ω–3 Long-chain polyunsaturated fatty acid intake and 12-y incidence of neovascular age-related macular degeneration and central geographic atrophy: AREDS report 30, a prospective cohort study from the Age-Related Eye Disease Study[1–4]

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ABSTRACT

Background: $\omega-3$ (n−3) Long-chain polyunsaturated fatty acids (LCPUFAs) affect processes implicated in vascular and neural retinal pathogenesis and thus may influence the risk of developing age-related macular degeneration (AMD).

Objective: We investigated whether $\omega-3$ LCPUFA intake was associated with a reduced likelihood of developing central geographic atrophy (CGA) and neovascular (NV) AMD.

Design: We undertook a nested cohort study within a multicenter phase 3 clinical trial, the Age-Related Eye Disease Study (AREDS), to study progression to advanced AMD in 1837 persons at moderate-to-high risk of this condition. The AREDS was designed to assess the clinical course, prognosis, risk factors, and nutrient-based treatments of AMD and ran from November 1992 to December 2005. We obtained baseline data on $\omega-3$ LCPUFA intake with a validated food-frequency questionnaire. Trained fundus graders ascertained AMD status from annual stereoscopic color photographs by using standardized methods at a single reading center across a 12-y period. We applied multivariable repeated-measures logistic regression with the incorporation of generalized estimating equation methods, because this permitted determination of progression to outcome at each visit.

Results: Participants who reported the highest $\omega-3$ LCPUFA intake (median: 0.11% of total energy intake) were 30% less likely than their peers to develop CGA and NV AMD. The respective odds ratios were 0.65 (95% CI: 0.45, 0.92; $P \leq 0.02$) and 0.68 (95% CI: 0.49, 0.94; $P \leq 0.02$).

Conclusions: The 12-y incidence of CGA and NV AMD in participants at moderate-to-high risk of these outcomes was lowest for those reporting the highest consumption of $\omega-3$ LCPUFAs. If these results are generalizable, they may guide the development of low-cost and easily implemented preventive interventions for progression to advanced AMD. This trial was registered at clinicaltrials.gov as NCT00594672. *Am J Clin Nutr* 2009;90:1601–7.

INTRODUCTION

Age-related macular degeneration (AMD) is a major cause of irreversible vision loss among people of Western European ancestry; estimates of the number of European and US residents living with sight-threatening AMD are 3.35 and 1.75 million, respectively (1). Over the next 20 y, these values are expected to increase by ≈50% if preventive interventions are not identified (1). Pharmacologic treatments for neovascular (NV) AMD exist, but they are limited in scope, are costly, and may result in complications as severe as end-stage disease. There is thus an unmet medical need to identify low-cost behavior-based treatment strategies for preventing progression to advanced AMD.

The Age-Related Eye Disease Study (AREDS), a large phase 3 clinical trial, has tested nutrient-based formulations as preventive interventions for AMD (2). The pathogenesis of AMD may have a primary inflammatory component (3, 4). Several recently characterized long-chain polyunsaturated fatty acid (LCPUFA)–derived mediators are implicated in immunomodulation and inflammatory responses (reviewed in references 5–8). Factors and processes affecting and resulting from ocular inflammation activate enzymes that cleave membrane-bound LCPUFAs and then convert free LCPUFAs (9) to potent mediators (eicosanoids, endocannabinoids, resolvins, lipoxins, docosatrienes, and neuroprotectins) with autocrine and paracrine effects on retinal inflammation, neovascularization, and cell survival (7, 8, 10). Intake and status of the principal dietary $\omega-3$ (n−3) LCPUFAs—docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)—thus have the capacity to alter inflammation-based processes implicated in AMD pathogenesis within neural and vascular retina (9, 11). Evidence of LCPUFA-AMD relations reported from large epidemiologic studies (9, 12–22) provides a reasonable basis for investigating the association of dietary $\omega-3$ LCPUFAs with advanced AMD. With this objective in mind, we analyzed data in a 12-y prospective study of >1800 participants in AREDS at

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Received February 5, 2009. Accepted for publication September 2, 2009. First published online October 7, 2009; doi: 10.3945/ajcn.2009.27594.

moderate-to-high risk of progression to advanced AMD and visual acuity of 20/32 (6/9.5, 0.2 logMAR) or better in at least one eye.

