The Relationship of Dietary Lipid Intake and Age-Related Macular Degeneration in a Case-Control Study

AREDS Report No. 20

*Age-Related Eye Disease Study Research Group**

Objective: To evaluate the association of lipid intake with baseline severity of age-related macular degeneration (AMD) in the Age-Related Eye Disease Study (AREDS).

Methods: Age-Related Eye Disease Study participants aged 60 to 80 years at enrollment (N=4519) provided estimates of habitual nutrient intake through a self-administered semiquantitative food frequency questionnaire. Stereoscopic color fundus photographs were used to categorize participants into 4 AMD severity groups and a control group (participants with <15 small drusen).

Results: Dietary total ω-3 long-chain polyunsaturated fatty acid (LCPUFA) intake was inversely associated with neovascular (NV) AMD (odds ratio [OR], 0.61; 95% confidence interval [CI], 0.41-0.90), as was docosahexaenoic acid, a retinal ω-3 LCPUFA (OR, 0.54; 95% CI, 0.36-0.80), comparing highest vs lowest quintile of intake, after adjustment for total energy intake and covariates. Higher fish consumption, both total and broiled/baked, was also inversely associated with NV AMD (OR, 0.61; 95% CI, 0.37-1.00 and OR, 0.65; 95% CI, 0.45-0.93, respectively). Dietary arachidonic acid was directly associated with NV AMD prevalence (OR, 1.54; 95% CI, 1.04-2.29). No statistically significant relationships existed for the other lipids or AMD groups.

Conclusion: Higher intake of ω-3 LCPUFAs and fish was associated with decreased likelihood of having NV AMD.

Arch Ophthalmol. 2007;125:671-679

THERE IS NO KNOWN METHOD to prevent the development of advanced age-related macular degeneration (AMD), the leading cause of irreversible vision loss in the United States among persons older than 65 years.[1-4] Identification of behaviorally modifiable risk factors is a promising approach with which to reduce the burden of AMD, a burden that will grow as the number of elderly persons in the population increases.[5-7] Nutrient-based preventive treatments for AMD development and progression were examined in a controlled randomized clinical trial in the Age-Related Eye Disease Study (AREDS).[8,9] The use of high doses of a combination of antioxidants (vitamin C, vitamin E, and beta carotene) and zinc reduced the risk of development of advanced AMD by about 25% in participants who had at least a moderate risk of developing AMD. The overall risk of moderate vision loss (>15 letters on the Early Treatment of Diabetic Retinopathy Study chart) was reduced by 19% at 5 years. In other analyses, AREDS baseline data were used to identify demographic, lifestyle, medical, and ocular factors associated with advanced AMD.

Bioactive molecules derived from diet may modify the risk of AMD onset and progression. Lipid-AMD relationships are plausible since certain lipids present in the retina have properties capable of modulating cellular damage that may be associated with advanced AMD.[10,11] We were particularly interested in the relationship of ω-3 and ω-6 long-chain polyunsaturated fatty acids (LCPUFAs) with AMD, since retinal concentrations of these compounds are dependent on and modifiable by diet.[12] Some studies have shown protective relationships of ω-3 LCPUFA[13,14] and ω-3 LCPUFA–rich food intake[5,13-16] with various stages of AMD. Although these relationships did not always persist after multivariable adjustments, measures of association remained in the direction of benefit. Monounsaturated fatty acid (MUFA)– and saturated fatty acid (SFA)–AMD relationships were also of interest as these nutrients are capable of modulating inflammation and signal transduction pathways associated with cell vi-

*The Writing Team for the Age-Related Eye Disease Study (AREDS) Research Group consisted of John Paul SanGiovanni, ScD; Emily Y. Chew, MD; Traci E. Clemons, PhD; Matthew D. Davis, MD; Frederick L. Ferris III, MD; Gary R. Gensler, MS; Natalie Kurinij, PhD; Anne S. Lindblad, PhD; Roy C. Milton, PhD; Johanna M. Seddon, MD; and Robert D. Sperduto, MD.
Group Information: A complete list of the members of the AREDS Research Group appears in *Arch Ophthalmol*. 2004;122:716-726.

ability. Although not always attaining statistical significance, measures of association for MUFA-AMD relationships have been in the direction of harm for people reporting highest vs lowest intake.[5,13-15] This is also the case for total SFA[5,13-15,17] and cholesterol intake.[5,14,17]

In this report, we evaluate the relationship of dietary lipid intake in the year prior to study enrollment with the baseline (enrollment) severity of AMD in AREDS. We examined these associations while considering the effect of nutrient- and nonnutrient-based predictors and correlates of drusen size and extent and pigment abnormalities associated with AMD, geographic atrophy, and neovascular (NV) AMD. An advantage of this case-control approach, compared with previous studies, was that it allowed us to efficiently recruit a large number of persons with advanced AMD.

METHODS

STUDY POPULATION

Details of the AREDS design and methods appear in earlier publications.[8,9,18] In summary, 11 retinal specialty clinics enrolled 4757 participants from 1992 through 1998. Participants were 55 to 80 years of age at enrollment and had best-corrected visual acuity of 20/32 or better in at least 1 eye. At least 1 eye of each participant was free from advanced AMD (defined as NV AMD or geographic atrophy involving the center of the macula) and any eye disease that could complicate assessment of AMD or lens opacity progression, and that eye could not have had previous ocular surgery (except cataract surgery). Potential participants were excluded for illness or disorders that would make long-term follow-up or compliance with the study protocol unlikely or difficult. Persons aged 55 through 59 years were recruited only if they had intermediate AMD (AREDS Category 3) or unilateral advanced AMD (AREDS Category 4).[8,18] The present analysis of 4519 persons excludes all 110 persons in this age group, because there were no age-matched controls for these cases. This analysis also excludes the 128 persons with bilateral aphakia for whom presurgical refractive error was not available.

