Circulating Omega-3 Fatty Acids and Neovascular Age-Related Macular Degeneration

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See the appendix for the members of the Nutritional AMD Treatment 2 Study Group.

Submitted: January 9, 2014
Accepted: February 12, 2014

Citation: Merle BMJ, Benlian P, Puche N, Bassols A, Delcourt C, Souied EH. Circulating omega-3 fatty acids and neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2014;55:2010–2019. DOI: 10.1167/iovs.14-13916

Purpose. We assessed the associations of serum, red blood cell membranes (RBCM) and dietary long-chain n-3 polyunsaturated fatty acids (LC-PUFAs) with neovascular age-related macular degeneration (AMD).

Methods. We included 290 patients of the Nutritional AMD Treatment 2 Study (NAT2) with neovascular AMD in one eye and early AMD lesions in the other eye, and 144 normal vision controls without AMD. Dietary intake of seafood was estimated by food frequency questionnaire. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) composition in serum and RBCM were determined by gas chromatography from 12-hour fasting blood samples and was expressed as percentages of total fatty acids profile. Logistic regressions estimated associations of neovascular AMD with dietary intake of seafood and circulating n-3 LC-PUFAs.

Results. Dietary oily fish and seafood intake were significantly lower in AMD patients than in controls. After adjustment for all potential confounders (age, sex, *CFH Y402H*, *ARMS2 A69S*, and *ApoE4* polymorphisms, plasma triglycerides, hypertension, hypercholesterolemia, and family history of AMD), serum EPA was associated significantly with a lower risk for neovascular AMD (odds ratio [OR] = 0.41; 95% confidence interval [CI], 0.22–0.77; $P = 0.005$). Analysis of RBCM revealed that EPA and EPA+DHA were associated significantly with a lower risk for 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).

Conclusions. The RBCM EPA and EPA+DHA, as long-term biomarkers of n-3 dietary PUFA status, were associated strongly with neovascular AMD and may represent an objective marker identifying subjects at high risk for neovascular AMD, who may most benefit from nutritional interventions. (http://www.controlled-trials.com/isrctn number, ISRCTN98246501.)

Keywords: age-related macular degeneration, omega-3 fatty acids, epidemiology, case-control study

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in industrialized countries.[1] It comprises two late forms associated with severe visual impairment (neovascular and atrophic AMD), generally preceded by early, asymptomatic, retinal abnormalities (drusen, pigmentary abnormalities). Treatments for neovascular AMD have been available for a few years. Although they stabilize vision, they are not curative, supporting the need for a targeted prevention toward high-risk asymptomatic subjects, identified by relevant biomarkers.

The condition of AMD is a multifactorial disease, involving genetic and environmental factors (in particular smoking and nutrition).[1] Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have important structural and protective functions in the retina.[2] The DHA reaches its highest concentration in the membranes of photoreceptors, and is important in photoreceptor differentiation and survival, as well as in retinal function.[2] The anti-inflammatory properties of EPA and DHA[2,3] are of particular interest in AMD, since inflammation appears to have a pivotal role in this condition.[4] Moreover, n-3 LC-PUFAs may increase the retinal density of macular pigment, which filters blue light, and has local antioxidant and anti-inflammatory activities.[5] Finally, derivatives of dietary n-3 LC-PUFAs, exhibit antiangiogenic properties in the retina.[6]

In 2008, a meta-analysis[7] of nine epidemiologic studies[8–16] showed a significantly reduced risk for AMD in subjects with high dietary intake of n-3 PUFAs and fish, the main food source of n-3 PUFAs. Since then, 10 additional studies have shown similar and consistent results.[17–26]

Dietary assessment methods rely on the subjects' memory and perceptions, and face the difficulties of the extreme day-to-

day variability of human diet, the hidden nature of many fats used for dressing and cooking, the bias in reporting due to social standards and nutritional recommendations, and the estimation of the nutritional content of foods. Because of the multiple difficulties of dietary assessment, circulating biomarkers may represent a more objective alternative for the assessment of nutritional status.[27] A better assessment of n-3 nutritional status could help identify high-risk subjects, who may benefit most from nutritional intervention. Such biomarkers also might be used to follow the efficacy of nutritional interventions in restoring adequate nutritional status.

Over the last 20 years, a number of biomarkers have been developed to assess the nutritional status in fatty acids according to different source tissues. Because of very limited capacity of endogenous synthesis, the body status of n-3 LC-PUFA mainly reflects dietary intake of these essential fatty acids. The shortest-term biomarkers of n-3 LC-PUFA body status are serum or plasma measurements, reflecting dietary intakes of the past few hours for triglycerides or of the past few days for cholesterol ester and phospholipid fatty acids carried within circulating lipoproteins. Red blood cell membranes (RBCM) and platelets are of particular interest, since they reflect longer-term overall dietary intake of n-3 LC-PUFA, incorporated within membrane phospholipids of bone marrow–derived cell lines during the past few months.[28] Because n-3 fatty acids may undergo variable interconversion after intestinal absorption, the omega-3 index (i.e., RBCM EPA+DHA) appears as an interesting long-term integrator of n-3 LC-PUFA body status.[29]

Circulating n-3 PUFAs have been evaluated in numerous studies, showing good correlation with dietary intake, and sensitivity to changes in dietary supplementation studies.[27] They have been used widely in association studies of n-3 PUFAs with a variety of health outcomes (cardiovascular diseases, obesity and diabetes, chronic inflammatory or neuro-psychiatric disorders, cancers, and so forth).[30–34] However, with regard to AMD, while many studies have reported associations with dietary intakes of n-3 PUFAs, very few data are available on associations of AMD with circulating biomarkers of *n*-3 PUFA status. Recently, we have shown that high plasma n-3 LC-PUFAs were associated significantly with a decreased risk for late AMD in elderly subjects from South of France.[35] This study used a single plasma measurement that represented a crude estimate of body fatty acid status. Measurement of n-3 PUFAs in RBCM may represent a better biomarker for longer term status, with a half-life of 120 days.[28]

In the present study, we reported the associations of dietary intake of seafood, and serum and RBCM n-3 LC-PUFAs with neovascular AMD in a French case-control study.