SUBJECTS AND METHODS

Details of the methods and study protocol were previously reported (23–25). AREDS includes the largest and longest-followed cohort of persons with NV AMD and central geographic atrophy (CGA) from a natural history study using standardized methods of fundus photography and outcome ascertainment. Before the study began, the protocol was approved by a Data and Safety Monitoring Committee and by the Institutional Review Board for each clinical center. Before enrollment, informed consent was obtained from all participants. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Study Design, Setting, and Participants

AREDS was a multicenter National Institutes of Health–administered natural history study and phase 3 clinical trial designed to assess the clinical course, prognosis, risk factors, and nutrient-based prevention and treatment of AMD. We enrolled participants from November 1992 to January 1998, completed the 5-y clinical trial in April 2001, and continued follow-up until December 2005 to evaluate the 4-y clinical course and progression of AMD for participants previously enrolled in the trial. We refer to the posttrial phase as the AREDS Follow-up Study. Eleven clinical sites enrolled 4757 US residents in 4 AMD categories as determined by the size and extent of drusen and abnormalities of the retinal pigment epithelium (RPE) in each eye, the presence of advanced AMD (determined by assessment of stereoscopic color photographs at a central reading center) (23), and visual acuity. The participants were aged 55–80 y at enrollment and had a best-corrected visual acuity of 20/32 or better in at least one eye. At least one eye was free of advanced AMD (defined as CGA or NV AMD) and any disease that could complicate assessment of AMD; the study eye(s) could not have had previous ocular surgery, except cataract extraction.

There were 2337 AREDS participants at moderate-to-high risk of progression to advanced AMD at enrollment. These individuals were classified through a standardized protocol (24) as having large drusen (n = 1562; AREDS group 3), geographic atrophy in one or both eyes (n = 118; AREDS group 4), or unilateral NV AMD (n = 657; AREDS group 5). By the end of the trial, 2231 (95.5%) participants had complete AMD data from annual fundus photographs. Of the 2211 participants at moderate-to-high risk of progression (AREDS Groups 3, 4, or 5) still living at the end of the clinical trial, 1929 (87.2%) agreed to participate in the follow-up study; 75 (3.9%) of these participants were subsequently lost to follow-up.

All participants analyzed in the present study had a visual acuity of 20/32 or better and were at moderate-to-high risk of progression to advanced AMD in their study eye(s); this was the complete cohort identified as AREDS category 3a (n = 1211) and category 4a (n = 626). Those from category 3a (large drusen and good vision) had bilateral large ($\geq$125 μm) drusen, extensive intermediate ($\geq$64 to $<$125 μm) drusen, and/or geographic atrophy that did not involve the center of the macula in at least one

TABLE 1
Subject characteristics distributed by advanced age-related macular degeneration (AMD) outcomes[1]

		Progression to CGA			Progression to NV AMD		
	Participants	No	Yes	P value[2]	No	Yes	P value[2]
	n (%)	n (%)	n (%)		n (%)	n (%)	
Total n	1837	1473	364		1254	583	
Age at enrollment				0.307			<0.001
60 to <65 y	305 (16.6)	250 (16.97)	55 (15.11)		235 (18.74)	70 (12.01)	
65 to <70 y	538 (29.29)	439 (29.80)	99 (27.20)		388 (30.94)	150 (25.73)	
≥70 y	994 (54.11)	784 (53.22)	210 (57.69)		631 (50.32)	363 (62.26)	
Female sex	992 (54)	790 (53.63)	202 (55.49)	0.523	652 (51.99)	340 (58.32)	0.011
AREDS treatment				0.083			0.124
Placebo	453 (24.66)	376 (25.53)	77 (21.15)		296 (23.60)	157 (26.93)	
AREDS formulations	1384 (75.34)	1097 (74.47)	287 (78.85)		958 (76.40)	426 (73.07)	
Smoking history				0.533			0.093
Never	732 (39.85)	596 (40.46)	136 (37.36)		516 (41.15)	216 (37.05)	
Former	933 (50.79)	742 (50.37)	191 (52.47)		631 (50.32)	302 (51.80)	
Current	172 (9.36)	135 (9.16)	37 (10.16)		107 (8.53)	65 (11.15)	
Baseline AMD category				0.809			<0.001
AREDS category 3a[3]	1211 (65.92)	973 (66.06)	238 (65.38)		903 (72.01)	308 (52.83)	
AREDS category 4a[4]	626 (34.08)	500 (33.94)	126 (34.62)		351 (27.99)	275 (47.17)	

[1] Data are from all Age-Related Eye Disease Study (AREDS) participants at moderate-to-high risk of progression to central geographic atrophy (CGA) and neovascular (NV) AMD with a visual acuity of 20/32 or better in at least one eye at enrollment (n = 1837). Advanced AMD was classified as CGA, choroidal neovascularization, or retinal pigment epithelium cell detachment including nondrusenoid retinal pigment epithelium detachment, serous sensory or hemorrhagic retinal detachment, subretinal hemorrhage, subretinal fibrosis, or evidence of confluent photocoagulation for NV AMD.

[2] Derived from chi-square tests on advanced AMD outcomes that were examined annually across a 12-y period.