STUDY GROUP DEFINITIONS

Persons were recruited for AREDS in 4 AMD categories determined by the size and extent of drusen in each eye, the presence of manifestations of advanced AMD, and visual acuity.[8] Based on reading center grading of stereoscopic photographs taken at enrollment, participants in this study were divided into 5 groups. The groups analyzed in this report, numbered in order of increasing severity of drusen or type of AMD, were defined as follows.[9] Subjects in group 1 (n=1115) were free of drusen or had nonextensive small (<63 µm) drusen. Group 1 represented our referent controls. Subjects in group 2 (n=1060) had at least 1 eye with 1 or more intermediate (63 µm-124 µm) drusen, extensive (cumulative area 1/12 diameter of AREDS standard disc area) small drusen, or pigment abnormalities (hyperpigmentation or hypopigmentation) associated with AMD. Subjects in group 3 (n=1568) had at least 1 eye with 1 or more large (≥125 µm) drusen or with extensive intermediate (65-124 µm) drusen. Subjects in group 4 (n=118) had at least 1 eye with definite geographic atrophy anywhere within 3000 µm of the fovea. Subjects in group 5 (n=658) had evidence of choroidal neovascularization or retinal pigment epithelial detachment in 1 eye (nondrusenoid retinal pigment epithelial detachment, serous sensory or hemorrhagic retinal detachment, subretinal hemorrhage, subretinal pigment epithelial hemorrhage, subretinal fibrosis) or scars of photocoagulation for AMD. The term *neovascular* was chosen as a simplified description of subjects in AMD group 5.

RECRUITMENT

Participants were recruited through various sources: medical records of AREDS clinics; referring physicians; patient lists from hospitals and health maintenance organizations; screenings at malls, fairs, senior centers, and other gathering places; public advertisements (radio, television, newspapers, flyers); and friends and family of participants and of clinical center staff. The estimated percentage of participants by recruitment source for AMD groups 1 and 2 differed from groups 3, 4, and 5 mainly for medical records (17% vs 63%), public advertisements (53% vs 24%), and friends and family of participants (13% vs 7%).[18]

PROCEDURES

Before study initiation, the protocol was approved by a data and safety monitoring committee and by the institutional review board for each clinical center. Informed consent was obtained from all participants prior to enrollment. Detailed questionnaires were administered to obtain demographic information, history of smoking and sunlight exposure, medical history, history of specific prescription drug and nonprescription medication use, and history of vitamin and mineral use. General physical and ophthalmic examinations included measurement of height, weight, blood pressure, manifest refraction, best-corrected visual acuity, intraocular pressure, slitlamp biomicroscopy, and ophthalmoscopy. Stereoscopic fundus photographs of the macula were taken in each clinical center and graded at a photograph reading center.[19]

At enrollment, subjects completed a self-administered, 90-item, semiquantitative, food frequency questionnaire (AREDS FFQ) containing a food list with commonly consumed sources of zinc and vitamins A, C, and E. The food list also contained items rich in a variety of other nutrients that have putative associations with AMD, such as lipids, macular xanthophylls (lutein/zeaxanthin), pro–vitamin A carotenoids, and vitamins and minerals with antioxidant properties. Subjects were asked how often, on average, they had consumed each food or beverage item during the past year. Average frequency of consumption was recorded across 9 levels that ranged from "never or less than once per month" to "2 or more per day." Average serving size was recorded as "small," "medium," or "large," with respect to standard examples.

The AREDS FFQ was based on the validated 1987 National Cancer Institute Health Habits and History Questionnaire version 2.1, which was modified for use in AREDS with data obtained from 2-day food records sampled from 78 study-eligible persons selected from the 11 AREDS clinics. The instrument was validated using a telephone-administered 24-hour dietary recall at 3- and 6-month postenrollment in 197 randomly selected participants.[20] Correlations of 24-hour recall data with the AREDS FFQ were corrected for attenuation with the method of Rosner and Willett.[21] Values for correlation coefficients are 0.64 for total SFA, 0.54 for MUFA, 0.45 for cholesterol, 0.36 for linoleic acid (LA), 0.27 for arachidonic acid (AA), 0.28 for α-linolenic acid, 0.35 for eicosapentaenoic acid (EPA), and 0.32 for docosahexaenoic acid (DHA).

Dietary intake data were processed with DIETSys software (version 3.0; National Cancer Institute, Information Management Services, Inc, Bethesda, Md, and Block Dietary Data Systems, Berkeley, Calif) at the Nutrition Coordinating Center, School of Public Health, University of Minnesota. The DIETSys system produced daily nutrient intake estimates for each sub-

Table 1. Multivariable Single-Nutrient ORs for AMD by Highest vs Lowest Energy-Adjusted Intake Quintiles of Dietary Lipids*

Nutrient	Model	Quintile 5 vs Quintile 1, OR (95% CI)			
		Group 2 ESD or NEID (n = 1060)	Group 3 EID or LD (n = 1568)	Group 4 GA (n = 118)	Group 5 NV AMD (n = 658)
ω-3 Fatty acids					
α-Linolenic acid (18:3 ω-3)	Age and sex	1.11 (0.85-1.46)	1.11 (0.87-1.43)	1.54 (0.86-2.74)	1.15 (0.84-1.59)
	AREDS Report 3	1.11 (0.85-1.46)	1.07 (0.83-1.38)	1.88 (0.77-1.61)	1.05 (0.74-1.47)
Eicosapentaenoic acid (20:5 ω-3)	Age and sex	1.08 (0.82-1.41)	0.86 (0.67-1.11)	0.81 (0.45-1.48)	0.62 (0.45-0.85)†
	AREDS Report 3	1.08 (0.83-1.41)	0.95 (0.73-1.23)	0.88 (0.37-2.09)	0.72 (0.51-1.01)‡
Docosahexaenoic acid(22:6 ω-3)	Age and sex	0.92 (0.70-1.20)	0.86 (0.67-1.11)	0.81 (0.43-1.51)	0.53 (0.38-0.74)†
	AREDS Report 3	0.90 (0.69-1.19)	0.96 (0.74-1.25)	0.99 (0.54-1.80)	0.60 (0.42-0.85)†
Total ω-3 LCPUFAs	Age and sex	1.06 (0.81-1.38)	0.86 (0.66-1.11)	0.98 (0.53-1.81)	0.56 (0.41-0.77)†
	AREDS Report 3	1.05 (0.80-1.37)	0.84 (0.73-1.23)	1.27 (0.50-3.22)	0.63 (0.45-0.89)†
ω-6 Fatty acids					
Linoleic acid (18:2 ω-6)	Age and sex	0.97 (0.74-1.27)	0.94 (0.73-1.21)	1.33 (0.70-2.52)	1.14 (0.82-1.58)
	AREDS Report 3	0.98 (0.75-1.28)	0.92 (0.71-1.19)	1.55 (0.62-3.87)	1.09 (0.77-1.54)
Arachidonic acid (20:4 ω-6)	Age and sex	0.86 (0.65-1.13)	1.10 (0.86-1.42)	1.18 (0.65-2.13)	1.29 (0.93-1.79)
	AREDS Report 3	0.85 (0.64-1.12)	1.13 (0.87-1.46)	1.11 (0.49-2.53)	1.09 (0.77-1.54)
Monounsaturated fatty acids					
	Age and sex	0.78 (0.59-1.03)	1.19 (0.92-1.54)	1.82 (0.91-3.64)	2.06 (1.48-2.86)†
	AREDS Report 3	0.77 (0.58-1.02)	1.09 (0.84-1.42)	1.34 (0.48-3.79)	1.80 (1.27-2.56)†
Saturated fatty acids					
	Age and sex	1.01 (0.77-1.34)	1.43 (1.11-1.85)‡	1.27 (0.65-2.50)	1.89 (1.35-2.64)†
	AREDS Report 3	1.00 (0.76-1.32)	1.30 (1.00-1.70)	0.78 (0.29-2.07)	1.56 (1.09-2.23)†
Dietary cholesterol					
	Age and sex	0.97 (0.74-1.27)	1.38 (1.08-1.77)‡	1.53 (0.83-2.83)	1.47 (1.07-2.03)†
	AREDS Report 3	0.97 (0.74-1.27)	1.34 (1.04-1.72)‡	1.15 (0.48-2.75)	1.16 (0.82-1.63)