Methods

Study Population

Cases. The 290 cases of neovascular AMD were included from Nutritional AMD Treatment 2 Study (NAT2) baseline examination.[36] The NAT2 study is a randomized, placebo-controlled, double-blind, parallel, comparative study. Patients were enrolled from December 2003 to October 2005 in a single center at the Department of Ophthalmology, Hôpital Intercommunal de Creteil, France. The study was reviewed and approved by the relevant institutional review board (CPP, Paris-Ile de France 5, Paris, France).

Eligible patients were affected by neovascular AMD in one eye and early AMD (any drusen or reticular pseudodrusen with or without pigmentary changes) in the other eye. Neovascular AMD was defined on the basis of fundus color pictures and fluorescein angiography examination. Inclusion criteria were age 55 years or older and younger than 85 years, and visual acuity better than +0.4 logarithm of minimum angle of resolution units in the study period.[36] The main exclusion criteria were: choroidal neovascularization (CNV) in both eyes or no CNV in either eye, wide central subfoveal atrophy of the study eye, and progressive ocular diseases (severe glaucoma or other severe retinopathy).[36]

Eye examination included best-corrected visual acuity, slit-lamp examination, fundus photography, and fluorescein angiography (Topcon501A; Topcon, Tokyo, Japan). The study was registered on the International Standard Randomized Controlled Trial Number Register and was allocated registration number ISRCTN98246501.

Controls. Controls were enrolled through local-newspapers calls for collaboration. A total of 144 men and women, aged 55 years or more, with normal visual acuity, no history of ocular diseases, and normal fundus examination and fundus photography, was recruited and examined at the Department of Ophthalmology of Creteil between 2002 and 2008. Controls were from the same geographical area as the AMD cases.

Written informed consent was obtained for all participants (cases and controls), as required by the French bioethical legislation and local ethic committee (CPP Henri Mondor). This study followed the tenets of the Declaration of Helsinki.

Biological Measurements of Fatty Acids

Overnight fasting blood samples were delivered to a single clinical chemistry laboratory (Hôpital Saint Antoine, APHP, Paris, France) within five hours and processed immediately as described.[36] For cases, blood samples collected at baseline examination (before any supplementation) were used for the present study. For controls, blood samples were obtained at the time of eye examination.

Fatty acid composition in serum and RBCM was determined by gas chromatography after they were transmethylated by diazomethane following a modified Dole's procedure.[37] Results for EPA and DHA content were expressed as a percentage of the total fatty acid profile in serum and RBCM, and were available for all participants ($n = 434$).

Other Biomarkers

Biological samples were collected in the same conditions and at time of fatty acid measurements. They included serum lipids and lipoproteins, and genetic polymorphisms validated as genetic markers of exudative AMD.

Serum total, high (HDL) and low (LDL) density lipoprotein-cholesterol, and triglycerides, were measured by enzymatic colorimetric and electrophoretic methods as described previously.[38] Genomic DNA was extracted from 10 mL blood leukocytes as described previously in AMD patients[39] and using the Illustra kit according to the manufacturer's protocol (GE Healthcare, Little Chalfont, Buckinghamshire, UK) in controls. Genotyping of *CFH* rs1061170, *ARM2/HTRA1* rs10490924, and *Apolipoprotein E2, 3, 4* alleles were performed by quantitative polymerase chain reaction allelic discrimination using reagents and conditions from Custom Taqman Single-Nucleotide Polymorphism Genotyping Assays (Applera, Corp., Saint Aubin, France), using ABI 7900HT (Applied Biosystems, Carlsbad, CA). Quality control of genotyping by Sanger sequencing and bioinformatics analysis were performed as described.[39]

Dietary Data

Dietary data were collected using a validated food frequency questionnaire (FFQ) that recorded the usual food intakes for

the last year.[16,40,41] The interview was conducted by trained technicians, by telephone, and lasted 45 to 60 minutes. The FFQ consists of 165 items and portions were estimated using a validated set of photographs. The set of photographs was given to the patient before the telephone interview. It was arranged by food type and meal pattern. In the analysis, the intakes were expressed in daily consumption in grams. The food composition table was REGAL[42] (Ciqual, Edinburgh, UK) expanded with carotenoid and fatty acid contents from the SU.VI.MAX table.[43] Total dietary intake of seafood is the sum of oily fish, white fish, and other seafood, and total dietary intake of fish is the sum of oily fish and white fish. Dietary data were available for 423 participants (97.4%).

Covariates

Socio-demographic factors and medical history were collected through face-to-face, standardized interviews at the same time as eye examination. They included age, sex, body mass index (BMI; weight [kg]/height^2 [m^2]), smoking status (never smoker or ever smoker), self-reported history of hypercholesterolemia, hypertension, diabetes, and family history of AMD, circulating biomarkers (serum total, HDL- and LDL-cholesterol, and triglycerides), and genetic biomarkers (*CFH* rs1061170, *ARM2/HTRA1* rs10490924, and *Apolipoprotein E2,* and *E4* alleles). All covariates were available for all participants (n = 434).

Statistical Analyses

Comparisons between neovascular AMD patients and controls were performed using the Pearson χ^2 for sex, Student's *t*-test for age, and logistic regression adjusted for age and sex for other variables.