[3] Participants in this category (large drusen and good vision) had bilateral large ($\geq$125 μm) drusen, extensive intermediate ($\geq$64 to $<$125 μm) drusen, and/or geographic atrophy that did not involve the center of the macula in at least one eye.

[4] Participants in this category had no advanced AMD (CGA or features of NV AMD) and good vision in the study eye; the other eye had definite lesions of advanced AMD.

eye. Category 4a participants had no advanced AMD (CGA or features of NV AMD) and good vision in the study eye; the other eye had definite lesions of advanced AMD. Advanced AMD was classified as CGA, choroidal neovascularization (CNV), or RPE detachment, including nondrusenoid RPE detachment, serous sensory or hemorrhagic retinal detachment, subretinal hemorrhage, subretinal fibrosis, or evidence of confluent photocoagulation for NV AMD. Median follow-up time after AREDS enrollment was 3663 d (mean ± SEM: 3240.37 ± 25.22 d). The primary outcome variables are progression to advanced CGA and/or NV AMD across the 12-y study period.

We applied data-quality filters to restrict our analytic sample to the most accurate reporters of dietary intake. The filters were based on total energy intake (TEI) values between the 1st and 99th percentiles of those from a nationally representative probability sample of US residents aged ≥71 y [Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1996, 1998]; the 1st percentile value was 677 kcal/d for women and 794 kcal/d for men, and the 99th percentile value was 1994 kcal/d for women and 2771 kcal/d for men (26, 27). This process yielded a final analytic sample of 1837 participants.

Procedures

We performed general physical and ophthalmic examinations to obtain information on demographic factors, environmental exposures, medical history, drug use, and habitual diet in the year before enrollment. Trained fundus graders who were masked to clinical and phenotypic information from previous years ascertained AMD from annual stereoscopic color images using a standardized and validated protocol at a single reading center. Retinal photographs were taken with a standardized protocol by AREDS-certified photographers using AREDS-certified cameras. Adjudication with a standardized protocol occurred when discrepancies emerged. Details on exposure and outcome ascertainment exist in *AREDS Reports 1* and *3* (23, 24).

We collected information on dietary LCPUFA intake at enrollment with a validated, self-administered, 90-item semiquantitative, food-frequency questionnaire based on the National Cancer Institute Health Habits and History Questionnaire (version 2.1) (19, 25). The University of Minnesota Nutrition Coordinating Center Food Composition Database (version 31) was used with the estimated quantity of nutrient intake and DietSys software (version 3.0; Block Dietary Data Systems, Berkeley, CA) to derive individual nutrient values for each questionnaire item. The instrument was validated by using a telephone-administered 24-h dietary recall 3 and 6 mo after enrollment in 197 randomly selected participants. Correlations of 24-h recall data with the AREDS FFQ were corrected for attenuation with the method of Rosner and Willett (28). The correlation coefficients were 0.35 for EPA and 0.32 for DHA. We computed nutrient densities (nutrient intake/TEI) to represent habitual intake. We defined LCPUFA variables as nutrient density scores of $\omega-3$ LCPUFA intake as classified into quintiles of the entire AREDS sample. The referent category was defined as intake in the lower 20% of the distribution.

Statistical modeling and analyses

All analyses were conducted with SAS statistical software (versions 9.1 and 9.2; SAS Institute Inc, Cary, NC). We examined

TABLE 2
$\omega-3$ Long-chain polyunsaturated fatty acid intake by advanced age-related macular degeneration (AMD) outcomes[1]

Quintile/median (% of TEI)	Participants	Progression to CGA			Progression to NV AMD		
		No	Yes	*P* value[2]	No	Yes	*P* value[2]
	n (%)	*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Total *n*	1837	1473	364		1254	583	
DHA				0.069			0.001
Quintile 1 (0.010)	381 (20.74)	303 (20.57)	78 (21.43)		237 (18.90)	144 (24.70)	
Quintile 2 (0.018)	350 (19.05)	290 (19.69)	60 (16.48)		239 (19.06)	111 (19.04)	
Quintile 3 (0.026)	372 (20.25)	287 (19.48)	85 (23.35)		268 (21.37)	104 (17.84)	
Quintile 4 (0.037)	392 (21.34)	305 (20.71)	87 (23.90)		254 (20.26)	138 (23.67)	
Quintile 5 (0.061)	342 (18.62)	288 (19.55)	54 (14.84)		256 (20.41)	86 (14.75)	
EPA				0.448			0.291
Quintile 1 (0.000)	364 (19.81)	286 (19.42)	78 (21.43)		235 (18.74)	129 (22.13)	
Quintile 2 (0.009)	388 (21.12)	311 (21.11)	77 (21.15)		271 (21.61)	117 (20.07)	
Quintile 3 (0.015)	366 (19.92)	285 (19.35)	81 (22.25)		243 (19.38)	123 (21.10)	
Quintile 4 (0.024)	371 (20.20)	306 (20.77)	65 (17.86)		257 (20.49)	114 (19.55)	
Quintile 5 (0.044)	348 (18.94)	285 (19.35)	63 (17.31)		248 (19.78)	100 (17.15)	
DHA+EPA				0.026			
Quintile 1 (0.013)	376 (20.47)	293 (19.89)	83 (22.80)		237 (18.90)	139 (23.84)	0.032
Quintile 2 (0.028)	360 (19.60)	302 (20.50)	58 (15.93)		250 (19.94)	110 (18.87)	
Quintile 3 (0.042)	374 (20.36)	283 (19.21)	91 (25.00)		259 (20.65)	115 (19.73)	
Quintile 4 (0.061)	384 (20.90)	310 (21.05)	74 (20.33)		255 (20.33)	129 (22.13)	
Quintile 5 (0.106)	343 (18.67)	285 (19.35)	58 (15.93)		253 (20.18)	90 (15.44)	

