments in the fellow eye. At baseline, PDT had been performed significantly more often in the DHA group than in the control group (67.3% vs. 54.8%; $P<0.05$) and laser photocoagulation was performed less frequently (50.5% vs, 59.1%; not significant).

The vast majority of patients were nonsmokers or former smokers. They had a past medical history mainly represented by cardiovascular and metabolic disorders: hyperlipidemia (60%), hypertension (58%), coronary heart disease (19%), and venous insufficiency or thrombosis (19%). Musculoskeletal and connective tissue disorders mainly were represented by osteoarthritis (41%), and gastrointestinal disorders mainly were represented by gastric acidity-related diseases (19%). Accordingly, most patients (98%) reported concomitant medications for these conditions. Hyperlipidemia or coronary heart disease was treated by statins in 79% of patients and by fibrates in 33% of patients.

At study entry, total energy, total fat, omega-3–to–omega-6 ratio, polyunsaturated fat, and DHA intakes estimated from the food frequency questionnaire did not differ between groups (Table 2). Plasma lipids and lipoprotein values were similar in both groups. Levels of EPA or DHA measured in serum and RBCM were consistent with PUFA and DHA intake estimated from the food frequency questionnaire. In summary, demographic and other baseline characteristics were similar in the DHA and placebo groups.

Postbaseline Analyses and Study Protocol Compliance

The major cause of deviation from the study protocol was premature withdrawal from the study, which occurred at a similar rate in the DHA and placebo groups (7.5% and 10.1%, respectively; $P = 0.59$; Fig 2). Other causes of major deviations included noncompliance with study treatment and use of nonpermitted medication. For the FAS survival analysis, 23 patients who were withdrawn prematurely had undergone at least 1 angiography after baseline with no CNV at the time of last angiography. Their data thus were censored at the time of last angiography. The PP population thus consisted of 232 patients (121 in the DHA group and 111 in the placebo group). Moreover, in the FAS, 25.1% of patients discontinued treatment during the study (Table 3, available at http://aaojournal.org). Overall compliance was calculated from cross-information of capsules taken (<80% and ≥80%) and treatment interruption (>20% and ≤20% of the treatment duration). Over the 3

Table 1. Baseline Clinical Characteristics of Nutritional AMD Treatment 2 Study Participants from the Full Analysis Set

	Full Analysis Set (n = 263)	
	Docosahexaenoic Acid Group (n = 134)	*Placebo Group* (n = 129)*
Mean age ± SD (yrs)	73.9±6.6	73.2±6.8
Gender (%)		
Male	31.3	39.5
Female	68.7	60.5
BMI (kg/m^2)	25.45±4.01	25.93±3.97
Mean age at AMD diagnosis ± SD (yrs)	71.1±7.4	70.2±7.9
Family history of AMD (%)	21.6	27.1
Ophthalmic evaluation of the study eye		
Mean visual acuity ± SD (logMAR units)	0.14±0.14	0.12±0.15
Cataract (% subjects)	61.2	62.0
AMD description in the study eye (%)		
Soft drusen		
Absent	0.7	0.0
<5	0.7	2.3
5–20	17.2	21.7
>20	81.3	76.0
Pigmentary changes (% subjects)	23.1	21.7
Stage of maculopathy		
1 (≥1 soft drusen or pigmentary changes)	77.6	78.3
2 (≥1 soft drusen with pigmentary changes)	22.4	21.7
Noncentral atrophy (% subjects)	18.7	12.4
Former treatment for AMD for fellow eye at baseline (%)†	91.0	86.0
Nutritional supplementation	54.9	58.6
All other therapy for fellow eye	88.5	84.7
PDT therapy for fellow eye	67.3	54.8
Laser treatment for fellow eye	50.5	59.1
Macular atrophy in the fellow eye at baseline (%)	16.4	11.6
Smoking history (%)		
Current smoker	6.7	8.5
Former smoker‡	14.2	17.1
Nonsmoker§	79.1	74.4
Past medical history (%)	97.0	98.4
Cardiovascular disorders	92.5	79.8
Metabolism and nutrition disorders	53.0	58.9
Musculoskeletal and connective tissue disorders	44.8	48.8
Gastrointestinal disorders	29.9	32.6
Concomitant medications (%)	97.8	99.2
Lipid-lowering agents	48.5	52.7
Agents acting on the renin-angiotensin system	41.8	35.7
Anti-inflammatory and anti-rheumatic products	15.7	28.7
Drugs used in diabetes	11.9	10.1

AMD = age-related macular degeneration; BMI = body mass index; logMAR = logarithm of the minimum angle of resolution; SD = standard deviation.
*No significant difference between the docosahexaenoic acid and placebo allocated group for all parameters except photodynamic therapy for fellow eye ($P<0.05$).
†Former treatment for AMD. Percent are given for subjects who received nutritional or other nonnutritional therapy.
‡Smoking cessation for fewer than 20 years.
§Smoking cessation for 20 years or more.

years, the proportion of compliant patients was similar in both groups; a minimum compliance of 78% was observed at years 1, 2, and 3.

