Original Investigation

Dietary ω-3 Polyunsaturated Fatty Acid Intake and Risk for Amyotrophic Lateral Sclerosis

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IMPORTANCE Amyotrophic lateral sclerosis (ALS) is a severe progressive disease that cannot be prevented or cured. Diet-derived long-chain polyunsaturated fatty acids (PUFAs) are incorporated in brain lipids and modulate oxidative and inflammatory processes and could thus affect ALS risk and progression.

OBJECTIVE To examine the association between ω-6 and ω-3 PUFA consumption and ALS risk.

DESIGN, SETTING, AND PARTICIPANTS Longitudinal analyses based on 1 002 082 participants (479 114 women and 522 968 men) in 5 prospective cohorts: the National Institutes of Health–AARP Diet and Health Study, the Cancer Prevention Study II Nutrition Cohort, the Health Professionals Follow-up Study, the Multiethnic Cohort Study, and the Nurses' Health Study. Diet was assessed via food frequency questionnaire developed or modified for each cohort. Participants were categorized into cohort-specific quintiles of intake of energy-adjusted dietary variables.

MAIN OUTCOMES AND MEASURES Cohort-specific multivariable-adjusted risk ratios (RRs) of ALS incidence or death estimated by Cox proportional hazards regression and pooled using random-effects methods.

RESULTS A total of 995 ALS cases were documented during the follow-up. A greater ω-3 PUFA intake was associated with a reduced risk for ALS. The pooled, multivariable-adjusted RR for the highest to the lowest quintile was 0.66 (95% CI, 0.53-0.81; $P < .001$ for trend). Consumption of both α-linolenic acid (RR, 0.73; 95% CI, 0.59-0.89; $P = .003$ for trend) and marine ω-3 PUFAs (RR, 0.84; 95% CI, 0.65-1.08; $P = .03$ for trend) contributed to this inverse association. Intakes of ω-6 PUFA were not associated with ALS risk.

CONCLUSIONS AND RELEVANCE Consumption of foods high in ω-3 PUFAs may help prevent or delay the onset of ALS.

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Editorial page 1085

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder with few effective treatments and a disease pathogenesis that is poorly understood.[1-3] Diet-derived polyunsaturated fatty acids (PUFAs) in brain neural plasma membranes can modulate oxidative stress, excitotoxicity, and inflammation,[4-6] mechanisms that have been implicated in the etiology of ALS and other neurodegenerative conditions.[1-3] In particular, ω-3 PUFAs have been found to have neuroprotective effects in animal models of aging[6] and brain ischemia.[7] Unexpectedly, however, pretreatment with high doses of eicosapentaenoic acid, a long-chain ω-3 PUFA, accelerated disease progression in a mouse model of ALS.[8] However, it is unclear to what extent this experimental result applies to human disease.

Data on the relation between PUFA intake and ALS risk are sparse. The results of 2 previous case-control studies[9,10] suggested lower ALS risk among individuals with high PUFA intake; however, to our knowledge, there are no prospective studies relating overall PUFA intake or ω-3 PUFA intake to ALS risk. Therefore, we conducted a pooled analysis of nearly 1000 cases of ALS occurring in 5 large prospective cohort studies, including the Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS), the Cancer Prevention Study II Nutrition Cohort (CPS-II Nutrition), the Multiethnic Cohort Study (MEC), and the National Institutes

of Health-AARP Diet and Health Study (NIH-AARP), to assess whether specific dietary PUFAs or total dietary fat intake affect ALS risk.

Methods

Study Populations

The HPFS began in 1986 when 51 529 male health professionals aged 40 to 65 years answered a mailed questionnaire pertaining to disease history and lifestyle characteristics.[11] The NHS includes 121 700 registered female nurses and began in 1976 when these women aged 35 to 55 years at baseline responded to a similar questionnaire.[12] Follow-up of both studies continues through biennial questionnaires where participants in each cohort report disease occurrence and information on risk factors for chronic disease including dietary variables. The CPS-II Nutrition cohort consists of a subpopulation of the full CPS-II cohort and includes 86 404 men and 97 786 women aged 50 to 79 years residing in 21 states with population-based cancer surveillance.[13,14] These men and women completed a mailed questionnaire in 1992 assessing various lifestyle and dietary factors. Updated exposure information was obtained in 1997 and biennially thereafter. The MEC cohort study consists of 96 937 men and 118 843 women aged 45 to 75 years with self-reported racial and ethnic backgrounds of African American, Japanese American, Latino, Native Hawaiian, and white.[15] At the study baseline in 1993-1996, participants who were living primarily in Hawaii and California (Los Angeles) completed a lifestyle and disease history questionnaire; additional mailings were sent every 5 years subsequently. The NIH-AARP Diet and Health Study consists of 340 148 men and 227 021 women aged 50 to 71 years residing in 6 states or 2 metropolitan areas that maintain high-quality cancer registries and began in 1995-1996 when participants completed a mailed food frequency questionnaire.[16] Of the original study population, approximately two-thirds completed a follow-up lifestyle questionnaire in 1996. All included studies were approved by the institutional review board at the institution where each study was conducted; the research presented here was approved by the institutional review boards at the Harvard School of Public Health and Brigham and Women's Hospital.