Associations of circulating n-3 PUFAs and fish intake with socio-demographic factors, medical history, dietary intake of seafood, and genetic polymorphisms were performed using Kruskal-Wallis ANOVA and Wilcoxon tests.

Associations of neovascular AMD with dietary intake of seafood and circulating n-3 PUFAs were estimated using logistic regression. Potentials confounders retained in the final multivariate model were factors associated significantly with neovascular AMD or n-3 PUFAs in our study (hypercholesterolemia, hypertension, family history of AMD, plasma triglycerides, and *CFH*, *ARMS2*, and *ApoE4* polymorphisms; $P < 0.05$). Dietary intake of seafood and circulating n-3 PUFAs variables were used as tertiles of distribution, the first tertile being the reference.

We also analyzed potential gene-environment interactions, and potential age- and sex-circulating n-3 PUFAs interactions. Interactions were introduced independently in the fully adjusted model and retained if they were significant ($P < 0.05$).

For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NY).

Results

Neovascular AMD patients were older than controls ($P < 0.0001$), but were not different regarding sex, smoking status, and BMI (Table 1). After adjustment for age and sex, neovascular AMD patients declared more frequently a family history of AMD ($P = 0.004$), hypercholesterolemia ($P = 0.004$), or hypertension ($P = 0.001$), both latter conditions being under stable corrective therapy. Frequency of self-declared diabetes did not differ between neovascular AMD patients and controls. Regarding genetic polymorphisms, *CFH Y402H* ($P < 0.0001$), *ARMS2 A69S* ($P < 0.0001$), and *ApoE4* ($P = 0.03$) polymorphisms were associated significantly with neovascular AMD. Neovascular AMD patients had lower plasma triglycerides than controls ($P = 0.0009$), while they had similar plasma total, HDL- and LDL-cholesterol (Table 1). Neovascular AMD patients had lower serum EPA ($P = 0.03$), RBCM EPA ($P < 0.001$), RBCM DHA ($P = 0.03$), and omega-3 index (RBCM EPA+DHA, $P = 0.001$) than controls, while they had serum DHA and EPA+DHA similar to controls after adjustment for age and sex (Table 1). Neovascular AMD patients had lower dietary intake of oily fish ($P = 0.02$) and total seafood ($P = 0.03$) than controls, but were not different regarding dietary intake of total fish, white fish, and other seafood (Table 1).

Table 2 presents the associations of fish intake and circulating n-3 fatty acids with socio-demographic factors, medical history, and genetic polymorphisms. Younger participants had a higher dietary intake of oily fish than older participants ($P = 0.0003$). Men had a higher dietary intake of total and oily fish (respectively, $P = 0.002$ and $P = 0.005$). Participants who declared hypertension had lower dietary intake of oily fish ($P = 0.003$). Participants with at least one allele E4 for *ApoE* polymorphism had higher dietary intake of total fish and oily fish (respectively, $P = 0.03$ and $P = 0.03$). Other socio-demographic factors, lifestyle, and AMD-related genetic polymorphisms were not associated with dietary intake of fish or seafood. Remarkably, none of the circulating n-3 LC-PUFAs appeared influenced by any of the socio-demographic, medical, or genetic risk factors for AMD analyzed herein.

As shown in Table 3, serum EPA, DHA, and EPA+DHA were associated significantly with all items of dietary intake of seafood (total fish, oily fish, white fish, other seafood, and total seafood). Subjects in the third tertile, for all seafood items had higher serum EPA, DHA, and EPA+DHA. The same trend was observed with RBCM EPA, DHA, and EPA+DHA, and reached statistical significance for all items of dietary intake of seafood except for RBCM DHA and white fish ($P = 0.08$). Of note, the median omega-3 index (i.e., RBCM EPA+DHA) was constantly >4, in subjects from the third tertile, for all seafood items.

As shown in Table 4, after adjustment for age and sex, dietary intake of total seafood and of total fish was associated inversely with neovascular AMD (respectively, $P = 0.05$ and $P = 0.04$). After adjustment for all potential confounders (age, sex, *CFH Y402H, ARMS2 A69S,* and *ApoE4* polymorphisms, plasma triglycerides, hypertension, hypercholesterolemia, and family history of AMD), these associations were no longer statistically significant. With regard to dietary intake of oily fish, white fish, or other seafood, associations were in the same direction, but did not reach statistical significance.

Associations of neovascular AMD with circulating n-3 PUFAs are shown in Table 5. After adjustment for age and sex, serum EPA was significantly associated with a lower risk for neovascular AMD (odds ratio [OR] = 0.59, $P = 0.04$), while serum DHA and EPA+DHA were not significantly associated with neovascular AMD. This association remained significant after adjustment for all potential confounders ($P = 0.005$).

With regard to RBCM n-3 PUFAs, after adjustment for age and sex, EPA and EPA+DHA were associated strongly with a lower risk for neovascular AMD (OR = 0.33, $P < 0.0001$ and OR = 0.44, $P = 0.002$, respectively) and after adjustment for all potential confounders, these associations remained significant (OR = 0.25, $P < 0.0001$ and OR = 0.52, $P = 0.03$, respectively). As in serum, DHA in RBCM was not associated significantly with neovascular AMD.

There was no detectable interaction between dietary intake of seafood or circulating n-3 PUFAs with *CFH*, *ARMS2* or *ApoE* genetic polymorphisms, age, or sex.