Occurrence of Choroidal Neovascularization in the Study Eye

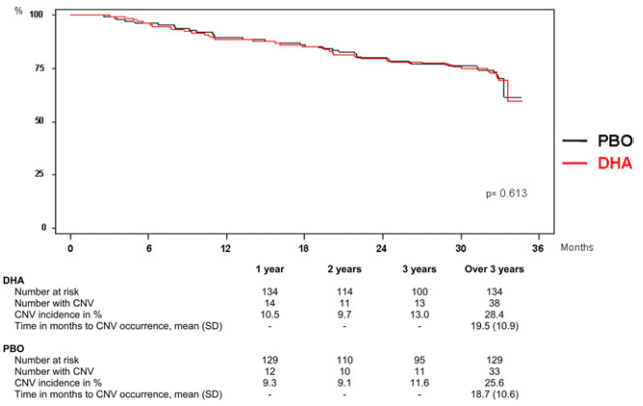
In the FAS, the mean time to occurrence of CNV was 19.1 months and was similar in the DHA and placebo groups (Fig 3). The overall proportion of patients in whom CNV developed over 3 years in the study eye was 27%, increasing progressively from 9% to 10% at year 1 and up to 12% to 13% at year 3. The first Cox model analysis taking into account the main adjustment factors (age at randomization, smoking status, and stage of maculopathy) confirmed no statistically significant difference between groups ($P = 0.613$; hazard ratio, 0.89; standard error, 0.272; 95% confidence interval, 0.55–1.42). Similar results were obtained with the

Table 2. Baseline Nutritional and Biological Characteristics of Nutritional AMD Treatment 2 Study Participants from the Full Analysis Set

	Full Analysis Set (n = 263)	
	Docosahexaenoic Acid Group (n = 134)	*Placebo Group* (n = 129)*
Food questionnaire (mean ± SD)		
Total energy (kcal/day)	1525±503	1518±502
Total fat (g/day)	60.4±25.4	58.8±24.7
Omega-3–to–omega-6 ratio	0.13±0.06	0.12±0.06
Polyunsaturated fat (g/day)	10.67±6.01	11.44±6.02
DHA intake (mg/day)	115±82	117±87
Laboratory data, median (5th–95th percentiles) or mean ± SD		
Triglycerides (mmol/l)	0.98 (0.49–2.31)	1.00 (0.46–2.17)
Total cholesterol (mmol/l)	5.78±1.10	5.65±0.91
HDL cholesterol (mmol/l)	1.80±0.52	1.76±0.56
LDL cholesterol (mmol/l)	3.84±1.06	3.73±0.87
Total cholesterol–to–HDL cholesterol	3.43±1.07	3.51±1.21
DHA in serum (% total fatty acids)†	1.26 (0.67–2.27)	1.29 (0.64–2.62)
EPA in serum (% total fatty acids)†	0.60 (0.25–1.31)	0.62 (0.25–1.64)
EPA plus DHA in serum (% total fatty acids)†	1.90 (1.14–3.42)	1.92 (0.96–3.95)
DHA in RBCM (% total fatty acids)†	3.04 (1.80–5.27)	3.21 (1.75–4.90)
EPA in RBCM (% total fatty acids)†	0.59 (0.30–1.09)	0.60 (0.32–1.16)
EPA plus DHA in RBCM (% total fatty acids)†	3.68 (2.23–6.16)	3.79 (1.78–5.71)

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HDL = high-density lipoprotein; LDL = low-density lipoprotein; RBCM = red blood cell membranes; SD = standard deviation.
*No significant difference between the DHA and placebo allocated groups for all parameters.
†Eicosapentaenoic acid and DHA content are expressed as the percentage of 1 fatty acid abundance relative to that of other medium- and long-chain fatty acids extracted from serum or red blood cell membranes.

second Cox model adjusting for additional factors (gender, body mass index, baseline RBCM DHA level, and family history of AMD). Adjustment factors had no significant association with time to occurrence of CNV, except for the stage of maculopathy at baseline ($P = 0.007$). In the PP population, the 3-year incidence of CNV also was similar in the DHA and placebo groups (29.8% and 29.7%, respectively; $P = 0.997$), and analyses using both Cox models gave similar results as those observed in the FAS population (first Cox model analysis on the PP population: $P = 0.774$; hazard ratio, 0.93; standard error, 0.289; 95% confidence interval, 0.58–1.50).



	1 year	2 years	3 years	Over 3 years
DHA				
Number at risk	134	114	100	134
Number with CNV	14	11	13	38
CNV incidence in %	10.5	9.7	13.0	28.4
Time in months to CNV occurrence, mean (SD)	-	-	-	19.5 (10.9)
PBO				
Number at risk	129	110	95	129
Number with CNV	12	10	11	33
CNV incidence in %	9.3	9.1	11.6	25.6
Time in months to CNV occurrence, mean (SD)	-	-	-	18.7 (10.6)

Figure 3. Graph and table showing time to occurrence of choroidal neovascularization (CNV) in the docosahexaenoic acid (DHA) and placebo (PBO) groups (full analysis set). **Top**, Survival curves showing the percentage of study participants remaining free of CNV across the study period. **Bottom**, Table showing total number of participants, number of participants with CNV, and incidence and time to occurrence of CNV at 1 year, 2 years, 3 years, and over the study period. SD = standard deviation.