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; CI, confidence interval; EID, extensive intermediate drusen; ESD, extensive small drusen; GA, geographic atrophy; LCPUFA, long-chain polyunsaturated fatty acid; LD, large drusen; NEID, nonextensive intermediate drusen; NV, neovascular; OR, odds ratio.

*The ORs are comparing each category of AMD with controls for highest vs lowest calorie-adjusted quintile of nutrient intake. All models include terms for total energy intake (represented as a continuous variable), age (60-65, 66-70, or 71-80 years), and sex. Estimates in models labeled as "AREDS Report 3" are also controlled for factors identified in AREDS Report 3.[18] For group 2, risk factors include angina (present/absent), arthritis (present/absent), and current use of hydrochlorothiazide (yes/no). For group 3, these factors include education (≤12 y, some college, or college degree), refractive error (hyperopia, mixed, or myopia), race (white/nonwhite), smoking history (ever≥6 mo/<6 mo or never), existing hypertension (systolic pressure ≥160 mm Hg or diastolic pressure ≥90 mm Hg or current antihypertensive medication use/absent), arthritis, hydrochlorothiazide, current use of diuretics (yes/no), and lens opacity (present/absent). For group 4, these factors include education, smoking history, current use of antacids (yes/no), and current use of thyroid hormones (yes/no). For group 5, these factors include body mass index (calculated as weight in kilograms divided by height in meters squared) (≤23.6, 23.7-30.9, or ≥31.0), education, refractive error, race, smoking history, existing hypertension, and lens opacity.

†Trend tests on quintile median nutrient values yielded *P* values ≤.01.

‡Trend tests on quintile median nutrient values yielded *P* values ≤.05.

ject by first multiplying the average age- and sex-adjusted portion size (derived from National Health and Nutrition Examination Survey II data) by the subject's reported serving size. The Nutrition Coordinating Center Food Composition Database (version 31, November 2000) was used with the estimated quantity of intake to derive individual nutrient values for each questionnaire item. Cumulative estimates for each nutrient were computed by summation of nutrient values across all foods and items.

STATISTICAL MODELING AND ANALYSES

Each of our 4 AMD groups (groups 2-5) was compared with the referent control group (group 1). Details of statistical modeling and analysis of nonnutritional risk factors appear in AREDS Report 3.[18] Briefly, demographic factors, medical history, treatment history, and ocular factors associated with AREDS AMD categories were identified through polychotomous logistic regression analyses. Factors significant at $P<.15$ in any group were retained for multivariate modeling. This involved model simplification with the χ^2 test of change in deviance and goodness-of-fit diagnostics with the likelihood ratio criterion. Final models from AREDS Report 3 were the starting point for the multivariate analyses described in this report. Habitual dietary intake of DHA, EPA, total ω-3 LCPUFAs, AA, MUFA, SFA, and cholesterol across the year prior to enrollment are the primary independent variables in these analyses.

We applied a staged modeling technique in which energy-adjusted lipid values were first evaluated with age- and sex-adjusted logistic models and then with the final logistic models from AREDS Report 3. Single-nutrient and multinutrient models were constructed. We adjusted nutrient intake values for total energy intake by computing nutrient densities (nutrient intake/total energy intake). We classified nutrient density values into quintiles with the lowest-intake quintile as the referent exposure category and included a term for total energy intake in all models. When nutrient density scores are modeled as such, the nutrient density coefficient represents the relation of nutrient composition with AMD status, independent of energy intake. To explore relationships between single dietary nutrients and AMD, we used models with each nutrient and all AREDS AMD group–specific factors identified in AREDS Report 3 (each AMD group had a unique set; these are listed in the footnote to **Table 1**). Single nutrients associated with AMD at *P* values ≤.05 in AREDS Report 3 final models were included in multinutrient models. We added alcohol intake to final multinutrient models because al-

Table 2. Multivariable Multinutrient ORs for NV AMD by Energy-Adjusted Intake of Dietary Lipids*