Table 1. Characteristics of Neovascular AMD Patients and Controls

Characteristics	Controls, $n = 144$	Neovascular AMD Patients, $n = 290$	Adjusted P^*
Sociodemographic factors			
Age, y, mean ± SD	67.7 ± 8.2	70.8 ± 7.59	<0.0001
Sex, *n* (%)			
Male	55 (38.2)	105 (36.2)	0.69
Female	89 (61.8)	185 (63.8)	
Smoking status, *n* (%)			
Never smoker	91 (63.2)	165 (56.9)	0.12
Ever smoker	53 (36.8)	125 (43.1)	
BMI, kg/m^2, mean ± SD	25.2 ± 3.7	25.7 ± 3.97	0.17
Self-reported medical history			
Hypercholesterolemia, *n* (%)			
No	102 (70.8)	147 (51.4)	0.0004
Yes	42 (29.2)	143 (49.3)	
Hypertension, *n* (%)			
No	102 (70.8)	149 (51.0)	0.001
Yes	42 (29.2)	141 (48.6)	
Diabetes, *n* (%)			
No	131 (91.0)	266 (91.7)	0.59
Yes	13 (9.0)	24 (8.3)	
Family history of AMD, *n* (%)			
No	125 (86.8)	222 (76.6)	0.004
Yes	19 (13.2)	68 (23.5)	
Genetic polymorphisms			
CFH Y402H, *n* (%)			
TT	56 (38.9)	63 (21.7)	<0.0001
CT	68 (47.2)	134 (46.2)	
CC	20 (13.9)	93 (32.1)	
ARMS2 A69S, *n* (%)			
GG	93 (64.6)	81 (27.9)	<0.0001
GT	46 (31.9)	133 (45.9)	
TT	5 (3.5)	76 (26.2)	
ApoE, *n* (%)			
At least 1 allele E2	18 (12.5)	53 (18.3)	0.12
At least 1 allele E4	39 (27.1)	48 (16.6)	0.03
Plasma lipids, mmol/L, median (fifth–95th percentiles) or mean ± SD			
Triglycerides	1.14 (0.57–2.30)	0.98 (0.48–2.17)	0.0009
HDL-cholesterol	1.83 ± 0.56	1.79 ± 0.55	0.48
LDL-cholesterol	3.91 (2.51–5.30)	3.64 (2.30–5.59)	0.29
Total cholesterol	5.85 ± 0.93	5.68 ± 1.04	0.16
Circulating omega 3 PUFA, % of fatty acids, median (fifth–95th percentiles)			
Serum EPA	0.74 (0.24–1.96)	0.60 (0.30–1.40)	0.03
Serum DHA	1.25 (0.63–2.00)	1.30 (0.60–2.40)	0.1
Serum EPA+DHA	1.99 (1.08–3.53)	1.90 (1.00–3.70)	0.78
Red blood cell membranes EPA	0.78 (0.29–1.47)	0.60 (0.30–1.20)	<0.0001
Red blood cell membranes DHA	3.51 (2.13–5.03)	3.20 (1.80–5.10)	0.03
Red blood cell membranes EPA+DHA	4.32 (2.63–6.48)	3.80 (2.10–5.90)	0.001
Dietary intake of seafood, g/d, median (fifth–95th percentiles)	$n = 139$	$n = 284$	
Total fish	19.9 (7.4–51.1)	17.1 (4.9–41.9)	0.05
Oily fish	8.2 (0.0–31.4)	5.5 (0.0–22.9)	0.02
White fish	9.9 (0.0–19.7)	9.9 (0.0–34.0)	0.68
Other seafood	1.8 (0.0–17.1)	0.7 (0.0–15.7)	0.16
Total seafood	22.7 (9.9–64.0)	20.4 (5.3–51.1)	0.03

* *P* Student's *t*-test for age, Pearson χ^2 for sex, and logistic regression adjusted for age and sex for other variables.

Table 2. Variations of Circulating n-3 PUFAs and Dietary Intake of Fish According to Socio-Demographic Factors, Lifestyle, and AMD-Related Genetic Polymorphisms