[1] Advanced AMD was classified as central geographic atrophy (CGA), choroidal neovascularization, or retinal pigment epithelium cell detachment including nondrusenoid retinal pigment epithelium detachment, serous sensory or hemorrhagic retinal detachment, subretinal hemorrhage, subretinal fibrosis, or evidence of confluent photocoagulation for neovascular (NV) AMD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TEI, total energy intake computed with the assumption of an intake of 2000 kcal/d.

[2] Derived from chi-square tests on advanced AMD outcomes examined annually across a 12-y period.

frequency distributions of potential covariates identified through empirical tests, literature reviews, and expert opinion.

We computed odds ratios in repeated-measures logistic regression models incorporating generalized estimating equation methods. This permitted determination of advanced AMD at each visit for each participant. All models included terms for baseline age in years (analyzed in categories as 71–80, 66–70, and 60–65 y), sex, and total energy intake (modeled as a continuous variable). In addition to age, smoking and the presence of advanced AMD in one eye have been the factors most strongly and consistently associated with progression to advanced AMD. As such, we ran additional models containing the original 3 variables, smoking history (ever smoked for ≥6 mo and never smoked ≥6 mo), AREDS baseline AMD severity category (3a, 4a; 23, 24), and AREDS treatment assignment (antioxidants, zinc, antioxidants + zinc, and placebo). We performed trend tests with the variables specified in age, sex, and energy-adjusted and final models using the calorie-adjusted median of LCPUFA intake value for a participant's intake quintile as the nutrient exposure.

RESULTS

Three hundred sixty-four of 1837 people (19.8%) progressed to CGA and 583 (31.7%) progressed to NV AMD across the 12-y follow-up period. Chi-square tests on variables in **Table 1** yielded P values <0.05 for the NV AMD outcome with advanced age, female sex, and AREDS baseline category 4a. These factors characterized persons who progressed to NV AMD more frequently than their peers. Information on dietary $\omega-3$ LCPUFA intake, distributed by advanced AMD endpoints, is shown in **Table 2**. The median value for DHA intake in quintile 3 (representing the median of all DHA intake values) was 0.026% of TEI, which is similar to the DHA value reported as the overall mean percentage of TEI from the National Health and Nutrition Examination (NHANES III; 0.03% TEI) and the 1994–1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII; 0.025% TEI). The AREDS median intake value for quintile 3 of EPA was 0.015% TEI; NHANES III reported 0.02% TEI and CSFII reported 0.013% TEI (27). CSFII and NHANES III are based on US national probability samples. There was a lower proportion of participants progressing to CGA and NV AMD among those who reported $\omega-3$ LCPUFA intakes in the highest quintile, relative to their peers (Table 2). Cross-tabulations of nonnutrient variables included in the final models by quintiles of $\omega-3$ LCPUFA intake are shown in **Table 3**. Of the potential predictors and correlates previously associated with advanced AMD that we investigated, only smoking history varied significantly with DHA+EPA intake; current smokers were less likely than their peers to report $\omega-3$ LCPUFA intakes in the top 20% (5th quintile) of the distribution.