Nutrient	Quintile	Median of %TEI	NV AMD/No AMD	OR (95% CI)	*P* Trend
ω-3 Fatty acids					
α-Linolenic acid	1	0.43	131/229	1 [Reference]	.82
	2	0.57	137/220	0.93 (0.67-1.32)	
	3	0.69	129/226	0.90 (0.64-1.27)	
	4	0.82	127/229	0.96 (0.68-1.36)	
	5	1.05	133/211	1.02 (0.72-1.44)	
Eicosapentaenoic acid	1	0.000	158/208	1 [Reference]	.05
	2	0.009	146/212	1.02 (0.74-1.43)	
	3	0.015	121/228	0.88 (0.62-1.24)	
	4	0.024	116/231	0.78 (0.55-1.10)	
	5	0.044	116/236	0.75 (0.52-1.08)	
Docosahexaenoic acid	1	0.010	163/198	1 [Reference]	.004
	2	0.018	135/213	0.85 (0.61-1.20)	
	3	0.026	120/240	0.65 (0.45-0.93)	
	4	0.037	131/224	0.75 (0.52-1.08)	
	5	0.061	108/240	0.54 (0.36-0.80)	
Total ω-3 LCPUFAs	1	0.013	163/206	1 [Reference]	.01
	2	0.028	137/207	0.91 (0.65-1.27)	
	3	0.042	125/239	0.72 (0.51-1.02)	
	4	0.061	121/226	0.77 (0.54-1.10)	
	5	0.110	111/237	0.61 (0.41-0.90)	
ω-6 Fatty acids					
Linoleic acid	1	4.54	124/221	1 [Reference]	.77
	2	6.12	132/223	0.96 (0.68-1.36)	
	3	7.32	138/235	0.97 (0.69-1.36)	
	4	8.59	127/209	1.08 (0.76-1.53)	
	5	10.71	136/227	1.01 (0.71-1.43)	
Arachidonic acid	1	0.021	117/215	1 [Reference]	.03
	2	0.032	127/230	0.99 (0.70-1.41)	
	3	0.041	134/236	1.20 (0.84-1.73)	
	4	0.051	127/222	1.23 (0.85-1.79)	
	5	0.070	152/212	1.54 (1.04-2.29)	
Monounsaturated fatty acids					.12
	1	8.20	103/245	1 [Reference]	
	2	11.03	115/213	1.13 (0.79-1.62)	
	3	12.87	134/216	1.13 (0.78-1.62)	
	4	14.62	133/222	1.06 (0.73-1.53)	
	5	17.23	172/219	1.38 (0.94-2.02)	
Saturated fatty acids					.14
	1	7.13	108/249	1 [Reference]	
	2	9.41	112/222	1.06 (0.75-1.52)	
	3	11.19	126/211	1.14 (0.80-1.63)	
	4	13.07	160/232	1.39 (0.98-1.97)	
	5	16.04	151/201	1.36 (0.94-1.97)	
Dietary cholesterol					.66
	1	53.74	117/250	1 [Reference]	
	2	74.00	118/220	0.98 (0.69-1.38)	
	3	92.10	116/234	0.88 (0.62-1.24)	
	4	113.40	153/200	1.28 (0.91-1.80)	
	5	157.52	152/211	1.14 (0.81-1.60)	

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; CI, confidence interval; LCPUFA, long-chain polyunsaturated fatty acid; NV, neovascular; OR, odds ratio; TEI, total energy intake.

*The ORs are comparing each category of NV AMD with controls for calorie-adjusted quintiles of nutrient intake. All models include terms for TEI (represented as a continuous variable), age (60-65, 66-70, or 71-80 years), and sex. Models also controlled for factors identified in AREDS Report 3[18] and noncolinear nutrients independently associated with NV AMD. These included body mass index (calculated as weight in kilograms divided by height in meters squared) (≤23.6, 23.7-30.9, or ≥31.0), education (≤12 y, some college, or college degree), refractive error (hyperopia, mixed, or myopia), race (white/nonwhite), smoking history (ever≥6 mo/<6 mo or never), hypertension (present/absent), lens opacity (present/absent), and quintiles of total ω-3 LCPUFA (excluding eicosapentaenoic acid and docosahexaenoic acid models), arachidonic acid, lutein/zeaxanthin, and alcohol intake.

cohol may alter lipid metabolism, transport, and bioavailability.[22,23] We also added lutein/zeaxanthin intake on conceptual,[24-26] historical,[27-29] and statistical grounds, as these dietary xanthophylls compose macular pigment, are associated with decreased likelihood of AMD among people reporting lowest dietary intakes, and persist herein in multivariable models. In cases where we observed differences in odds between highest- and lowest-nutrient quintiles, we conducted trend tests with quintile medians modeled as a continuous variable. To examine the relationship of ω-3 LCPUFA–rich food with AMD, total and types of fish intake were added to these same models in place of ω-3 LCPUFA nutrient values.

Table 3. Multivariable Multinutrient ORs for NV AMD by Frequency of Consumption for Foods Rich in ω-3 LCPUFAs*

Fish Intake (Medium Serving†)	AMD Group, No. (%)		OR (95% CI)	*P* Trend
	NV AMD (n = 657)	No AMD (n = 1115)		
Total fish				.01
<1 medium serving/mo	47 (12)	51 (5)	1 [Reference]	
1-3 medium serving/mo	163 (25)	218 (20)	0.83 (0.50-1.40)	
1 medium serving/wk	13 (2)	22 (2)	0.76 (0.32-1.84)	
>1-2 medium servings/wk	202 (31)	343 (31)	0.75 (0.46-1.23)	
>2 medium servings/wk	232 (35)	481 (43)	0.61 (0.37-1.00)	
Tuna or tuna casserole				.06
<1 medium serving/mo	215 (33)	293 (27)	1 [Reference]	
1-3 medium serving/mo	293 (45)	511 (46)	0.83 (0.64-1.08)	
1 medium serving/wk	69 (11)	134 (12)	0.83 (0.57-1.22)	
>1 medium serving/wk	80 (12)	177 (16)	0.72 (0.50-1.04)	
Baked or broiled fish				.02
<1 medium serving/mo	203 (31)	260 (23)	1 [Reference]	
1-3 medium serving/mo	277 (42)	459 (41)	0.85 (0.65-1.11)	
1 medium serving/wk	84 (13)	172 (15)	0.78 (0.54-1.13)	
>1 medium serving/wk	93 (14)	224 (20)	0.65 (0.45-0.93)	
Fried fish				.56
<1 medium serving/mo	338 (51)	598 (54)	1 [Reference]	
1-3 medium serving/mo	205 (31)	369 (33)	0.94 (0.74-1.21)	
1 medium serving/wk	64 (10)	79 (7)	1.54 (1.02-2.33)	
>1 medium serving/wk	50 (8)	69 (7)	1.19 (0.75-1.89)	
Oysters				.18
<1 medium serving/mo	612 (93)	1026 (92)	1 [Reference]	
1-3 medium serving/mo	40 (6)	81 (7)	0.76 (0.48-1.20)	
1 medium serving/wk	3 (0)	2 (0)	2.38 (0.36-15.89)	
>1 medium serving/wk	2 (0)	6 (0)	0.30 (0.05-1.69)	
Other shellfish				.08
<1 medium serving/mo	395 (60)	596 (54)	1 [Reference]	
1-3 medium serving/mo	229 (35)	429 (38)	0.85 (0.67-1.07)	
1 medium serving/wk	10 (2)	25 (2)	0.58 (0.26-1.31)	
>1 medium serving/wk	23 (3)	65 (6)	0.70 (0.40-1.22)	

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; LCPUFA, long-chain polyunsaturated fatty acid; NV, neovascular; OR, odds ratio.