Characteristics	*n*	Serum EPA+DHA (% of Fatty Acids) Median (Fifth–95th Percentiles)	RBCM EPA+DHA (% of Fatty Acids) Median (Fifth–95th Percentiles)	*n*	Total Fish (g/d) Median (Fifth–95th Percentiles)	Oily Fish (g/d) Median (Fifth–95th Percentiles)	White Fish (g/d) Median (Fifth–95th Percentiles)
Sociodemographic factors							
Age, y							
<70	203	2.04 (1.15–3.70)	4.10 (2.47–5.83)	199	19.7 (5.3–51.1)	8.2 (0.0–31.4)	9.9 (2.5–19.7)
≥0	231	1.90 (0.90–3.60)	3.86 (2.11–6.02)	224	17.0 (4.9–42.6)	5.0 (0.0–22.9)	9.9 (0.0–38.4)
*P**		0.11	0.20		0.05	0.0003	0.84
Sex							
Men	160	1.91 (1.05–3.70)	4.00 (2.45–5.86)	157	19.9 (4.9–58.3)	7.9 (0.0–31.4)	9.9 (0.0–39.4)
Women	274	1.91 (1.00–3.70)	4.00 (2.10–6.20)	266	15.7 (5.0–41.3)	5.4 (0.0–22.9)	9.9 (0.0–26.4)
P		0.61	0.71		0.002	0.005	0.25
Smoking status							
Never smoker	256	1.93 (1.11–3.70)	4.00 (2.20–6.40)	248	16.6 (5.0–42.6)	5.7 (0.0–21.4)	9.9 (2.5–24.1)
Ever smoker	178	1.91 (0.90–3.70)	4.00 (2.40–5.80)	175	19.7 (4.9–53.4)	7.9 (0.0–31.4)	9.9 (0.0–39.4)
P		0.32	0.72		0.06	0.17	0.31
BMI, kg/m^2							
<25	218	2.00 (1.02–4.10)	4.05 (2.30–5.83)	211	19.7 (4.9–51.1)	5.7 (0.0–25.7)	9.9 (0.0–34.0)
≥25	214	1.90 (1.00–3.53)	4.00 (2.30–6.20)	212	17.8 (4.9–42.6)	7.5 (0.0–25.7)	9.9 (0.0–26.4)
P		0.30	0.61		0.71	0.67	0.31
Medical history							
Hypercholesterolemia							
No	245	1.90 (1.00–3.53)	4.03 (2.40–5.90)	237	18.4 (5.3–50.9)	7.9 (0.0–25.7)	9.9 (2.5–38.4)
Yes	189	2.00 (1.05–3.70)	4.00 (2.20–6.00)	186	21.0 (5.0–50.7)	5.5 (0.0–25.7)	19.0 (4.9–42.6)
P		0.95	0.62		0.44	0.31	0.70
Hypertension							
No	251	2.00 (1.00–3.60)	4.07 (2.40–6.20)	246	18.9 (5.3–45.4)	7.9 (0.0–25.7)	9.9 (0.0–34.0)
Yes	183	1.90 (1.05–3.70)	4.00 (2.20–5.90)	177	17.7 (4.9–47.2)	5.0 (0.0–22.9)	9.9 (0.0–26.9)
P		0.26	0.35		0.14	0.003	0.89
Diabetes							
No	397	2.00 (1.02–3.70)	4.03 (2.20–6.00)	386	18.9 (5.0–47.2)	7.5 (0.0–25.7)	9.9 (0.0–28.6)
Yes	37	1.60 (0.90–3.20)	3.50 (2.47–6.29)	37	15.7 (2.5–42.6)	5.4 (0.0–31.4)	9.9 (0.0–24.1)
P		0.05	0.09		0.47	0.90	0.40
Family history of AMD							
No	347	1.90 (1.02–3.60)	4.07 (2.39–5.90)	337	19.5 (4.9–45.4)	7.5 (0.0–25.7)	9.9 (0.0–34.0)
Yes	87	2.00 (1.00–3.90)	3.90 (2.20–6.20)	86	16.5 (7.1–47.3)	5.0 (0.0–25.7)	9.9 (2.5–23.3)
P		0.47	0.21		0.51	0.17	0.67
Genetic polymorphisms							
CFH Y402H							
CC	113	1.90 (1.10–4.00)	3.80 (2.20–6.29)	109	19.7 (4.9–42.6)	5.7 (0.0–25.7)	9.9 (2.5–34.0)
CT	202	1.96 (1.08–3.90)	4.10 (2.40–6.02)	198	19.7 (5.7–50.9)	7.9 (0.0–27.9)	9.9 (0.0–39.4)
TT	119	1.90 (0.90–3.00)	4.07 (2.10–5.70)	116	15.6 (3.6–48.3)	5.5 (0.0–22.9)	9.9 (0.0–19.7)
P		0.40	0.49		0.13	0.86	0.09
ARMS2 A69S							
GG	174	1.90 (1.02–3.90)	4.14 (2.50–6.02)	169	19.7 (4.9–51.1)	7.9 (0.0–31.4)	9.9 (0.0–34.0)
GT	179	2.00 (1.00–3.70)	4.03 (2.00–6.20)	176	17.9 (4.9–41.1)	5.7 (0.0–22.9)	9.9 (0.0–19.7)
TT	81	1.90 (1.20–3.10)	3.80 (2.60–5.62)	78	19.5 (5.0–58.0)	6.6 (0.0–31.4)	9.9 (0.0–39.4)
P		0.63	0.27		0.66	0.77	0.65
ApoE							
At least 1 E2 allele	71	1.90 (0.90–3.50)	3.75 (2.00–5.80)	69	19.9 (7.1–50.9)	7.9 (0.0–22.9)	9.9 (2.5–39.4)
No E2 allele	363	1.95 (1.10–3.70)	4.07 (2.40–6.00)	354	17.8 (4.9–45.1)	5.7 (0.0–25.7)	9.9 (0.0–26.4)
P		0.21	0.10		0.16	0.80	0.10
At least 1 E4 allele	87	1.90 (0.90–3.70)	4.10 (2.00–6.02)	84	19.8 (7.3–58.0)	7.9 (0.0–31.4)	9.9 (2.5–39.4)
No E4 allele	347	1.91 (1.10–3.70)	4.00 (2.30–6.00)	339	17.8 (4.9–42.6)	5.7 (0.0–25.7)	9.9 (0.0–24.1)
P		0.88	0.96		0.03	0.03	0.18

* *P* for Wilcoxon test or Kruskal-Wallis ANOVA.

TABLE 3. Variations of Circulating n-3 PUFAs According to Dietary Intake of Seafood