The findings of repeated-measures logistic regression analyses are shown in **Table 4**. The results from final models did not change appreciably from those obtained in models specified solely with age-, sex-, and energy-adjustment. Participants reporting the highest DHA, EPA, or DHA+EPA intakes were ≈30% less likely to progress to CGA and NV AMD as their peers reporting the lowest intake. Respective ORs for comparison of progression to CGA in the highest compared with the

TABLE 3
Subject characteristics by quintiles of reported $\omega-3$ long-chain polyunsaturated fatty acid intake[1]

		DHA+EPA quintile					
	Participants	1	2	3	4	5	P value[2]
	n (%)						
Total n	1837	376	360	374	384	343	
Quintile median (% of TEI)		0.013	0.028	0.042	0.061	0.106	
Age at enrollment [n (%)]							0.337
60 to <65 y	305 (16.60)	58 (15.43)	65 (18.06)	64 (17.11)	52 (13.54)	66 (19.24)	
65 to <70 y	538 (29.29)	106 (28.19)	103 (28.61)	123 (32.89)	113 (29.43)	93 (27.11)	
≥70 y	994 (54.11)	212 (56.38)	192 (53.33)	187 (50.00)	219 (57.03)	184 (53.64)	
Female sex [n (%)]	992 (54.00)	206 (54.79)	198 (55.00)	211 (56.42)	198 (51.56)	179 (52.19)	0.646
AREDS treatment [n (%)]							0.860
Placebo	453 (24.66)	96 (25.53)	92 (25.56)	91 (24.33)	97 (25.26)	77 (22.45)	
AREDS formulations	1384 (75.34)	280 (74.47)	268 (74.44)	283 (75.67)	287 (74.74)	266 (77.55)	
Smoking history [n (%)]							0.014
Never	732 (39.85)	144 (38.30)	142 (39.44)	163 (43.58)	155 (40.36)	128 (37.32)	
Former	933 (50.79)	182 (48.40)	185 (51.39)	173 (46.26)	197 (51.30)	196 (57.14)	
Current	172 (9.36)	50 (13.30)	33 (9.17)	38 (10.16)	32 (8.33)	19 (5.54)	
Baseline AMD category [n (%)]							0.081
AREDS category 3a[3]	1211 (65.92)	229 (60.90)	230 (63.89)	254 (67.91)	258 (67.19)	240 (69.97)	
AREDS category 4a[4]	626 (34.08)	147 (39.10)	130 (36.11)	120 (32.09)	126 (32.81)	103 (30.03)	
Progression to CGA [n (%)]	364 (19.81)	83 (22.07)	58 (16.11)	91 (24.33)	74 (19.27)	58 (16.91)	0.026
Progression to NV AMD [n (%)]	583 (31.74)	139 (36.97)	110 (30.56)	115 (30.75)	129 (33.59)	90 (26.24)	0.032

[1] EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AREDS, Age-Related Eye Disease Study; CGA, central geographic atrophy; NV, neovascular; TEI, total energy intake computed with the assumption of an intake of 2000 kcal/d; AMD, age-related macular degeneration.

[2] Derived from chi-square tests.

[3] Participants in this category (large drusen and acuity of 20/32 or better) had bilateral large (≥125 μm) drusen, extensive intermediate (≥64 to <125 μm) drusen, and/or geographic atrophy that did not involve the center of the macula in at least one eye.

[4] Participants in this category had no advanced AMD (CGA or features of NV AMD) and acuity of 20/32 or better in the study eye; the other eye had definite lesions of advanced AMD.

TABLE 4
Odds ratios (ORs) and 95% CIs for the 12-y progression to central geographic atrophy (CGA) and neovascular age-related macular degeneration (NV AMD)[1]