End Point Definition

Follow-up of ALS in the CPS-II Nutrition, MEC, and NIH-AARP studies was done through a search of the National Death Index. The vital status of the participants in these studies was determined by automated linkage with the National Death Index. The underlying and contributing causes of death were coded according to the *International Classification of Diseases, Ninth Revision*. All individuals with code 335.2 (motor neuron disease) listed as the underlying or contributing cause of death were considered to have had ALS. In a previous validation study,[17] it was found that ALS was the primary diagnosis listed on death certificates in most instances where code 335.2 was listed as a cause or contributory cause of death.[17]

In the NHS and HPFS, incident ALS was also documented. In each biennial follow-up questionnaire, participants were asked to report a specific list of medically diagnosed conditions (initially not including ALS) and any other major illness. Amyotrophic lateral sclerosis was added to the list of specific conditions on the NHS questionnaires in 1992 and onwards and on the HPFS questionnaires in 2000 and onwards. We requested permission to contact the treating neurologist and for release of relevant medical records from participants who reported a diagnosis of ALS on the open question on major illnesses or on the specific question. Because of the rapidly progressive nature of the disease (median survival, 1.5-3 years),[15-17] many participants with ALS died before we could send the release request for medical records so the request was sent to the closest family member. After obtaining written permission, we asked the treating neurologists to complete a questionnaire to confirm the diagnosis of ALS and to rate the certainty of the diagnosis (definite, probable, or possible) and send medical records. Starting in 2004, the questionnaire was modified to include the El Escorial criteria. The final confirmation for our study purposes was made by a neurologist with experience in ALS diagnosis based on the review of medical records. We relied on the diagnosis made by the treating neurologist if the information in the medical record was insufficient or if it could not be obtained. Only participants with definite and probable ALS were included as cases in the primary analyses. When we were unable to confirm (ie, obtain a copy of the medical record or the neurologist's questionnaire) incident self-reported ALS, we classified the participant as having possible ALS and excluded him or her from the primary analysis unless death occurred during follow-up and ALS was listed on the death certificate.

Assessment of Diet and Other Covariates

We assessed participant diet using semiquantitative food frequency questionnaires (FFQs), which were developed or modified specifically for each cohort.[11,12,14-16,18] Participants reported habitual intake of each food on a scale ranging from never or less than 1 time per month to 6 servings or more per day. Nutrient intakes were then estimated by multiplying frequency of consumption by the specified portion size. Each study provided information on energy intake and macronutrient intakes including consumption of saturated, monounsaturated, and specific dietary PUFAs.

In the NIH-AARP and MEC studies, diet was assessed at baseline, whereas in the HPFS and NHS, diet was assessed at baseline and updated every 4 years. Because additional food items were added to NHS questionnaires after 1980, we considered 1984 as baseline. For CPS-II Nutrition, baseline for this study was in 1999, when dietary information on specific PUFAs was assessed using a modified version of the FFQ used in the HPFS and NHS. Individual validation and reproducibility studies for macronutrient intake, including fat subtype, were conducted for each dietary assessment instrument in subsets of participants from each study by comparing the FFQ estimates with intake estimated from multiple diet records (HPFS and NHS) or 24-hour recall (CPS-II Nutrition, MEC, and NIH-AARP studies) and with the fatty acid composition of adipose