Dietary Intake of Seafood	Tertile (Range, g/d)	SERUM (% of Fatty Acids) Median (Fifth–95th Percentiles)						RBCM (% of Fatty Acids) Median (Fifth–95th Percentiles)					
		EPA	P*	DHA	P	EPA+DHA	P	EPA	P	DHA	P	EPA+DHA	P
Total fish	1, n = 151 (0–12.8)	0.60 (0.22–1.20)	<0.0001	1.20 (0.60–2.20)	0.0004	1.77 (0.90–3.10)	<0.0001	0.60 (0.29–1.00)	<0.0001	3.00 (1.70–5.10)	<0.0001	3.70 (1.90–5.70)	<0.0001
	2, n = 147 (12.8–23.0)	0.70 (0.20–1.60)		1.30 (0.63–2.40)		2.00 (1.00–3.52)		0.60 (0.30–1.18)		3.22 (2.00–4.90)		3.92 (2.40–5.83)	
	3, n = 125 (23.0–139.0)	0.76 (0.40–2.20)		1.40 (0.73–2.38)		2.20 (1.20–4.77)		0.80 (0.40–1.60)		3.70 (2.37–5.30)		4.50 (2.90–6.68)	
Oily fish	1, n = 198 (0–5.4)	0.60 (0.23–1.34)	0.0008	1.20 (0.60–2.20)	0.02	1.80 (1.00–3.40)	0.002	0.60 (0.24–1.12)	<0.0001	3.00 (1.60–5.10)	0.0001	3.70 (2.00–5.77)	<0.0001
	2, n = 125 (5.4–12.0)	0.75 (0.20–2.00)		1.30 (0.60–2.60)		2.00 (0.90–4.00)		0.79 (0.40–1.40)		3.40 (2.20–5.00)		4.29 (2.60–6.20)	
	3, n = 100 (12.0–100.0)	0.70 (0.30–2.10)		1.37 (0.80–2.31)		2.20 (1.24–4.65)		0.71 (0.31–1.60)		3.71 (2.28–5.30)		4.55 (2.81–6.70)	
White fish	1, n = 156 (0–9.0)	0.60 (0.22–1.23)	0.004	1.20 (0.60–2.10)	0.002	1.82 (0.90–3.10)	<0.0001	0.60 (0.30–1.10)	0.0002	3.20 (1.81–5.10)	0.08	3.86 (2.20–5.80)	0.01
	2, n = 135 (9.0–14.0)	0.70 (0.24–1.70)		1.30 (0.70–2.40)		1.90 (1.00–3.70)		0.60 (0.29–1.20)		3.30 (1.90–4.80)		3.90 (2.20–5.80)	
	3, n = 132 (14.0–69.0)	0.70 (0.25–2.15)		1.40 (0.70–2.38)		2.20 (1.10–4.00)		0.70 (0.40–1.60)		3.55 (1.80–5.30)		4.30 (2.39–6.40)	
Other seafood	1, n = 254 (0–2.6)	0.60 (0.20–1.40)	0.05	1.27 (0.63–2.20)	0.01	1.90 (1.0–3.41)	0.002	0.60 (2.29–1.16)	0.008	3.20 (1.80–5.32)	0.03	3.80 (2.10–6.29)	0.003
	2, n = 86 (2.6–7.0)	0.67 (0.29–1.82)		1.23 (0.60–2.30)		1.90 (1.00–4.13)		0.61 (0.33–1.40)		3.32 (2.00–4.96)		4.10 (2.60–5.70)	
	3, n = 83 (7.0–62.9)	0.73 (0.30–2.00)		1.40 (0.80–2.40)		2.20 (1.20–4.00)		0.70 (0.33–1.56)		3.67 (2.20–4.90)		4.50 (2.60–5.80)	
Total seafood	1, n = 142 (0–15.7)	0.60 (0.25–1.10)	<0.0001	1.17 (0.60–2.20)	<0.0001	1.70 (1.00–3.10)	<0.0001	0.57 (0.28–0.98)	<0.001	3.00 (1.80–5.10)	0.001	3.65 (2.10–5.70)	<0.0001
	2, n = 142 (15.7–26.0)	0.60 (0.18–1.42)		1.29 (0.60–2.10)		1.90 (0.90–3.41)		0.60 (0.30–1.12)		3.29 (1.80–4.94)		3.91 (2.30–5.83)	
	3, n = 139 (26.0–155.4)	0.80 (0.40–2.20)		1.40 (0.71–2.40)		2.26 (1.20–4.40)		0.80 (0.40–1.60)		3.70 (2.20–5.10)		4.50 (2.60–6.40)	

* P for Kruskal-Wallis ANOVA.

DISCUSSION

In the present study, a high RBCM EPA+DHA index (omega-3 index) was significantly associated with a 48% reduction of the odds of neovascular AMD. The associations of neovascular AMD with EPA status also appeared particularly strong (OR = 0.25, $P < 0.0001$ for RBCM EPA and OR = 0.41 $P = 0.005$ for serum EPA).

In the present study, the results of seafood consumption are consistent with previous dietary studies. Although AMD patients had significantly lower oily fish and seafood intake than controls, associations did not reach statistical significance after adjustment for all potential confounders. Among published case-control studies reporting associations between fish consumption and AMD, one found a significant association,[18] whereas 3 studies, including the Age-Relate Eye Disease Study (AREDS), showed no significant association.[11–13] Moreover, in 2008, a meta-analysis estimated that the risk for late AMD was reduced by 38% in participants with high dietary intakes of n-3 LC-PUFAs.[7] Since then, 4 large prospective[20,21,24,26] and 4 large cross-sectional[18,19,23,25] dietary studies published consistent and similar results.

The present results for serum EPA+DHA are consistent with the only published study on plasma n-3 LC-PUFAs in AMD, from the population-based Alienor Study.[35] This study showed a 33% decreased risk for neovascular AMD in subjects with high plasma n-3 LC-PUFAs; however, not reaching statistical significance (OR = 0.67, $P = 0.08$).[35] Interestingly, AMD risk was found here, in a new and independent sample of the French population, in the same range (OR = 0.74, $P = 0.35$) for serum EPA+DHA. In the Alienor study, plasma EPA was not associated with neovascular AMD ($P = 0.51$), while plasma DHA was borderline with neovascular AMD ($P = 0.06$). In the present study, we found a significant association with serum EPA ($P = 0.005$), but not with serum DHA ($P = 0.81$).