Nutrient and quintile of intake	Energy intake	Models for progression to CGA: CGA/No CGA	Age, sex, TEI-adjusted OR (95% CI)[2]	*P*	Final model OR (95% CI)[2]	*P*	Models for progression to NV AMD: NV/No NV	Age, sex, TEI-adjusted OR (95% CI)[2]	*P*	Final model OR (95% CI)[2]	*P*
	% of TEI	*n/n*					*n/n*				
DHA											
1	0.010	78/303	1.00		1.00		144/237	1.00		1.00	
2	0.018	60/290	0.80 (0.55, 1.14)	0.219	0.80 (0.56, 1.15)	0.225	111/239	0.84 (0.63, 1.14)	0.265	0.89 (0.66, 1.20)	0.431
3	0.026	85/287	1.14 (0.82, 1.58)	0.437	1.15 (0.83, 1.59)	0.417	104/268	0.70 (0.51, 0.94)	0.019	0.75 (0.55, 1.02)	0.065
4	0.037	87/305	1.04 (0.74, 1.44)	0.837	1.04 (0.75, 1.44)	0.832	138/254	0.84 (0.63, 1.11)	0.219	0.89 (0.67, 1.19)	0.443
5	0.061	54/288	0.70 (0.48, 1.00)	0.052	0.68 (0.47, 0.99)	0.042	86/256	0.59 (0.43, 0.83)	0.002	0.66 (0.47, 0.92)	0.014
P for trend[3]			0.125		0.099			0.0005		0.026	
EPA											
1	0.000	78/286	1.00		1.00		129/235	1.00		1.00	
2	0.009	77/311	0.90 (0.65, 1.26)	0.557	0.90 (0.64, 1.25)	0.528	117/271	0.71 (0.53, 0.96)	0.028	0.73 (0.54, 0.98)	0.038
3	0.015	81/285	1.04 (0.74, 1.46)	0.820	1.04 (0.74, 1.45)	0.817	123/243	0.89 (0.66, 1.20)	0.447	0.97 (0.72, 1.31)	0.834
4	0.024	65/306	0.75 (0.53, 1.08)	0.122	0.74 (0.52, 1.06)	0.104	114/257	0.71 (0.52, 0.96)	0.028	0.76 (0.55, 1.03)	0.079
5	0.044	63/285	0.71 (0.50, 1.02)	0.067	0.70 (0.49, 1.00)	0.051	100/248	0.66 (0.48, 0.90)	0.010	0.71 (0.51, 0.98)	0.039
P for trend[3]			0.033		0.024			0.019		0.068	
DHA+EPA											
1	0.013	83/293	1.00		1.00		139/237	1.00		1.00	
2	0.028	58/302	0.69 (0.48, 0.99)	0.047	0.70 (0.49, 1.00)	0.051	110/250	0.76 (0.56, 1.02)	0.065	0.79 (0.58, 1.06)	0.111
3	0.042	91/283	1.14 (0.83, 1.56)	0.426	1.14 (0.83, 1.56)	0.417	115/259	0.79 (0.58, 1.06)	0.116	0.85 (0.63, 1.15)	0.299
4	0.061	74/310	0.78 (0.56, 1.10)	0.159	0.78 (0.56, 1.10)	0.157	129/255	0.77 (0.58, 1.04)	0.086	0.83 (0.62, 1.13)	0.235
5	0.106	58/285	0.66 (0.46, 0.94)	0.021	0.65 (0.45, 0.92)	0.016	90/253	0.62 (0.44, 0.85)	0.004	0.68 (0.49, 0.94)	0.020
P for trend[3]			0.038		0.028			0.010		0.044	

[1] Advanced AMD was classified as CGA, choroidal neovascularization, or retinal pigment epithelium cell detachment including nondrusenoid retinal pigment epithelium detachment, serous sensory or hemorrhagic retinal detachment, subretinal hemorrhage, subretinal fibrosis, or evidence of confluent photocoagulation for NV AMD. Multivariable repeated-measures logistic regression with generalized estimating equation methods was used to analyze the data. Participants in AMD category 3a (large drusen and acuity of 20/32 or better) had bilateral large ($\geq$125 μm) drusen, extensive intermediate ($\geq$64 to $<$125 μm) drusen, and/or geographic atrophy that did not involve the center of the macula in at least one eye. Participants in AMD category 4a had no advanced AMD (CGA or features of NV AMD) and good vision in the study eye; the other eye had definite lesions of advanced AMD. Final models contained terms for age, sex, total energy intake (TEI), smoking history, AMD status at enrollment, and Age-Related Eye Disease Study clinical trial treatment. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] For $\omega-3$ long-chain polyunsaturated fatty acid intakes from logistic regression models comparing energy-adjusted long-chain polyunsaturated fatty acid intake quintiles (quintile 1: reference).

[3] Computed by using median values for intake quintiles.

lowest quintiles of DHA, EPA, and DHA+EPA were as follows: 0.68 (95% CI: 0.47, 0.99; $P \leq 0.042$), 0.70 (95% CI: 0.49, 1.00; $P \leq 0.051$), and 0.65 (95% CI: 0.45, 0.92; $P \leq 0.016$). Corresponding ORs for the NV AMD endpoint are as follows: 0.66 (95% CI: 0.47, 0.92; $P \leq 0.014$), 0.71 (95% CI: 0.51, 0.98; $P \leq 0.039$), and 0.68 (95% CI: 0.49, 0.94; $P \leq 0.020$). We saw significant trends for a reduced likelihood of developing CGA with increased EPA ($P \leq 0.024$) and DHA+EPA ($P \leq 0.028$). Trend tests on NV AMD attained significance for DHA ($P \leq 0.026$) and DHA+EPA ($P \leq 0.044$). Where any LCPUFA-AMD relation existed, the magnitude of association was greatest for the fifth (highest reported intake) compared with the first (lowest reported intake) intake quintile comparisons.

DISCUSSION

Relations between $\omega-3$ LCPUFA intake and the prevalence (25) and incidence (19) of advanced AMD in past AREDS studies existed in the present cohort. Our results showed that, in a large 12-y longitudinal study of elderly people at moderate-to-high risk of progression to advanced AMD, persons who reported the highest intake of $\omega-3$ LCPUFAs were 30% less likely to develop CGA and NV AMD than were their peers who reported the lowest levels of intake of $\omega-3$ LCPUFAs. Although experimental studies are needed to make conclusive inferences, these results support the proposition that $\omega-3$ LCPUFAs may play key roles in modulating processes implicated in the pathogenesis of AMD (9, 11).