or red cell membranes.[11,18-24] In the HPFS, correlations for eicosapentaenoic acid (EPA) and ω-6 fatty acids between reported FFQ intake and proportion in adipose tissue were 0.47 and 0.50, respectively.[20] In the NHS, the correlations between the average of four 7-day diet records and baseline FFQ for total, saturated, monounsaturated, and polyunsaturated fats were 0.57, 0.68, 0.58, and 0.48, respectively,[24] while correlation between FFQ-estimated dietary PUFA and red blood cell concentration was 0.41 for EPA, 0.27 for linoleic acid, and 0.54 for docosapentaenoic acid (DPA).[25] In CPS-II Nutrition, correlations between dietary assessment via FFQ with four 24-hour diet recalls ranged from 0.42 to 0.66 for dietary fat and fat subtypes.[18] Correlations between FFQs administered in 1992 and 1997 were 0.54 or greater for total dietary fat and fat subtype intake in men and in women.[18] In the MEC study, energy-adjusted correlations for dietary fat intakes estimated from FFQs and three 24-hour dietary recalls ranged from 0.31 to 0.77.[23] In the NIH-AARP, energy-adjusted correlations for intakes of total, saturated, monounsaturated, and polyunsaturated fat estimated from FFQs and two 24-hour recalls were 0.53 or greater in both sexes.[26]

Information on other covariates of interest, including smoking status, height, weight, education level, and physical activity, was collected at baseline for all cohorts.

Statistical Analysis

A total of 54 756 (5.2%) persons with extreme energy intake (3 SDs greater than or less than the study-specific mean on a $\log_e$ scale; roughly corresponding to total caloric intake of <500 and ≥4500 kcal/d) were excluded from the analysis. In the NIH-AARP, we excluded participants who reported serious illness at baseline (n = 20 188, 3.6%). Person-years of follow-up were calculated from study baseline to the earliest of time of ALS symptom onset (in the NHS and HPFS), death, loss to follow-up, or the end of follow-up. End of follow-up was June 2008 for the NHS, December 2008 for the HPFS, December 2008 for CPS-II Nutrition, December 2007 for the MEC study, and December 2008 for the NIH-AARP.

Within each cohort, we energy adjusted all nutrients using the residual method.[27] We categorized participants into cohort-specific quintiles for all dietary-fat variables because differences in intake across studies could reflect true dietary differences or differences in dietary assessment. We calculated marine ω-3 PUFAs as the sum of EPA, docosahexaenoic acid (DHA), and DPA because high correlations between intakes of theses PUFAs prevent assessment of their independent effects. We applied Cox proportional hazards regression stratified by age in years to calculate cohort-specific hazard ratios and associated 95% CIs for each study separately. For the CPS-II Nutrition, MEC study, and NIH-AARP, analyses were conducted in men and women separately. We pooled estimates using DerSimonian and Laird methods for random effects and assessed heterogeneity using Q statistics.[28] Within study-specific multivariate Cox models, we adjusted for potential confounders, such as body mass index (BMI [continuous], calculated as weight in kilograms divided by height in meters squared), physical activity (approximate tertiles corresponding to low, medium, and high activity levels), education level (<high school, high school, or >high school), smoking status (never a smoker, past, or current), vitamin E intake (quartiles), and total major carotenoid intake (quartiles), as uniformly as possible across all studies.[29-33] We tested for trend across all categorical analyses by modeling as a continuous covariate a new variable where participants in a certain category were assigned the median value for that category. To address the possibility that participants could be experiencing symptoms of ALS at the time of questionnaire completion, we conducted a lagged analysis where we excluded the first 4 years of follow-up in each cohort.

To address potential nonlinearity, we assessed the effect of each PUFA on the risk for ALS semiparametrically using restricted cubic splines. To create these estimates, we pooled studies into a single data set and each spline model was stratified by study, sex, age, and year of questionnaire. We additionally adjusted for a similar set of covariates as the study-specific models using quintiles (total vitamin E, total carotenoid intake, BMI, physical activity, education level, and energy intake).

We assessed effect modification by age (<median and ≥median age), smoking status (current or nonsmoker), sex, vitamin E intake (<median and ≥median intake), and low BMI (<22 and ≥22). We performed sensitivity analyses using cumulative averages of intake of PUFA from available cohorts (the NHS and HPFS); however, the results remain unchanged so they are not presented.

In secondary analysis, we modeled fat subtypes as a percentage of total energy using nutrient-density models simultaneously adjusted for total energy intake, percentage of energy from protein, and percentage of energy from all other types of dietary fat.[34] Coefficients from these models could be interpreted as the effect of substituting a specified percentage of energy from fat with the same percentage of energy from carbohydrates.[27,34] We assessed nutrient densities continuously and pooled risk ratios using DerSimonian and Laird methods for random effects.

All statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc) and graphics were generated using R software version 2.11 (The R Foundation for Statistical Computing).

Results

A total of 995 individuals with ALS were documented among 1 002 082 participants (479 114 women and 522 968 men) during study follow-up, ranging from approximately 9 to 24 years. Sex-specific estimates of median dietary ω-3 and ω-6 PUFA intake were consistent across studies. For men, median ω-3 PUFA intake ranged from 1.40 to 1.85 g/d and median ω-6 PUFA intake ranged from 11.82 to 15.73 g/d. For women, median ω-3 PUFA intake ranged from 1.14 to 1.43 g/d and median ω-6 PUFA intake ranged from 8.94 to 12.01 g/d. The relation between ω-3 PUFA intake and potential ALS risk factors is shown in **Table 1** and the correlations between different PUFAs in the eTable in the Supplement.

Overall, total ω-3 PUFA intake was associated with a 34% reduced risk for ALS in the multivariable model comparing the highest with the lowest quintile (multivariable pooled relative risk [RR], 0.66; 95% CI, 0.53-0.81; $P < .001$ for trend; **Table 2**)

Table 1. Selected Age-Adjusted Characteristics[a]

Characteristic	Total No. of ALS Cases (Men/Women)	Baseline Cohort Size[b]	Median Length of Follow-up, y	Mean (SD) Quintile of Total ω-3 Fatty Acid Intake 1	2	3	4	5
Nurses' Health Study, 1984 to 2008	81 (0/81)	92 059	24					
Age, y[c]				61 (7)	61 (7)	61 (7)	60 (7)	60.72 (7)
BMI				26 (5)	26 (5)	27 (5)	27 (5)	27 (5)
Current smokers, %				15	13	12	12	13
High physical activity level, %				27	29	30	30	29
Total vitamin E intake, mg/d				68 (100)	70 (101)	72 (101)	74 (103)	79 (107)
Total major carotenoid intake, mg/d				13 (6)	14 (7)	15 (7)	15 (7)	16 (8)
Total ω-6 fatty acid intake, g/d				7 (2)	8 (2)	8 (2)	9 (2)	10 (3)
Health Professionals Follow-up Study, 1986 to 2008	63 (63/0)	51 529	21					
Age, y[c]				55 (10)	54 (10)	54 (10)	54 (10)	55 (10)
BMI				25 (3)	25 (3)	25 (3)	26 (3)	26 (7)
Current smokers, %				12	10	9	7	8
High physical activity level, %				30	33	34	34	36
Total vitamin E intake, mg/d				43 (80)	47 (84)	50 (85)	50 (86)	57 (93)
Total major carotenoid intake, mg/d				14 (7)	16 (8)	17 (8)	19 (9)	20 (11)
Total ω-6 fatty acid intake, g/d				10 (3)	11 (3)	12 (3)	13 (3)	15 (4)
Cancer Prevention Study II Nutrition Cohort, 1999 to 2008	142 (71/71)	151 347	9					
Age, y[c]				69 (3)	69 (6)	70 (6)	70 (6)	70.26 (6)
BMI				25 (5)	27 (5)	26 (6)	26 (4)	26.35 (4)
Current smokers, %				5	4	4	4	4
High physical activity level, %				22	24	25	25	26
<High school education, %				27	32	30	27	25
Total vitamin E intake, mg/d				98 (99)	96 (98)	96 (98)	96 (98)	98 (98)
Total major carotenoid intake, mg/d				11 (5)	12 (6)	13 (6)	13 (6)	14 (7)
Total ω-6 fatty acid intake, g/d				7 (2)	9 (2)	11 (2)	12 (2)	15 (4)
Multiethnic Cohort Study, 1993-1997 to 2007	140 (83/57)	215 688	14					
Age, y[c]				61 (9)	60 (9)	60 (9)	60 (9)	59 (9)
BMI				25 (4)	26 (5)	26 (5)	26 (5)	26 (6)
Current smokers, %				14	14	15	17	18
High physical activity level, %				25	26	29	33	37
<High school education, %				44	44	44	43	42
Total vitamin E intake, mg/d				64 (126)	61 (128)	60 (122)	59 (122)	59 (122)
Total major carotenoid intake, mg/d				11 (8)	11 (7)	12 (7)	12 (7)	13 (8)
Total ω-6 fatty acid intake, g/d				9 (2)	12 (2)	14 (2)	16 (2)	19 (3)
NIH-AARP Diet and Health Study	568 (384/184)	546 214	11					
Age, y[c]				61 (5)	62 (5)	62 (5)	62 (5)	62 (5)
BMI				27 (5)	27 (5)	27 (5)	27 (5)	28 (5)
Current smokers, %				12	12	11	12	12
High physical activity level, %				20	19	19	19	19
<High school education, %				29	27	26	24	23
Total vitamin E intake, mg/d				87 (108)	83 (106)	80 (104)	79 (103)	80 (104)
Total major carotenoid intake, mg/d				15 (11)	16 (10)	17 (10)	17 (10)	18 (12)
Total ω-6 fatty acid intake, g/d				9 (3)	11 (3)	13 (3)	15 (3)	18 (4)