To our knowledge, the present study is the first case-control study reporting associations of RBCM n-3 long-chain fatty acids with neovascular AMD. We showed significant and strong associations of neovascular AMD with RBCM EPA and RBCM EPA+DHA. As expected, association with AMD was stronger for RBCM than serum measurements, because EPA or DHA measured in RBCM are more stable and longer-term biomarkers of body LC-PUFAs homeostasis and less influenced by lifestyle or other endogenous factors than EPA+DHA in serum or plasma.[28]

In the present study, associations of neovascular AMD with circulating EPA (in serum and RBCM) were markedly stronger than with circulating DHA. This could reflect differences in endogenous metabolism of n-3 LC-PUFA, which could be visible more readily through circulating EPA than through circulating DHA. For example, there is high interindividual variability with different tissue-specific rates of EPA/DHA interconversion, depending on age, sex, nutritional, or metabolic conditions.[29] Moreover, although DHA is quantitatively more abundant than EPA in serum or cell membranes, changes in serum and RBCM EPA are more pronounced than serum or RBCM DHA, with changes in dietary intakes of EPA+DHA, even in subjects taking n-3 LC-PUFA oral supplements exclusively enriched in DHA.[29] Alternately, the protective role of EPA is supported by oxidative metabolism by cyclooxygenases and lipoxygenases to produce eicosanoids with vasoregulatory and anti-inflammatory properties in the retina.[2] The EPA also is the precursor of docosapentaenoic acid (DPA), which is known to be the potential precursor of n-3 very long chain PUFAs (VLC-PUFAs), including 24:5 n-3 fatty acid, the most abundant VLC-PUFA present in the retina.[44] A recent study has observed a decreased of some n-3 VLC-PUFAs (notably 24:5 n-3) in early and intermediate AMD retinas as

Table 4. Associations of Dietary Intake of Seafood With Neovascular AMD

Dietary Intake of Seafood	Tertile	Range, g/d	Model 1*			Model 2†		
			OR	95% CI	*P* for Trend	OR	95% CI	*P* for Trend
Total fish	1	0–12.8	1.00	Ref	0.04	1.00	Ref	0.21
	2	12.8–23.0	0.63	0.38–1.05		0.55	0.30–1.00	
	3	23.0–139.0	0.57	0.34–0.97		0.69	0.37–1.29	
Oily fish	1	0–5.4	1.00	Ref	0.13	1.00	Ref	0.56
	2	5.4–12.0	0.85	0.52–1.39		0.99	0.55–1.80	
	3	12.0–100.0	0.67	0.40–1.12		0.82	0.44–1.53	
White fish	1	0–9.0	1.00	Ref	0.34	1.00	Ref	0.17
	2	9.0–14.0	1.00	0.60–1.67		1.25	0.68–2.29	
	3	14.0–69.0	0.79	0.47–1.29		0.63	0.34–1.15	
Other seafood	1	0–2.6	1.00	Ref	0.10	1.00	Ref	0.64
	2	2.6–7.0	0.60	0.36–1.01		0.59	0.32–1.11	
	3	7.0–62.9	0.71	0.42–1.20		0.98	0.52–1.86	
Total seafood	1	0–15.7	1.00	Ref	0.05	1.00	Ref	0.22
	2	15.7–26.0	0.60	0.36–1.01		0.50	0.27–0.92	
	3	26.0–155.4	0.59	0.35–0.99		0.68	0.36–1.28	

* Model 1, OR estimated using logistic regression adjusted for age and sex; AMD patients, $n = 284$; controls, $n = 139$.

† Model 2, OR estimated using logistic regression adjusted for age, sex, *CFH Y402H*, *ARMS2 A69S*, and *ApoE4* polymorphisms, plasma triglycerides, hypertension, hypercholesterolemia, and family history of AMD; AMD patients, $n = 284$; controls, $n = 139$.

compared to age-matched control.[44] Finally, two randomized, prospective, placebo-controlled, clinical trials have tested the efficiency of oral n-3 LC-PUFAs supplementation on late AMD development.[36,45] First, the NAT2 study found no effect of a three-year oral EPA+DHA (1:3, EPA:DHA [mg/mg ratio] from fish-oil) on progression from early AMD to neovascular AMD, in the second eye of patients with unilateral neovascular AMD at baseline.[36] Second, AREDS2 primary analyses showed that addition of lutein+zeaxanthin, EPA+DHA (2:1, EPA:DHA [mg/mg ratio] from ethyl esters) or both to the AREDS formulation did not further reduce the 5-year risk of progression from early to late AMD (geographic or neovascular AMD).[45] Remarkably, in placebo groups from both trials, incidence of late AMD at follow-up was lower than that expected from observational studies, suggesting that trial-effects (e.g., healthy lifestyle, unreported self-supplementation in LC-PUFA, and so forth) might have reduced statistical study power in both randomized trials. Therefore, these two recent clinical trials, may not challenge more than one decade of observational studies in favor of a protective effect of dietary n-3 PUFAs on AMD. The AREDS study recently published that 5 years after the clinical trial end, the beneficial effects of the AREDS formulation persisted for development of neovascular AMD, suggesting a potential long-term effect of nutritional factors involved in

Table 5. Associations of Circulating n-3 PUFAs With Neovascular AMD.