Existing treatments for advanced AMD are invasive, costly, and limited in scope and may result in blinding complications. Nutrient-based interventions with dietary supplements have shown promise in reducing the likelihood of progression to advanced AMD (2). A 4000-person, 5-y randomized clinical trial designed to examine the efficacy of $\omega-3$ LCPUFAs for this purpose is now underway (www.areds2.org). Rationale for the trial was based on studies that have consistently yielded measures of association in the direction of benefit for persons reporting the highest levels of consumption of $\omega-3$ LCPUFAs and $\omega-3$ LCPUFA-rich foods (9, 12–22). AMD may have an inflammatory etiology (3, 4), and work with in vivo model systems has shown that dietary $\omega-3$ LCPUFAs have the capacity to affect pathologic inflammatory processes in the retina via modulation of potent lipid-based mediators (29). There is emerging evidence on the immunoregulatory effect of EPA- and

DHA-based lipoxygenase metabolites, but our data did not permit us to examine this concept (30).

Our study had some limitations. The observational design did not allow us to conclusively rule out the effect of unmeasured factors that vary simultaneously with LCPUFA intake and progression to advanced AMD. In some populations, the frequency of $\omega-3$ LCPUFA-rich food consumption may be a proxy for a healthier lifestyle. We did not have data on physical activity or metabolic rate; however, because persons who smoke are less likely to practice beneficial health-related behaviors, we examined the distribution of $\omega-3$ LCPUFA intake by smoking status. In our sample, persons who smoked were more likely than their peers to report low $\omega-3$ LCPUFA intakes. Another issue of potential concern was whether we measured the effect of nutrients in addition to DHA and EPA that are concentrated in $\omega-3$ LCPUFA-rich food. The fact that DHA is the most abundant resident LCPUFA in the retina (31) and is selectively accreted and retained there (32) (EPA is the precursor to DHA) strengthened our inferences. Nonetheless, we examined the dietary sources of DHA and EPA and the variance that >25 nutrients shared with these LCPUFAs. The main sources of omega-3 LCPUFAs in the diets of the AREDS participants were fish and seafood. Fish intake contributed 93% of EPA and 71% of DHA (19). We applied an empirical approach with multivariable regression models to examine shared variance (colinearity) in our nutrient data (19). To evaluate the potential confounding effect of nutrient-nutrient associations, we computed partial correlation coefficients from linear regressions of log-transformed nutrient scores for all 25 nutrients on DHA, EPA, and DHA +EPA. After all measured nutrients that varied independently with $\omega-3$ LCPUFA intake were controlled for, only EPA and DHA contributed >5% to the variance in $\omega-3$ LCPUFA intake; there were no variables other than EPA associated with DHA, and vice versa; the results indicate that the $\omega-3$ LCPUFA intake data likely reflect actual $\omega-3$ LCPUFA intakes. As such, we believe that it was unlikely that EPA and DHA were markers for other nutrients in our sample.

Our prospective sampling scheme reduced the likelihood of differential misclassification of dietary intake, because participants were all at moderate-to-high risk of progression at enrollment. Most participants probably did not know they were at risk of progression to advanced AMD when the dietary data were obtained. Whereas those with more advanced disease, and thus at a higher risk of progression, may have been more likely to modify their habitual diet, there were few existing health recommendations or commercial claims for preventive uses of $\omega-3$ LCPUFA intake for AMD at the time of data collection. Our study had many other strengths, including low attrition, a long follow-up period, and standardized procedures for data collection and exposure/outcome measurements. Outcomes were ascertained by using annually collected fundus photographs taken by AREDS-certified photographers and -cameras. The process included masked gradings by a small group of trained graders working at a single center who used photographic standards and a validated protocol. Discordant findings between graders were adjudicated by the director of the reading center. The final phenotypes in a large subset of these data were also further evaluated and validated by the center director. Graders performed periodic reliability tests with a standard photo set to reduce and correct diagnostic drift.

We report the first evidence that a 12-y incidence of CGA and NV AMD is associated with dietary $\omega-3$ LCPUFA intake. These results are supported by current knowledge from model systems and clinical populations. We conclude that, our results—if confirmed by other studies and extended by clinical trials—may guide the development of low-cost, easily implemented, and widely accepted interventions to prevent the progression to advanced AMD.

We thank the AREDS participants for their essential contributions. We acknowledge the AREDS Operations Committee (Gary Gensler, Roy Milton, Frederick L Ferris III, Anne Lindblad, Alice Henning, Wendy McBee, Diana Tull, and Natalie Kurinij) and the AREDS Research Group.