Incidence of Choroidal Neovascularization as a Function of Eicosapentaenoic Acid plus Docosahexaenoic Acid Levels

A large variability in DHA and EPA levels in serum and RBCM was observed in both groups (Table 4). In the DHA-allocated group, DHA and EPA increased markedly and consistently in both serum and RBCM from as early as 6 months after treatment initiation, further increasing significantly from 6 months to the 3-year follow-up (not shown). Of note, relative EPA increase from baseline was more pronounced than DHA in serum or RBCM. Median RBCM levels of EPA plus DHA also increased modestly, but significantly, in the placebo group at the 3-year follow-up. This mainly was the result of a mild but significant increase in RBCM level of EPA at 6 months and 3 years.

As shown in Figure 4, baseline tertiles of EPA plus DHA levels in plasma and RBCM were similar in both groups. However, despite a strong and significant postbaseline increase in DHA and EPA levels in the DHA group, there was a broad postbaseline overlap between the lower (first) tertiles of serum EPA plus DHA levels in the DHA group and the higher (third) tertiles in the placebo group (Fig 4). The picture was similar for EPA plus DHA in RBCM, a prespecified secondary efficacy end point. Remarkably, in the DHA group only, patients from the higher tertile clearly departed from the lower tertiles, with more than doubling of EPA plus DHA levels in serum and RBCM from baseline to follow-up. This prompted an investigation of whether steadily high DHA levels on the long term could be protective against CNV occurrence in AMD patients receiving continuous oral supplementation enriched in DHA over 3 years.

Table 4. Docosahexaenoic Acid and Eicosapentaenoic Acid Content in Serum and Red Blood Cell Membranes in Patients from the Full Analysis Set with Documented Docosahexaenoic Acid and Eicosapentaenoic Acid Measures from Baseline to Follow-up (n = 230)

	Docosahexaenoic Acid Group (n = 118)		Placebo Group (n = 112)		*P* Value, Docosahexaenoic Acid Group versus Placebo Group
	Median (5th–95th Percentile)	*% Change from Visit 1 (P Value)*	*Median (5th–95th Percentile)*	*% Change from Visit 1 (P Value)*	
DHA in serum					
Visit 1	1.26 (0.70–2.28)	—	1.29 (0.63–2.62)	—	0.230
Visit 2	2.22 (1.28–3.32)	+73% (<0.001)	1.24 (0.61–2.19)	–9% (0.029)	<0.001
Visit 5	2.38 (1.31–3.56)	+89% (<0.001)	1.28 (0.65–2.17)	−6% (0.317)	<0.001
EPA in serum					
Visit 1	0.60 (0.25–1.31)	—	0.62 (0.27–1.85)	—	0.154
Visit 2	1.28 (0.49–2.56)	+113% (<0.001)	0.69 (0.30–1.66)	+14% (0.252)	<0.001
Visit 5	1.47 (0.56–2.59)	+120% (<0.001)	0.72 (0.25–1.97)	+15% (0.083)	<0.001
EPA plus DHA in serum					
Visit 1	1.90 (1.14–3.46	—	1.91 (0.96–4.07)	—	0.759
Visit 2	3.55 (1.81–5.18)	+82% (<0.001)	1.90 (1.00–3.31)	−1% (0.496)	<0.001
Visit 5	3.89 (2.05–6.10)	+95% (<0.001)	2.02 (0.98–4.09)	+1% (0.018)	<0.001
DHA in RBCM					
Visit 1	3.03 (1.61–5.22)	—	3.26 (1.90–4.90)	—	0.383
Visit 2	4.71 (3.06–6.94)	+45% (<0.001)	3.08 (1.74–4.94)	−7% (0.094)	<0.001
Visit 5	5.21 (2.75–7.04)	+58% (<0.001)	3.27 (2.11–4.95)	+5% (0.238)	<0.001
EPA in RBCM					
Visit 1	0.58 (0.30–1.09)	—	0.61 (0.33–1.16)	—	0.646
Visit 2	1.20 (0.55–1.87)	+99% (<0.001)	0.66 (0.36–1.26)	+12% (0.006)	<0.001
Visit 5	1.55 (0.62–2.51)	+137% (<0.001)	0.80 (0.38–1.59)	+24% (<0.001)	<0.001
EPA + DHA in RBCM					
Visit 1	3.67 (2.10–6.00)	—	3.93 (2.48–5.84)	—	0.377
Visit 2	6.09 (3.85–8.58)	+55% (<0.001)	3.72 (2.19–5.69)	−6% (0.272)	<0.001
Visit 5	6.77 (4.04–9.21)	+70% (<0.001)	4.19 (2.67–6.14)	+9% (0.007)	<0.001

— = no % change; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; RBCM = red blood cell membrane.
Eicosapentaenoic acid and DHA content are expressed as the percentage of 1 fatty acid abundance relative to that of other medium- and long-chain fatty acids extracted from serum or RBCM.

As a secondary prespecified outcome, the AUC of EPA plus DHA levels measured at baseline, 6 months, and follow-up in serum and RBCM were analyzed as surrogates of long-term DHA supplementation status. The risk of CNV developing in the study eye was assessed from the Cox model by comparing CNV incidence in the lower and higher tertiles for AUC of EPA plus DHA levels over 3 years in both groups (Fig 5). Only in the DHA group, a 68% risk reduction of CNV was observed for 3-year EPA plus DHA contents in RBCM ($P = 0.047$) and in serum, although this last association did not reach statistical significance ($P = 0.06$). In the group of patients allocated to DHA, the 3-year incidence of CNV was 14.3% (4/28) in the higher tertile of AUC of levels of EPA plus DHA in RBCM versus 32.5% (13/40) in the lower tertile. Similarly, CNV incidence in the higher tertile of AUC of serum levels of DHA plus EPA was 11.1% (3/27) compared with 32.5% (13/40) in the lower tertile. This suggested that a subgroup of AMD patients who exhibited sustained high EPA plus DHA content in serum and membranes seemed to be protected from CNV after 3-year continuous fixed-dose DHA-enriched oral supplementation.