*The ORs are comparing each category of NV AMD with controls for levels of fin fish and shellfish intake. Point estimates and CIs are adjusted for factors discussed in the footnote to Table 2; in Table 3, model levels of marine product consumption were entered in place of ω-3 LCPUFA nutrient variables (eicosapentaenoic acid, docosahexaenoic acid, and total ω-3 LCPUFAs).

†A medium serving for all fin fish and total fish is approximately 4 oz (about 115 g). A medium serving size for shellfish is approximately 3 oz (about 85 g).

RESULTS

Table 1 gives odds ratios (ORs) for sex-, age-, and calorie-adjusted and multivariable analyses of single nutrients with each AREDS AMD group. Values for AMD outcomes represent comparisons of the persons within the highest-intake quintile vs persons within the lowest-intake quintile. For multivariable models, protective relationships with NV AMD were observed for DHA (OR, 0.60; 95% confidence interval [CI], 0.42-0.85) and total ω-3 LCPUFAs (OR, 0.63; 95% CI, 0.45-0.89). Odds of NV AMD were increased in the highest-intake group for MUFA (OR, 1.80; 95% CI, 1.27-2.56) and SFAs (OR, 1.56; 95% CI, 1.09-2.23). The relationship of supplement years with AMD was negligible (data not shown) for all vitamins and minerals and was therefore not considered in subsequent models. Because the only multivariable lipid-AMD relationship observed outside of the NV AMD group was an increased likelihood of group 3 with increased cholesterol intake (OR, 1.34; 95% CI, 1.04-1.72), the remaining sections of this report will present multinutrient models for the NV AMD group only.

Table 2 gives ORs for NV AMD by quintiles of lipid intake; models contain terms for all nutritional factors that were statistically associated both with NV AMD and factors from AREDS Report 3. Nutritional factors included total energy intake, DHA, AA, lutein/zeaxanthin, and alcohol. Factors from AREDS Report 3 included age, race, sex, education, body mass index, refractive error, smoking history, hypertension, and lens opacity. Docosahexaenoic acid (OR, 0.54; 95% CI, 0.36-0.80; *P* trend = .004) and total ω-3 LCPUFA (OR, 0.61; 95% CI, 0.41-0.90; *P* trend = .01) intake persisted as independent factors in these models. Magnitude and precision of the ORs for DHA and total ω-3 LCPUFAs were not altered appreciably compared with multivariable single-nutrient models. Odds of NV AMD were significantly increased among participants classified in the highest quintile of AA intake (OR, 1.54; 95% CI, 1.04-2.29; *P* trend = .03). In these multinutrient models, neither ORs nor trends for NV AMD attained statistical significance for intake of MUFA, total SFAs, or cholesterol. As such, final models did not include terms for these factors (*P* > .10 for all comparisons).

Table 3 gives ORs for multivariable relationships of NV AMD with reported intake of ω-3 LCPUFA–rich foods.

Table 4. Multivariable Multinutrient ORs of Eicosapentaenoic and Docosahexaenoic Acids for NV AMD by Quintiles of Arachidonic Acid Intake*

Nutrient	Quintile	Median of %TEI	NV AMD/No AMD	OR (95% CI)	*P* Trend
Arachidonic acid quintiles 1 and 2					
Eicosapentaenoic acid	1	0.002	84/122	1 [Reference]	.06
	2	0.010	76/104	1.17 (0.74-1.86)	
	3	0.017	38/84	0.87 (0.51-1.49)	
	4	0.027	32/90	0.70 (0.40-1.22)	
	5	0.054	14/45	0.58 (0.28-1.23)	
Docosahexaenoic acid	1	0.010	112/151	1 [Reference]	.02
	2	0.020	67/108	1.09 (0.70-1.70)	
	3	0.029	33/87	0.66 (0.39-1.11)	
	4	0.040	24/65	0.66 (0.36-1.22)	
	5	0.073	8/34	0.46 (0.30-1.14)	
Arachidonic acid quintiles 4 and 5					
Eicosapentaenoic acid	1	0.000	42/51	1 [Reference]	.50
	2	0.011	49/75	1.05 (0.56-1.96)	
	3	0.018	56/83	1.11 (0.60-2.03)	
	4	0.027	57/90	0.93 (0.51-1.70)	
	5	0.049	75/135	0.89 (0.50-1.61)	
Docosahexaenoic acid	1	0.013	19/22	1 [Reference]	.20
	2	0.021	40/60	1.00 (0.44-2.30)	
	3	0.030	65/92	1.12 (0.51-2.45)	
	4	0.042	77/110	1.07 (0.50-2.28)	
	5	0.078	78/150	0.78 (0.36-1.66)	

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; NV, neovascular; OR, odds ratio.
*The ORs are comparing each category of NV AMD with controls for calorie-adjusted quintiles of nutrient intake by levels of arachidonic acid intake. Point estimates and CIs are adjusted for factors for group 5 discussed in the footnote to Table 2.

Based on results from the FFQ, intake of fish and shellfish contributed 93% of EPA and 71% of DHA intake. Odds ratios are relative to participants who reported consumption of a medium-size portion of the particular type of fish or shellfish less than 1 time per month in the year prior to AREDS enrollment. After controlling for caloric intake and covariates discussed in the preceding section, total fish intake of more than 2 medium servings per week was associated with lower odds of NV AMD (OR, 0.61; 95% CI, 0.37-1.00). Participants who consumed 4 oz of broiled/baked fish more than 1 time per week were also less likely to have a diagnosis of NV AMD (OR, 0.65; 95% CI, 0.45-0.93). Results were similar for tuna intake, although they did not attain statistical significance. The magnitude of ORs for these relationships was similar to that observed for ω-3 LCPUFAs.

Table 4 contains results of our final models for EPA and DHA run within upper (quintiles 4 and 5) and lower strata (quintiles 1 and 2) of AA. Arachidonic acid (C20:4ω-6) is a major LCPUFA of the ω-6 family. Major sources of AA in the diets of AREDS participants were beef and pork (32%), turkey and chicken (25%), eggs (20%), and fish (11%). Among participants reporting lower levels of AA intake, there was a significant trend for increased risk of NV AMD with decreasing DHA.