The authors' responsibilities were as follows—JPS, EA, and EYC: developed the concept and structure of the project; RDS, TEC, and ADM: helped to refine the manuscript; GFR: provided essential guidance on statistical aspects of the project; and EA and JPS: conducted data analyses. All authors contributed to the data interpretation and composition of this report. None of the authors had a conflict of interest.

REFERENCES

1. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol 2004;122: 564–72.
2. Age-Related Eye Disease Study Group. A randomized, placebo-controlled clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 2001;119:1417–36.
3. Donoso LA, Kim D, Frost A, Callahan A, Hageman G. The role of inflammation in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2006;51:137–52.
4. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. Prog Retin Eye Res 2001; 20:385–414.
5. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 2008;8:349–61.
6. Weylandt KH, Kang JX. Rethinking lipid mediators. Lancet 2005;366: 618–20.
7. Bazan NG. Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. Trends Neurosci 2006;29:263–71.
8. Bazan NG. Neurotrophins induce neuroprotective signaling in the retinal pigment epithelial cell by activating the synthesis of the anti-inflammatory and anti-apoptotic neuroprotectin D1. Adv Exp Med Biol 2008;613:39–44.
9. SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Prog Retin Eye Res 2005;24:87–138.
10. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci USA 2004;101:8491–6 Epub 2004 May 19.
11. SanGiovanni JP, Mehta S, Mehta S. Variation in lipid-associated genes as they relate to risk of advanced age-related macular degeneration. World Rev Nutr Diet 2009;99:105–58.
12. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. Arch Ophthalmol 2003;121:1728–37.
13. Seddon JM, Rosner B, Sperduto RD, et al. Dietary fat and risk for advanced age-related macular degeneration. Arch Ophthalmol 2001;119: 1191–9.
14. Mares-Perlman JA, Brady WE, Klein R, VandenLangenberg GM, Klein BE, Palta M. Dietary fat and age-related maculopathy. Arch Ophthalmol 1995;113:743–8.
15. Cho E, Hung S, Willett WC, et al. Prospective study of dietary fat and the risk of age-related macular degeneration. Am J Clin Nutr 2001;73: 209–18.
16. Smith W, Mitchell P, Leeder SR. Dietary fat and fish intake and age-related maculopathy. Arch Ophthalmol 2000;118:401–4.

17. Heuberger RA, Mares-Perlman JA, Klein R, et al. Relationship of dietary fat to age-related maculopathy in the Third National Health and Nutrition Examination Survey. Arch Ophthalmol 2001;119:1833–8.
18. Chong EW, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Dietary omega-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: a systematic review and meta-analysis. Arch Ophthalmol 2008;126:826–33.
19. SanGiovanni JP, Chew EY, Agron E, et al. The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS Report No. 23. Arch Ophthalmol 2008;126:1274–9.
20. Augood C, Chakravarthy U, Young I, et al. Oily fish consumption, dietary docosahexaenoic acid and eicosapentaenoic acid intakes, and associations with neovascular age-related macular degeneration. Am J Clin Nutr 2008;88:398–406.
21. Chong EW, Robman LD, Simpson JA, et al. Fat consumption and its association with age-related macular degeneration. Arch Ophthalmol 2009;127:674–80.
22. Tan JS, Wang JJ, Flood V, Mitchell P. Dietary fatty acids and the 10-year incidence of age-related macular degeneration: the Blue Mountains Eye Study. Arch Ophthalmol 2009;127:656–65.
23. Age-Related Eye Disease Study Group. The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. Control Clin Trials 1999;20:573–600.
24. Age-Related Eye Disease Study Group. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. Ophthalmology 2000;107:2224–32.
25. SanGiovanni JP, Chew EY, Clemons TE, et al. The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS Report No. 20. Arch Ophthalmol 2007;125:671–9.
26. US Department of Agriculture, Agricultural Research Service. USDA Nationwide Food Surveys, 1935-1998. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=14392 (cited 30 August 2009).
27. Food and Nutrition Board, Institute of Medicine of the National Academies. Dietary reference intake for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academies Press, 2005.
28. Rosner B, Willett WC. Interval estimates for correlation coefficients corrected for within-person variation: implications for study design and hypothesis testing. Am J Epidemiol 1988;127:377–86.
29. Connor KM, SanGiovanni JP, Lofqvist C, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med 2007;13:868–73.
30. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol 2008;153 (suppl 1):S200–15.
31. Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 1983;22:79–131.
32. Neuringer M, ed. The relationship of fatty acid composition to fuction in the retina and visual system. Adelaide, South Australia: Ross Laboratories, 1993.