Other Ocular Efficacy End Points in the Study Eye

In the FAS, a decrease in mean visual acuity (in logMAR units) was observed in the study eye in both groups throughout the study (Table 5). Most patients had stable vision or a decrease of fewer than 15 letters on the ETDRS chart at 3 years (82.2% in the DHA group vs. 85.7% in the placebo group). At the last visit, there were no significant differences between DHA and placebo groups for changes from baseline in visual acuity measured in logMAR units and for the proportion of patients with a decrease of more than 15 letters on the ETDRS chart.

Drusen were assessed only in the population of patients where CNV had not occurred in the study eye during the study, because drusen could not be analyzed clearly after onset of CNV. The mean number of small, intermediate, and large drusen was slightly lower in the DHA group than in the placebo group at visits 1 and 5. The mean number of intermediate drusen decreased slightly in both groups between visits 1 and 5. The mean number of small and large drusen remained relatively stable in both groups between visits 1 and 5. The evolution of the number of drusen was not significantly different between the 2 groups, whatever the size of the drusen. The progression of the total area of all drusen was not significantly different between groups. At baseline, 94 patients presented reticular drusen in the NAT2 study.[32] The conversion of reticular drusen to wet AMD was not different between DHA and placebo groups. Similarly, progression to geographic atrophy was not significantly different between the 2 groups, whatever the size of the drusen at baseline.

Safety

Adverse Events. The frequency of adverse events (AEs) was not significantly different in the DHA and placebo groups (Table 6, available at http://aaojournal.org). Five patients in the DHA group and 2 patients in the placebo group experienced at least 1 treatment-emergent AE (gastrointestinal disorders, allergic dermatitis, or

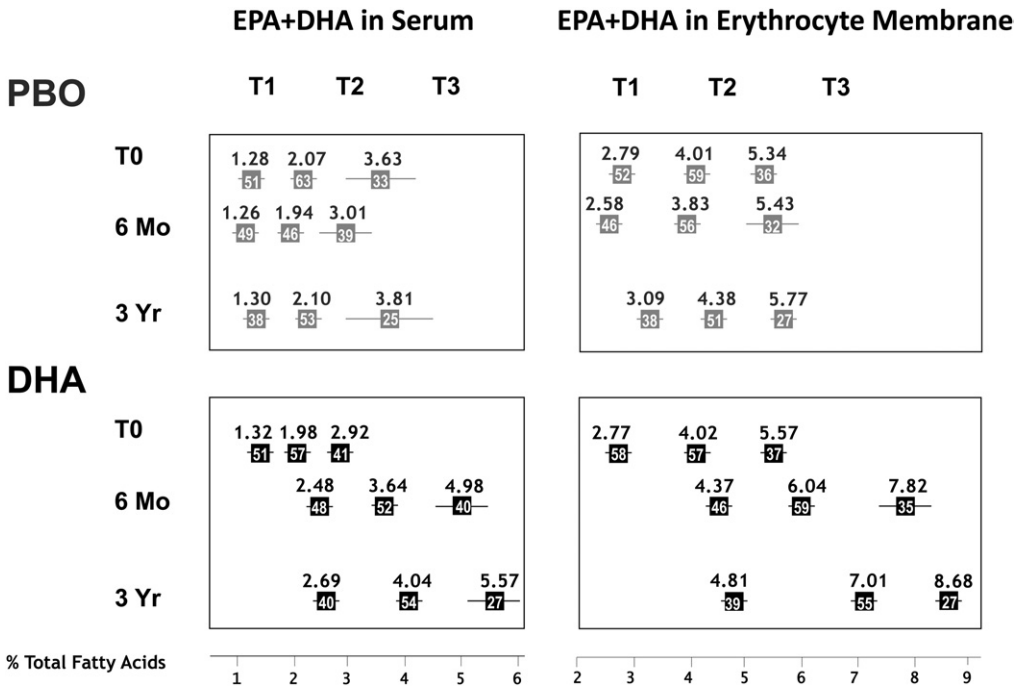


Figure 4. Graphs showing the distribution by tertiles of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) levels in placebo (PBO) and DHA groups at each time point of the study. T1, T2, and T3 = first, second, and third tertiles of EPA plus DHA levels, respectively, measured at baseline (T0), 6 months (6 Mo) and 3 years (3 Yr). Numbers above symbols represent the mean for each tertile of levels. Lines across symbols represent the standard deviation. Numbers within symbols represent the number of individuals in each group. **Top**, Distribution of EPA plus DHA levels observed in (**Left**) serum and (**Right**) red blood cell membrane (RBCM) in the PBO group. **Bottom**, Distribution of EPA plus DHA levels observed in (**Left**) serum and (**Right**) RBCM in the DHA group.

breath odor) considered to be probably related to the study treatment.