COMMENT

This report extends the findings of AREDS Report 3 to include lipid- and ω-3 LCPUFA–rich food-based factors. Higher levels of total ω-3 LCPUFA and ω-3 LCPUFA–rich food intake were associated with a lower likelihood of NV AMD after statistical control for nutrient- and nonnutrient-based predictors and correlates. Participants with reported ω-3 LCPUFA intake in the highest quintile were 40% less likely to be in the NV AMD group than participants with reported intake in the lowest quintile. Of ω-3 LCPUFAs, the strongest effects were observed for DHA intake. Trends of decreasing odds with increasing nutrient intake existed for EPA, DHA, and total ω-3 LCPUFAs. Relative to participants consuming less than 1 medium serving of broiled or baked fish per month, the magnitude and precision of the OR for ω-3 LCPUFA–rich food intake of 1 or more 4-oz servings of this food per week were similar to those for total ω-3 LCPUFA. Participants with reported intake in the highest quintile for AA, an ω-6 LCPUFA, were 1.5 times more likely to be in the NV AMD group than participants with intake in the lowest quintile. We found no associations of other lipid intake with NV AMD when essential fatty acids (α-linolenic acid and LA) and MUFA, SFA, and cholesterol intake were modeled as primary independent variables and adjusted for DHA, AA, alcohol, lutein/zeaxanthin, and the set of AMD group-specific covariates from AREDS Report 3.

INTERPRETATION OF FINDINGS IN THE CONTEXT OF THE EVIDENCE BASE

Our study results are concordant with those in the extant literature for sight-threatening forms of AMD; ORs for comparisons of high vs low ω-3 LCPUFA and fish intake have been in the direction of benefit. The magnitude of effect for ω-3 LCPUFA–NV AMD ORs that we observed is also in the range of those reported previously. The Dietary An-

cillary Study of the Eye Disease Case Control Study researchers describe a trend for decreasing odds of advanced (exudative) AMD among subjects reporting highest ω-3 LCPUFA and fish intake.[13] We did not observe a relationship of DHA intake with geographic atrophy. In the Progression of Age-Related Macular Degeneration Study, the only published prospective study to our knowledge examining the relationship of lipid intake with progression to advanced AMD (defined as NV AMD or geographic atrophy), people reporting fish intake at frequencies of 2 or more times per week had a lower likelihood of progression to advanced AMD ($P<.05$) if they also reported lower levels of LA intake.[15] The likelihood of having any AMD (with visual loss of 20/30 or worse in at least 1 eye) was reduced among people reporting highest DHA, tuna, and total fish intake within a prospective sample from the Nurses' Health Study and the Health Professionals Follow-up Study.[14] When modeled simultaneously with intake of other dietary lipids, ω-3 LCPUFA–AMD relationships did not remain statistically significant, although they were in the direction of benefit (risk ratios for highest vs lowest of DHA and EPA intake were 0.8; 95% CI 0.5-1.1).[14]

Three population-based studies examined the relationship of fish intake with "late" (NV or atrophic) AMD as classified by the Wisconsin Age-Related Maculopathy Grading System.[30,31] Each of these studies had fewer than 55 cases of late AMD; however, although not statistically significant, ORs were in the direction of benefit. Our results were also within the range of those from other studies for MUFA[5,6,13-15] and SFA[5,6,13-15,17] intake.

INTAKE-STATUS-FUNCTION RELATIONSHIPS

Docosahexaenoic acid, EPA, and AA are major dietary LCPUFAs; DHA and AA are major structural LCPUFAs of retinal photoreceptor outer segments and vascular tissue.[32-34] The amount of DHA in retinal tissue[35,36] and plasma[37] is modifiable by and dependent on dietary intake. Long-chain polyunsaturated fatty acids may be obtained through diet or biosynthesized from essential fatty acids. α-Linoleic acid (18:3ω-3) is the essential fatty acid precursor to the ω-3 LCPUFAs and LA (18:2ω-6) is the precursor to ω-6 LCPUFAs. Intake levels of neither precursor attained statistical significance when modeled as the single nutrient in the final calorie-adjusted multivariable model for NV AMD.

While our models simultaneously controlling for ω-3 and ω-6 LCPUFAs yielded significant results, it is important to consider the potential for effect modification within levels of these nutritional variables, as they act as substrates in the same biochemical pathways for production of potent lipid mediators. Two studies suggest an effect modification of fish/ω-3 LCPUFA intake with LA intake in advanced AMD; both reported that the ORs for these factors were in the direction of benefit among subjects reporting lowest levels of LA intake.[13,15] In our analyses, a direction of benefit was seen for DHA with NV AMD among AREDS subjects in the highest levels (fourth and fifth quintiles) of LA (OR, 0.31; 95% CI, 0.11-0.97). However, evaluation of effect modification by another ω-6 fatty acid, AA, did yield a trend for a benefit among subjects in the lower level of AA intake (Table 4).

PUTATIVE MECHANISMS

Long-chain polyunsaturated fatty acids are effected by and influence processes implicated in the pathogenesis of vascular and neural retinal diseases.[10] Docosahexaenoic acid and EPA may serve as protective agents in the retina because of their influence on gene expression,[38-41] retinal cell differentiation,[42-44] and survival.[42-46] Free LCPUFAs, liberated when phospholipase A2 hydrolyzes DHA and AA from their primary storage forms in retinal tissue, are the precursors and substrates to families of bioactive molecules (eicosanoids,[47-51] endocannabinoids,[52,53] and LCPUFA-based autocoids[11,54]) that act as potent regulators of retinal vascular function, cell survival, inflammation, and energy balance. Long-chain polyunsaturated fatty acids also affect factors and processes driving key events in the angiogenic cascade.[10,13] Docosahexaenoic acid may also be involved in rhodopsin regeneration[55] and work in signaling cascades to enhance activation of membrane-bound retinal proteins.[12,56-58]

CAVEATS

There are inherent limitations in the nature of this case-control sampling design. However, we believe a number of factors increase the strength of our inferences. The AREDS sample contains a large number of participants with NV AMD and dietary intake data. All data were collected with a standardized protocol by centrally trained staff. Trained graders at a reading center ascertained the AMD phenotype based on fundus photographs using a standardized grading system.

The potential for erroneous selection of nonnutrient-based variables is discussed in AREDS Report 3.[18] Because all nonnutritional factors associated with NV AMD have been reported in previously published studies, we applied the simplifying assumption that we were unlikely to model factors identified through spurious relationships. However, the possibility of a consistent bias across studies cannot be excluded.