Abbreviations: ALS, amyotrophic lateral sclerosis; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); NIH, National Institutes of Health.

[a] Values are standardized to the age distribution of the specific study population.

[b] Prior to exclusion for implausible energy intake.

[c] Value is not age adjusted.

Table 2. Multivariable-Adjusted RRs of ALS According to Quintile of Baseline Intake of ω-3 and ω-6 Fatty Acids

Fatty Acid Type	Quintile 1 [Reference]	2	3	4	5	*P* for Trend[a]	*P* for Heterogeneity[b]
Total ω-3 fatty acids, median, g/d	0.94	1.21	1.43	1.68	2.11		
No. of cases	246	200	199	195	154		
Age-adjusted RR (95% CI)[c]	1.00	0.81 (0.67-0.98)	0.78 (0.61-0.99)	0.75 (0.57-0.99)	0.62 (0.50-0.78)	<.001	.15
Multivariable-adjusted RR (95% CI)[d]	1.00	0.83 (0.69-1.01)	0.81 (0.64-1.03)	0.78 (0.60-1.03)	0.66 (0.53-0.81)	<.001	.21
Total ω-6 fatty acids, median, g/d	7.65	10.14	12.20	14.51	18.38		
No. of cases	221	197	202	188	186		
Age-adjusted RR (95% CI)[c]	1.00	0.90 (0.73-1.10)	0.92 (0.72-1.17)	0.87 (0.72-1.06)	0.87 (0.72-1.07)	.15	.36
Multivariable-adjusted RR (95% CI)[d]	1.00	0.92 (0.75-1.13)	0.94 (0.73-1.22)	0.90 (0.74-1.10)	0.88 (0.72-1.08)	.22	.38

Abbreviations: ALS, amyotrophic lateral sclerosis; RR, relative risk.

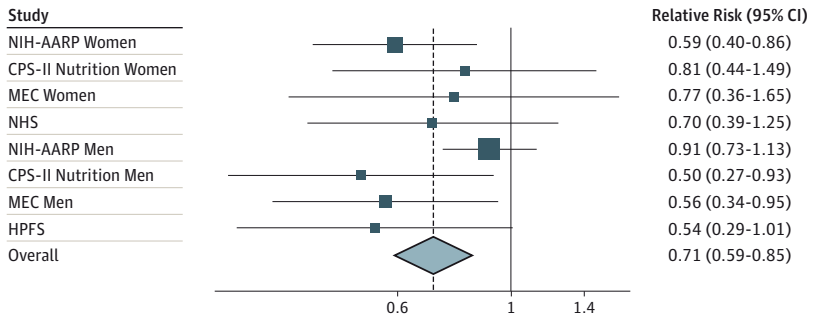
[a] Calculated using median value for each quintile.

[b] Calculated from the Q statistic and is used to quantify differences between studies.

[c] Adjusted for age (years) and sex.

[d] Adjusted for age (years), sex, smoking status (never, past, or current), total vitamin E intake (quartiles), total major carotenoid intake (quartiles), body mass index (calculated as weight in kilograms divided by height in meters squared; <23, 23-<25, 25-<30, or ≥30), physical activity (low, average, or high), education level (<high school, high school, or >high school), and energy intake (quintiles).

Figure 1. Study-Specific and Pooled Multivariable Relative Risks



Study-specific and pooled multivariable relative risks and 95% CIs of amyotrophic lateral sclerosis for a 1 g/d increase in intake of ω-3 polyunsaturated fatty acid. The squares and horizontal lines correspond to the study-specific multivariable relative risk and 95% CI, respectively. The inverse of the variance is used to calculate study weight and is represented by the area of the square. The diamond displays the pooled multivariable relative risk and 95% CI. We observed no significant effect modification by sex (*P* = .74). CPS-II Nutrition indicates the Cancer Prevention Study II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, the Multiethnic Cohort Study; NHS, the Nurses' Health Study; and NIH-AARP, the National Institutes of Health–AARP Diet and Health Study.

and results were relatively consistent across studies (**Figure 1**). Total ω-6 PUFA intake was not associated with ALS risk (multivariable pooled RR comparing the highest with lowest quintile, 0.88; 95% CI, 0.72-1.08; *P* = 0.22 for trend).