Approximately 50% of all reported AEs were ocular, mainly represented in both groups by a worsening of macular degeneration, cataract, and reduced visual acuity in the fellow eye. In the fellow eye previously treated for advanced exudative AMD at baseline, best-corrected visual acuity decreased further over the study period: from 1.042±0.496 logMAR at baseline up to

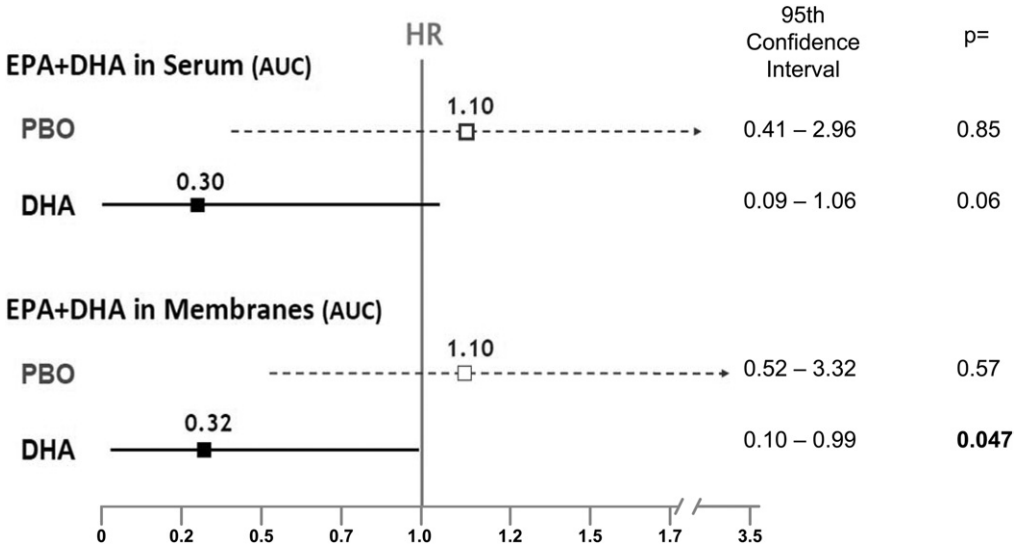


Figure 5. Graph showing the hazard ratio (HR) for 3-year choroidal neovascularization (CNV) incidence as a function of the area under the receiver operating characteristic curve (AUC) of polyunsaturated fatty acid levels measured in serum and red blood cell membranes over the study period. Numbers above symbols represent the HR for CNV incidence computed from the Cox model of the higher versus the lower tertile of the AUC of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) levels over 3 years, in (**Top**) serum and (**Bottom**) red blood cell membranes. Placebo (PBO) group = open symbols; DHA group = filled symbols; lines across symbols = 95% confidence interval; dashed lines = PBO group; solid lines = DHA group.

Table 5. Changes from Baseline in Visual Acuity in the Full Analysis Set (n = 263) and Changes in Number and Area of Drusen in Study Eye

	Docosahexaenoic Acid Group	Placebo Group	*P* Value, Docosahexaenoic Acid versus Placebo Group
Best-corrected visual acuity decrease from baseline, mean ± SD (logMAR)			
At month 6	0.040±0.122	0.007±0.118	
At year 1	0.037±0.173	0.008±0.122	
At year 2	0.086±0.231	0.057±0.201	
At year 3	0.155±0.297	0.116±0.258	0.311
Proportion of subjects with a decrease of more than 15 letters on ETDRS chart, no. observed/total (%)			
At month 6	4/131 (3.1%)	2/126 (1.6%)	
At year 1	7/131 (5.3%)	1/123 (0.8%)	
At year 2	13/120 (10.8%)	11/116 (9.5%)	
At year 3	21/118 (17.8%)	16/112 (14.3%)	0.469
No. and area of drusen during the study, mean ± SD*			
No. of small drusen			
At baseline	30.5±43.2 (n = 96)	38.1±47.1 (n = 96)	
At year 3	32.3±34.7 (n = 86)	40.9±37.8 (n = 83)	0.270
No. of intermediate drusen			
At baseline	47.3±51.5 (n = 96)	54.2±57.5 (n = 96)	
At year 3	40.7±40.1 (n = 86)	51.9±46.7 (n = 83)	0.763
No. of large drusen			
At baseline	49.8±46.3 (n = 96)	57.4±53.4 (n = 96)	
At year 3	50.8±47.0 (n = 86)	60.6±53.0 (n = 83)	0.423
Total area of all drusen (μm^2)			
At baseline	1 614 594±1 855 703 (n = 96)	1 820 091±1 830 451 (n = 96)	
At year 3	1 889 351±2 112 253 (n = 86)	2 006 937±2 040 908 (n = 83)	0.851

ETDRS = Early Treatment Diabetic Retinopathy Study; logMAR = logarithm of the minimum angle of resolution; SD = standard deviation.
*Number and area of drusen are from the full analysis set excluding subjects with new vessels in the study eye (n = 192). Small drusen, <63 μm; intermediate drusen, between 63 and 125 μm; large drusen, >125 μm.