The sampling scheme for our clinic-based case-control design may have increased the probability of exposure misclassification among subjects. The Eye Disease Case Control Study[13] researchers restricted their analysis exclusively to newly diagnosed AMD cases and found results similar to our own. Exposure misclassification may have occurred if (1) the accuracy of dietary recall varied between participants with eye disease and those in the comparison group (groups were defined on the basis of eye disease and this may have influenced classification of exposure) or (2) some participants with severe eye disease altered their diets in the years immediately prior to enrollment to conform to "healthy diet" recommendations. We would expect these conditions to modify our results toward no association.

The possible effect of selection bias also needs to be considered. Participants in AREDS classified with no AMD or lower severity of AMD (groups 1 and 2) were more likely than members of groups 3, 4, and 5 to volunteer in response to public advertisements. These people were also more likely to be enrolled in the study through familial association with participants in groups 3 and 4.

As such, there were baseline group differences in the distributions of some demographic, behavioral, and medical factors previously associated with AMD. Our multivariable models included many of these potential confounders, but if this sampling scheme yielded imbalances in unknown and therefore unmeasured (behavioral and lifestyle) factors associated with diet and AMD, our findings could have been biased.

Nutrient intake values may partially reflect a more general lifestyle or socioeconomic-demographic construct associated with NV AMD. As such, we performed a number of analyses to evaluate the potential for effect modification. The strongest and most consistent factors associated with sight-threatening AMD (age and smoking) were not associated with ω-3 LCPUFA intake. Participants with a college education were almost twice as likely to be in a higher quintile of DHA intake than people with 12 or fewer years of education after adjusting for AREDS Report 3 factors (OR, 1.8; 95% CI, 1.6-2.1). While sample-restricted analyses compromised power of the statistical test, we observed no changes in the direction of previously observed lipid-AMD relationships when data were analyzed within each stratum. Observations were similar for other factors (eg, strata of race and body mass index).

Adding LCPUFAs, lutein/zeaxanthin, and alcohol to the model for NV AMD had a negligible effect on the magnitude and precision of ORs for factors from AREDS Report 3 that were associated with NV AMD (age, education, refractive error, race, smoking, and hypertension). Body mass index and lens opacity did not persist as statistically significant risk factors, although point estimates were changed only minimally. To evaluate the potential confounding impact of nutrient-nutrient relationships, we obtained partial correlation coefficients from linear regressions of log-transformed nutrient scores for all nutrients on each nutrient. The partial correlation coefficient allowed us to quantify the magnitude of relationships and also to determine the potential for colinearity. After controlling for all measured nutrients that varied independently with ω-3 LCPUFA intake, only EPA and DHA contributed more than 5% to variance in ω-3 LCPUFAs; these results indicate that the estimate of ω-3 LCPUFA intake is likely to reflect actual ω-3 LCPUFA intake. We believe that it is unlikely that EPA and DHA are markers for other nutrients.

CONCLUSIONS

This AREDS report provides evidence that people reporting highest levels of ω-3 LCPUFA intake have a decreased likelihood of having NV AMD relative to people reporting lowest levels of intake. Because increased intake of AA is also associated with an increased likelihood of having NV AMD, it is important to consider the balance and composition of dietary LCPUFAs from the ω-3 and ω-6 families. These results and those from other observational analytic investigations[13] suggest that modifying diet to include more foods rich in ω-3 LCPUFAs could result in a reduction in the risk of having NV AMD. In addition to carefully designed observational analytic designs, clinical trials would provide unique information on whether dietary intervention or supplementation with ω-3 LCPUFAs may help prevent the development of advanced AMD.

Submitted for Publication: May 12, 2006; final revision received September 14, 2006; accepted September 14, 2006.
Correspondence: AREDS Coordinating Center, The EMMES Corporation, 401 N Washington St, Suite 700, Rockville, MD 20850-1707 (aredspub@emmes.com).
Financial Disclosure: None reported.
Funding/Support: This report was supported by contracts from the National Eye Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Md.

REFERENCES

1. National Advisory Eye Council. *Vision Research—A National Plan, 1999-2003.* Washington, DC: US Dept of Health and Human Services; 1999. NIH Publication 99-4120.
2. *Prevent Blindness AmericaVision Problems in the US: Prevalence of Adult Vision Impairment and Age-Related Eye Disease in America.* Chicago, Ill: Prevent Blindness America; 2002.
3. Friedman DS, O'Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol.* 2004;122:564-572.
4. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology.* 1992;99:933-943.
5. Smith W, Mitchell P, Leeder SR. Dietary fat and fish intake and age-related maculopathy. *Arch Ophthalmol.* 2000;118:401-404.
6. Seddon JM. Epidemiology of age-related macular degeneration. In: Ryan SJ, ed. *Medical Retina.* Vol 2. 3rd ed. St Louis, Mo: CV Mosby; 2001:1039-1050.
7. Snow KK, Seddon JM. Do age-related macular degeneration and cardiovascular disease share common antecedents? *Ophthalmic Epidemiol.* 1999;6:125-143.
8. The Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study (AREDS): design implications. AREDS report No. 1. *Control Clin Trials.* 1999;20:573-600.
9. The Age-Related Eye Disease Study Research 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;119:1417-1436.
10. 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.
11. 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 U S A.* 2004;101:8491-8496.
12. Salem N Jr, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids.* 2001;36:945-959.
13. Seddon JM, Rosner B, Sperduto RD, et al. Dietary fat and risk for advanced age-related macular degeneration. *Arch Ophthalmol.* 2001;119:1191-1199.
14. 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;73:209-218.
15. 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;121:1728-1737.
16. Heuberger RA, Mares-Perlman JA, Klein R, Klein BE, Millen AE, Palta M. Relationship of dietary fat to age-related maculopathy in the Third National Health and Nutrition Examination Survey. *Arch Ophthalmol.* 2001;119:1833-1838.
17. Mares-Perlman JA, Brady WE, Klein R, VandenLangenberg GM, Klein BE, Palta M. Dietary fat and age-related maculopathy. *Arch Ophthalmol.* 1995;113:743-748.
18. The Age-Related Eye Disease Study Research 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;107:2224-2232.
19. The Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study report number 6. *Am J Ophthalmol.* 2001;132:668-681.
20. Kurinij N, Gensler G, Milton R; AREDS Research Group. Development and validation of a food frequency questionnaire in a randomized trial of eye disease.