	Tertile	Range, % of Fatty Acids	Model 1*			Model 2†		
			OR	95% CI	*P* for Trend	OR	95% CI	*P* for Trend
Serum								
EPA	1	0–0.5	1.00	Ref	0.04	1.00	Ref	0.005
	2	0.5–0.9	0.61	0.37–1.00		0.50	0.27–0.91	
	3	0.9–3.7	0.59	0.36–0.98		0.41	0.22–0.77	
DHA	1	0–1.1	1.00	Ref	0.46	1.00	Ref	0.81
	2	1.1–1.5	0.66	0.40–1.07		0.69	0.39–1.24	
	3	1.5–3.9	1.23	0.74–2.04		1.10	0.60–2.01	
EPA+DHA	1	0–1.7	1.00	Ref	0.87	1.00	Ref	0.35
	2	1.7–2.4	1.10	0.67–1.80		0.95	0.53–1.72	
	3	2.4–7.5	0.96	0.58–1.59		0.74	0.40–1.38	
RBCM								
EPA	1	0–0.5	1.00	Ref	<0.0001	1.00	Ref	<0.0001
	2	0.5–0.8	0.63	0.37–1.09		0.46	0.24–0.87	
	3	0.8–3.4	0.33	0.20–0.55		0.25	0.13–0.47	
DHA	1	0–2.9	1.00	Ref	0.09	1.00	Ref	0.37
	2	2.9–3.9	0.51	0.31–0.83		0.59	0.33–1.07	
	3	3.9–7.3	0.64	0.38–1.07		0.76	0.41–1.39	
EPA+DHA	1	0–3.5	1.00	Ref	0.002	1.00	Ref	0.03
	2	3.5–4.6	0.53	0.32–0.89		0.60	0.33–1.10	
	3	4.6–9.3	0.44	0.27–0.74		0.52	0.29–0.94	

* Model 1, OR estimated using logistic regression adjusted for age and sex; AMD patients, $n = 290$; controls, $n = 144$.

† Model 2, OR estimated using logistic regression adjusted for age, sex, *CFH Y402H*, *ARMS2 A69S*, and *ApoE4* polymorphisms, plasma triglycerides, hypertension, hypercholesterolemia and family history of AMD; AMD patients, $n = 290$; controls, $n = 144$.

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AMD pathogenesis.[46] Moreover, in the NAT2 study, the 3-year incidence of CNV was reduced significantly (hazard ratio [HR], 0.32; 95% confidence interval [CI], 0.10–0.99; $P = 0.047$) in patients achieving the highest RBCM EPA+DHA (omega-3 index > 8) over 3 years.[36] From these combined results, it seems to be relevant to analyze n-3 RBCM EPA+DHA status in AMD. Biological status of n-3 PUFAs could help identify those subjects at risk for AMD, and RBCM n-3 PUFAs appear more relevant as a biomarker of AMD.

Strength of our study was the combined use of biological data, mainly EPA+DHA RBCM measurements with dietary assessment of n-3 PUFA status, in the same groups of individuals affected or not with AMD. Indeed, from differences in well-established risk factors (age, medical history, *CFH*, *ARMS2*, and *APOE* polymorphisms) found with a group of normal vision/normal fundus individuals, the AMD group seemed as typical of a population of patients with exudative AMD. Although apparently paradoxical, that triglycerides were found significantly lower in AMD patients despite them being more numerous with dyslipidemia, may be somewhat expected since the whole population had plasma triglyceride concentrations within the normal range, including AMD patients regularly taking lipid-lowering medications. Finally, the omega-3 index (EPA+DHA index) measured in RBCM is a very good biomarker of n-3 PUFAs status in humans and recognized as a risk factor in cardiovascular diseases.[47] In the future, it may prove useful in the clinical setting, for the identification of AMD patients deficient in n-3 LC-PUFAs, which may benefit the most from nutritional intervention.

Selection of controls always is a concern in case-control studies, selection bias being difficult to avoid.[48] In the present study, controls were selected from the general population, in the same geographic area as cases. They were not aware of the specific objectives of the study, before the interview and blood sample. When we compared cases and controls, they were not different for sex, smoking, BMI, diabetes, and plasma cholesterol. However, cases were older than controls. Also, hypercholesterolemia and hypertension were more frequent in cases, which is partially consistent with previous studies.[49] Our two groups also were comparable for dietary intakes. To limit the potential bias due to differences in age, hypertension, or hypercholesterolemia, we used multivariate modeling. However, despite that we adjusted our analyses for these potential confounders, as well as major AMD-related genes, we cannot exclude residual confounding as in all epidemiologic studies.

Also, as our study focused on neovascular AMD cases only, our results can be generalized only to this type of AMD.

In conclusion, from the present report, elderly individuals with high RBCM levels of EPA+DHA, a long-term marker of intracellular LC-PUFAs, have a strongly reduced risk for neovascular AMD. This suggests the RBCM EPA+DHA index to be considered as added to the list of clinically relevant biomarkers of AMD.

Acknowledgments

The authors thank all physicians, nurses, and patients from Creteil University Eye Clinic; all biologists, in particular Claude Wolf, for scientific advice and support, all laboratory technicians, particularly Dominique Farabos, Myriam Mahe, and Dominique Labaud, for excellent technical assistance, from Biochimie B Laboratory from Saint Antoine Hospital.

Supported by Laboratoire Bausch & Lomb, Clinical Research, Montpellier, France, and by a grant from Fédération des aveubles et handicaps visuels de France and from Fondation Nestlé France (BMJM).

Disclosure: **B.M.J. Merle**, Fédération des aveugles et handicapés visuels de France (F), Fondation Nestlé France (F), Laboratoires Théa (R), Bausch & Lomb (R); **P. Benlian**, Bausch & Lomb (R); **N. Puche**, None; **A. Bassols**, Bausch & Lomb (E); **C. Delcourt**, Laboratoires Théa (C), Bausch & Lomb (C, R), Novartis (C), Laboratoires Théa (R); **E.H. Souied**, Bausch & Lomb (F, C, R), Laboratoires Théa (C), Laboratoires Théa (R)

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