1.231±0.431 logMAR at follow-up. In the severely affected fellow eye, the proportion of patients from the DHA group experiencing a decrease of more than 15 letters on the ETDRS chart was significantly higher (40.7%) than in the placebo group (22.3%; $P = 0.003$) at follow-up. Conversely, the percentage of patients in whom a cataract developed, who experienced a worsening of cataract, or who had cataract surgery in the study eye at follow-up was significantly lower in the DHA than in the placebo group compared with baseline (50.0% vs. 62.5%; $P = 0.032$).

The most frequently reported nonocular AEs included nausea, osteoarthritis, hypertension, hypercholesterolemia, diabetes mellitus, and anxiety. A total of 42 serious AEs were reported in 31 patients in the DHA group and 39 serious AEs were reported in 30 patients in the placebo group, all of which were considered to be unlikely to be related to the study treatment, except for 2 undetermined serious AEs (pulmonary embolism in the DHA group and cerebral hemorrhage in the placebo group). Three patients in the DHA group and 6 patients in the placebo group died during the study. All deaths were considered unlikely to be related to the study protocol or treatment.

Plasma Lipids and Lipoproteins. Moderate but significant changes from baseline in fasting plasma lipids and lipoproteins were observed in the DHA group, as expected in normolipidemic patients taking fish oil (Table 6, available at http://aaojournal.org). Although low-density lipoprotein cholesterol levels decreased slightly by 3% to 4% ($P = 0.008$), HDL cholesterol levels increased significantly by 10% to 13% ($P = 0.001$). This resulted in the absence of significant variation in total cholesterol, but in a significant reduction in the atherogenic total cholesterol/HDL cholesterol index ($P<0.001$). Plasma triglycerides did not change consistently over the study period in the DHA group. These DHA-related changes contrasted with the absence of significant changes in the placebo group throughout the study period.

Discussion

The treatment of exudative AMD has improved dramatically with the recent introduction of angiogenesis inhibitors. Because these treatments show some efficacy limitations, the prevention or delay of complete vision loss remains of paramount importance. Many observational studies have suggested that nutritional interventions may reduce the incidence of AMD.[2,7,14,30,31] Previous open-label studies also support the hypothesis of a beneficial role of oral DHA supplementation in prevention of AMD.[33,34] To the authors' knowledge, the NAT2 is the first randomized double-blind study exploring the potential of a long-term oral PUFA supplement enriched in DHA to prevent or slow down the development of CNV in a homogenous group of patients with a typical and severe form of AMD.

The ongoing Age-Related Eye Disease Study 2 is a large, multicenter, randomized trial designed to assess the effects of oral supplementation with high doses of macular xanthophylls (lutein 10 mg and zeaxanthin 2 mg), omega-3 long-chain PUFAs (DHA/EPA, 1:2, 1000 mg), or both in com-

bination with vitamin and antioxidant supplements, on the progression to late AMD. Here, DHA was given at a daily dose of 840 mg in the form of fish-oil containing a DHA-to-EPA ratio of 3:1 (3 capsules, equivalent to eating approximately 100 g of oily fish/day) for 3 years. This dose (1100 mg EPA plus DHA) is approximately twice the recommended nutritional daily intake of EPA plus DHA (500 mg) in France.[35] This corresponds to the total daily dose—although with a higher DHA-to-EPA ratio—proposed in large randomized controlled trials using omega-3 fatty acids in the prevention of cardiovascular disease.[36]

A homogeneous group of 300 patients affected with exudative AMD in one eye and early lesions (mainly drusen) in the study eye was selected. These patients had a mean age of 73.5 years and were at high risk of CNV developing in the study eye within 3 years. It should be noted that 98.1% of patients had more than 5 soft drusen and 78.7% of patients more than 20 large drusen in the study eye at study entry. Both treatment groups were comparable for demographic characteristics and ocular characteristics of the study eye. Other clinical features (age, gender ratio, smoking status, family history) were characteristic of an exudative AMD population.[37] Of note, the monocentric design of the study offered an opportunity to assess the dietary pattern and PUFA levels in a homogeneous population of patients with advanced exudative AMD. It seemed that despite normal daily energy intake for elderly patients (mean ± SD, 1522± 501 kcal/day), NAT2 study participants ate less DHA (mean ± SD, 116±85 mg/day) than the 500 mg/day recommended for daily energy intake in the general French population.[35] Moreover, the omega-6–to–omega-3 ratio of daily PUFA intake was at least twice that recommended.[38] The deficit in daily DHA intake and imbalance in omega-3 fatty acid intake, estimated from the food frequency questionnaire, was reflected in the serum and membrane EPA and DHA measurements at study entry. The overall RBCM DHA content (mean ± SD, 3.27±0.71%) was less than the 4% average RBCM DHA content found in younger healthy adults.[39]

Despite the fact that the study enrolled elderly participants, overall yearly compliance with study products exceeded 78%. Only 10 individuals (3.8%) did not comply at all, whereas approximately 70% were fully compliant over the 3-year study period, confirmed by the doubling in DHA plus EPA serum levels in patients allocated oral DHA.

Times to onset and incidence of CNV in the study eye were not significantly different between groups, despite a significant increase in DHA and EPA levels in RBCM. Oral DHA did not modify the rate of visual acuity changes over 3 years in the DHA group compared with the placebo group. Most patients (>82%) maintained stable vision or had a fewer than 15-letter loss on the ETDRS chart against baseline. No marked changes from baseline in the progression of drusen in the study eye were observed throughout the study in either group.