Paper presented at: The International Conference on Dietary Assessment Methods; May 7, 1998; Phoenix, Ariz.
21. 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;127:377-386.
22. Lands WEM, Pawlosky RJ, Salem N. Alcoholism, antioxidant status, and essential fatty acids. In: Papas AM, ed. *Antioxidant Status, Diet, Nutrition, and Health*. Boca Raton, Fla: CRC Press; 1999:299-344.
23. Pawlosky RJ, Salem N Jr. Ethanol exposure causes a decrease in docosahexaenoic acid and an increase in docosapentaenoic acid in feline brains and retinas. *Am J Clin Nutr*. 1995;61:1284-1289.
24. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu Rev Nutr*. 2003;23:171-201.
25. Neuringer M, Sandstrom MM, Johnson EJ, Snodderly DM. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2004;45:3234-3243.
26. Leung IY, Sandstrom MM, Zucker CL, Neuringer M, Max Snodderly D. Nutritional manipulation of primate retinas, IV: effects of n–3 fatty acids, lutein, and zeaxanthin on S-cones and rods in the foveal region. *Exp Eye Res*. 2005;81:513-529.
27. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA*. 1994;272:1413-1420.
28. Mares-Perlman JA, Fisher AI, Klein R, et al. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol*. 2001;153:424-432.
29. Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand*. 2002;80:368-371.
30. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128-1134.
31. Klein R, Meuer SM, Moss SE, Klein BE. Detection of drusen and early signs of age-related maculopathy using a nonmydriatic camera and a standard fundus camera. *Ophthalmology*. 1992;99:1686-1692.
32. Neuringer M. The relationship of fatty acid composition to function in the retina and visual system. In: Dobbing J, ed. *Lipids, Learning, and the Brain: Fats in Infant Formulas, Report of the 103rd Ross Conference on Pediatric Research*. Columbus, Ohio: Ross Laboratories; 1993:134-163.
33. Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res*. 1983;22:79-131.
34. Lecomte M, Paget C, Ruggiero D, Wiernsperger N, Lagarde M. Docosahexaenoic acid is a major n-3 polyunsaturated fatty acid in bovine retinal microvessels. *J Neurochem*. 1996;66:2160-2167.
35. Lin DS, Anderson GJ, Connor WE, Neuringer M. Effect of dietary N-3 fatty acids upon the phospholipid molecular species of the monkey retina. *Invest Ophthalmol Vis Sci*. 1994;35:794-803.
36. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc Natl Acad Sci U S A*. 1986;83:4021-4025.
37. Sanders TA, Hinds A, Pereira CC. Influence of n-3 fatty acids on blood lipids in normal subjects. *J Intern Med Suppl*. 1989;731:99-104.
38. de Urquiza AM, Liu S, Sjoberg M, et al. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science*. 2000;290:2140-2144.
39. Lin Q, Ruuska SE, Shaw NS, Dong D, Noy N. Ligand selectivity of the peroxisome proliferator-activated receptor alpha. *Biochemistry*. 1999;38:185-190.
40. Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, Wahli W. Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol Cell*. 1993;77:67-76.
41. Yu K, Bayona W, Kallen CB, et al. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J Biol Chem*. 1995;270:23975-23983.
42. Politi LE, Rotstein NP, Carri NG. Effect of GDNF on neuroblast proliferation and photoreceptor survival: additive protection with docosahexaenoic acid. *Invest Ophthalmol Vis Sci*. 2001;42:3008-3015.
43. Rotstein NP, Aveldano MI, Barrantes FJ, Roccamo AM, Politi LE. Apoptosis of retinal photoreceptors during development in vitro: protective effect of docosahexaenoic acid. *J Neurochem*. 1997;69:504-513.
44. Rotstein NP, Politi LE, Aveldano MI. Docosahexaenoic acid promotes differentiation of developing photoreceptors in culture. *Invest Ophthalmol Vis Sci*. 1998;39:2750-2758.
45. Rotstein NP, Aveldano MI, Barrantes FJ, Politi LE. Docosahexaenoic acid is required for the survival of rat retinal photoreceptors in vitro. *J Neurochem*. 1996;66:1851-1859.
46. Kim HY, Akbar M, Kim KY. Inhibition of neuronal apoptosis by polyunsaturated fatty acids. *J Mol Neurosci*. 2001;16:223-227, discussion 279-284.
47. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids*. 2001;36:1007-1024.
48. Rose DP, Connolly JM, Rayburn J, Coleman M. Influence of diets containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in nude mice. *J Natl Cancer Inst*. 1995;87:587-592.
49. Rose DP, Connolly JM. Antiangiogenicity of docosahexaenoic acid and its role in the suppression of breast cancer cell growth in nude mice. *Int J Oncol*. 1999;15:1011-1015.
50. Badawi AF, El-Sohemy A, Stephen LL, Ghoshal AK, Archer MC. The effect of dietary n-3 and n-6 polyunsaturated fatty acids on the expression of cyclooxygenase 1 and 2 and levels of p21ras in rat mammary glands. *Carcinogenesis*. 1998;19:905-910.
51. Hamid R, Singh J, Reddy BS, Cohen LA. Inhibition by dietary menhaden oil of cyclooxygenase-1 and -2 in N-nitrosomethylurea-induced rat mammary tumors. *Int J Oncol*. 1999;14:523-528.
52. Yazulla S, Studholme KM, McIntosh HH, et al. Immunocytochemical localization of cannabinoid CB1 receptor and fatty acid amide hydrolase in rat retina. *J Comp Neurol*. 1999;415:80-90.
53. Bisogno T, Delton-Vandenbroucke I, Milone A, Lagarde M, Di Marzo V. Biosynthesis and inactivation of N-arachidonoylethanolamine (anandamide) and N-docosahexaenoylethanolamine in bovine retina. *Arch Biochem Biophys*. 1999;370:300-307.
54. Serhan CN, Arita M, Hong S, Gotlinger K. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids*. 2004;39:1125-1132.
55. Chen Y, Houghton LA, Brenna JT, Noy N. Docosahexaenoic acid modulates the interactions of the interphotoreceptor retinoid-binding protein with 11-cis-retinal. *J Biol Chem*. 1996;271:20507-20515.
56. Litman BJ, Mitchell DC. A role for phospholipid polyunsaturation in modulating membrane protein function. *Lipids*. 1996;31(suppl):S193-S197.
57. Litman BJ, Niu SL, Polozova A, Mitchell DC. The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: visual transduction. *J Mol Neurosci*. 2001;16:237-242, discussion 279-284.
58. Niu SL, Mitchell DC, Lim SY, et al. Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid deficiency. *J Biol Chem*. 2004;279:31098-31104.