Analysis of a prespecified secondary efficacy parameter identified a small subgroup (highest tertile of DHA plus EPA levels in RBCM over 3 years), among patients allocated the DHA-enriched supplement. This subgroup of patients achieving high levels of EPA plus DHA in RBCM over a 3-year follow-up had nearly 70% less risk of developing CNV than those remaining in the lower tertile ($P = 0.047$).

There are several possible explanations for the lack of efficacy on the primary outcome in this study. First, the power of the study may have been insufficient to demonstrate a significant effect of the DHA-enriched PUFA supplement. The overall 3-year CNV incidence of 27% was slightly lower (30%–36%) than those previously reported in the French population.[29] Choroidal neovascularization developed in a total of 71 patients instead of the 78 patients expected. More events may be needed to demonstrate a significant difference.

Of the 300 enrolled subjects, only 263 (87.7%) could be included in the statistical analysis, mainly because of the patients' decisions or other health-related constraints leading to interruption of their participation to the study before the first follow-up visit. This may have reduced the statistical power for detecting a difference between the 2 groups further. Low statistical power is reflected by the wide confidence intervals of the hazard ratios, which show that a true effect of up to 45% cannot be totally excluded (hazard ratio, 0.89; 95% confidence interval, 0.551–0.42 for the main analysis). Because percentage of subjects and reasons for interrupting were similar in both groups, interruption is unlikely to have introduced a bias in the estimation of the difference between the 2 groups. In addition, the study may have been too short, considering the stage of AMD in the study eye at baseline and highest rate of CNV occurrence within the last 3 months of the study (Fig 3). Over the 3 years of study duration, 31.6% of patients did not comply fully with the study treatment in the DHA-allocated group, thus reducing any potential treatment benefit in this group.

Second, unexpected results were observed for EPA levels in the placebo group. In this group, EPA levels in RBCM increased significantly by 12% at 6 months and increased further in the long-term: up to 24% after 3 years. These changes were not significant for DHA either in serum or RBCM at early or follow-up time points. However, EPA changes were strong enough to drive a significant EPA plus DHA increase in serum or RBCM at 3 years. Because EPA is a major constituent of seafood,[15] because it may convert into DHA mainly in the liver, and because EPA plus DHA level in RBCM is well correlated with long-term dietary EPA and DHA intake,[39] this suggests that a significant proportion of patients might have increased their daily consumption of fish, marine food, or omega-3–containing derivatives after study enrollment (Fig 6, available at http://aaojournal.org). At 3 years of follow-up, mean EPA plus DHA levels of serum and RBCM in the first tertile of the DHA group (2.69% and 4.81%, respectively) were lower than in the third tertile of the placebo group (3.81% and 5.77%, respectively; Fig 4), suggesting that approximately 25% of patients in the DHA group had either not taken DHA (poor compliance) without disclosing to investigators or did not respond to oral DHA supplementation. In addition, 47 patients from the placebo group may have taken omega-3 fatty acid-containing oral supplements without informing investigators, as seen from an increase in RBCM levels of DHA exceeding 38% and in RBCM levels of EPA

exceeding 75% between 2 visits (threshold defined from the Nutritional AMD Treatment 1 study).[20] Furthermore, the EPA plus DHA content in RBCM exceeded 4% in the placebo group at follow-up, reaching values regarded as protective in cardiovascular diseases.[39] The 25.6% CNV incidence observed in the placebo group was lower than the expected 33% rate used for study power calculation. This suggests a possible trial participation effect, a placebo effect, or both in patients anxious about short-term complete loss of vision.[40] Hence, a slight increase in EPA or DHA intake related to behavioral changes resulting from participating in a placebo-controlled trial might have been sufficient to slow down the development of CNV.

Furthermore, because there is no edible fatty acid with a neutral biological effect, olive oil was selected as the placebo because it is part of a staple diet and has no deleterious effect on the retina. However, it was reported recently that a diet rich in omega-3 fatty acids and olive oil may reduce the risk of early and late AMD, respectively.[41] In the present study, it cannot be excluded that olive oil might have had a beneficial effect on AMD progression, explaining, at least partly, the relatively low occurrence of CNV in the placebo group. Finally, the lack of repeating the frequency food questionnaire during this 3-year interval could be considered as a limitation of the study, but frequency food questionnaire analysis was not a predefined secondary outcome. In future studies investigating oral supplementation with omega-3 PUFA in AMD, care should be taken in monitoring dietary and other behavioral changes.

Third, DHA may have a protective effect at an earlier stage of the disease. In the present study, a significant effect of the stage of AMD was found with regard to 3-year CNV onset ($P = 0.022$, first Cox model, and $P = 0.008$, second Cox model). Indeed, in a large cohort of 38 022 women free of AMD at baseline, regular consumption of DHA, EPA, and fish was associated with a lower AMD incidence and may have a role in preventing early AMD.[14]

Despite these limitations, there remained an apparent contradiction between the consistency of observational studies across several populations and the findings from a randomized trial on the role of oral DHA on the development of exudative AMD. Because NAT2 participants received a fixed oral dose of DHA and EPA supplementation without antioxidants, xanthophylls, or zinc over 3 years, whether increasing long-term circulating and cellular EPA and DHA amount could influence choroidal angiogenesis was tested. A secondary analysis from the present study identified a small subgroup of patients achieving steadily high overall and more specifically cellular DHA levels who seemed to be protected against developing choroidal neovessels. The 3-year incidence of CNV in patients with permanently high EPA plus DHA levels was significantly lower than incidence in patients with permanently low EPA plus DHA levels: 14.3% versus 32.5% (hazard ratio, 0.32; 95% confidence interval, 0.10–0.99; $P = 0.047$). A similar reduction in CNV risk (>65%) was reported consistently in large human cohort studies in subgroups of patients with the highest daily DHA intake.[8–14,31–33] This may suggest that some individuals may achieve a cellular EPA and DHA content sufficient to display biological effects in the retinal microvasculature, as seen in atherosclerotic and cardiovascular disease.[42] Docosahexaenoic acid is a precursor of various derivatives (resolvins, neuroprotectins, or maresins) shown in animal models to display specific anti-inflammatory and antiangiogenic properties.[43,44] The NAT2 study was focused on DHA, but the dose of EPA delivered in this study may still be clinically significant. The recent literature emphasizes that EPA is likely to be a precursor for very long-chain PUFAs in the retina and that abnormalities of very long-chain PUFAs may be related to AMD risk.[45] Provided that unknown or unmeasured confounding factors may affect any subgroup analysis and awaiting for results from upcoming randomized trials, this finding, if confirmed, could present novel preventive strategies targeted at increasing endogenous EPA and DHA levels in exudative AMD.

Safety follow-up showed that DHA was well tolerated at a daily dose of 840 mg over 3 years in a study powered to detect an AE rate of more than 1%. Most AEs were minor and were unlikely related to the study treatment. Serious AEs resulted from common morbidity (mainly cancer) seen in an elderly population. Plasma lipoprotein profiles improved over the study period. Plasma HDL cholesterol increased by more than 10%, thereby reducing the atherogenic index, in agreement with lipoprotein changes observed in large cardiovascular trials.[46]

The proportion of patients with a worsening cataract was lower in the DHA group than in the placebo group. Ocular adverse events were mainly those expected from a group of elderly subjects with exudative AMD at the stage of legal blindness in 1 eye previously treated before the era of angiogenesis inhibitors. Safety analysis of visual acuity in the severely affected fellow eye showed that the proportion of patients who lost more than 15 letters on the ETDRS chart was higher in the DHA group (40.7%) than in the placebo group (22.3%). Because this study began before the era of anti-VEGF therapy, most of the fellow eyes were treated by PDT. Worsening of visual acuity was common among patients with exudative AMD treated by PDT.[47,48] Since then, the emergence of anti-VEGF therapies during the study radically changed the prognosis of exudative AMD. Most of these cases initially were affected with occult CNV. Photodynamic therapy studies demonstrated that, at 24 months, a loss of more than 15 letters was observed in 55% of patients treated by PDT and in 68% of patients without treatment.[47,48] Therefore, the observation that 40.7% of patients in the current DHA group had lost more than 15 letters falls within the expected range after PDT. Fellow eyes were treated unequally in both groups without randomization for previous treatment. It should be noted that PDT was performed significantly more often in the DHA group than in the control group (67.3% vs. 54.8%; $P<0.05$). However, laser photocoagulation usually is performed on extrafoveal lesions and can stabilize exudation. At baseline, laser photocoagulation was performed more frequently in the placebo group than in the DHA group (59.1% vs. 50.5%). Thus, significant differences in treatment of patients in the 2 groups explain the differences in final better-corrected visual acuity in the fellow eye. Furthermore, worsening of better-corrected visual acuity was the

common feature of exudative AMD before the era of anti-VEGF and was not considered to be a treatment-emergent AE.

In conclusion, DHA-enriched supplementation at a daily dose of 840 mg over 3 years in patients with exudative AMD in one eye and macular drusen in the other eye (study eye) had no effect on preventing the onset of exudative AMD in the study eye. Under the study conditions, the overall 3-year incidence of CNV was lower than expected from observational cohort studies. Interestingly, patients with permanently high RBCM levels of EPA plus DHA seemed to be at lower risk for CNV developing. Further analyses of nutritional interactions with genetic factors may identify potential effects of EPA plus DHA supplementation in specific subgroups.

Acknowledgment. The authors thank Dr. Alain Platel for reviewing and discussing the manuscript.

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Footnotes and Financial Disclosures

Originally received: August 6, 2012.
Final revision: January 2, 2013.
Accepted: January 2, 2013.
Available online: ●●●. Manuscript no. 2012-1201.

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*A full listing of the Nutritional AMD Treatment 2 Study Group is available at http://aaojournal.org.

Presented at: Association for Research in Vision and Ophthalmology Annual Meeting, 2012; and the Macula Society Annual Meeting, 2012.

Financial Disclosure(s):
The author(s) have made the following disclosure(s):
Eric H. Souied: Consultant and lecturer—Laboratoire Bausch & Lomb Chauvin
Pascale Benlian: Financial support and lecturer—Laboratoire Bausch & Lomb Chauvin
Cécile Delcourt: Consultant and financial support—Laboratoire Bausch & Lomb Chauvin; Consultant and financial support—Laboratoires Théa; Consultant – Novartis
Sponsored by Laboratoire Bausch & Lomb Chauvin, Montpellier, France.

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