Individually, intakes of α-linolenic acid (ALA) and marine ω-3 PUFA were each associated with lower ALS risk (**Table 3**). The pooled multivariable RR comparing individuals in the highest quintile with those in the lowest was 0.73 for ALA (95% CI, 0.59-0.89; *P* = .003 for trend) and 0.84 for marine ω-3 PUFA (95% CI, 0.65-1.08; *P* = .03 for trend). We also detected marginally nonsignificant inverse trends across quintiles of dietary arachidonic acid intake (*P* = .07 for trend). However, on control for total ω-3 PUFA intake, arachidonic acid was not associated with ALS. No such attenuation was observed for ALA or marine ω-3 PUFA following adjustment for total ω-6 intake. Linoleic acid intake appeared not to be associated with ALS risk.

In semiparametric analyses using restricted cubic splines, we detected significant inverse linear relationships between intake of total ω-3 and ALA and marginally significant inverse trends for marine ω-3 PUFA (**Figure 2**; for total ω-3: *P* < .001; for ALA: *P* = .002; for marine ω-3: *P* = .06). Intakes of linoleic acid and arachidonic acid were not associated with ALS in semiparametric analyses (all *P* > .20).

In the lagged analysis where we excluded the first 4 years of follow-up in each cohort, we also observed similar associations between total ω-3 PUFA intake and ALA and ALS risk. The RR comparing the highest with the lowest quintile was 0.64 (95% CI, 0.51-0.81; *P* = .001 for trend) for total ω-3 and 0.73 (95%

Table 3. Multivariable-Adjusted Relative Risks of ALS According to Quintile of Intake of Individual Fatty Acids

Fatty Acid Type	Quintile 1 [Reference]	2	3	4	5	*P* for Trend[a]	*P* for Heterogeneity[b]
Linoleic acid, median, g	8.00	10.16	11.68	13.32	16.21		
No. of cases	228	190	203	186	187		
Age-adjusted RR (95% CI)[c]	1.00	0.83 (0.64-1.07)	0.87 (0.67-1.14)	0.84 (0.69-1.02)	0.82 (0.65-1.04)	.09	.18
Multivariable-adjusted RR (95% CI)[d]	1.00	0.85 (0.62-1.10)	0.90 (0.69-1.17)	0.84 (0.67-1.05)	0.83 (0.66-1.05)	.10	.18
Arachidonic acid, median, g	0.07	0.08	0.10	0.13	0.18		
No. of cases	229	225	194	173	175		
Age-adjusted RR (95% CI)[c]	1.00	0.99 (0.81-1.21)	0.87 (0.66-1.16)	0.73 (0.51-1.03)	0.80 (0.61-1.04)	.01	.16
Multivariable-adjusted RR (95% CI)[d]	1.00	1.00 (0.81-1.23)	0.90 (0.67-1.20)	0.76 (0.52-1.09)	0.86 (0.64-1.12)	.07	.12
α-Linolenic acid, median, g	0.82	1.06	1.27	1.50	1.93		
No. of cases	235	202	211	184	162		
Age-adjusted RR (95% CI)[c]	1.00	0.85 (0.67-1.08)	0.89 (0.71-1.12)	0.76 (0.58-1.00)	0.70 (0.57-0.86)	.001	.28
Multivariable-adjusted RR (95% CI)[d]	1.00	0.88 (0.70-1.11)	0.93 (0.74-1.17)	0.79 (0.61-1.04)	0.73 (0.59-0.89)	.003	.34
Marine ω-3, median; g	0.04	0.07	0.11	0.17	0.30		
No. of cases	195	218	224	193	164		
Age-adjusted RR (95% CI)[c]	1.00	1.15 (0.94-1.39)	1.15 (0.95-1.40)	1.03 (0.81-1.30)	0.82 (0.63-1.08)	.04	.18
Multivariable-adjusted RR (95% CI)[d]	1.00	1.16 (0.96-1.41)	1.16 (0.95-1.41)	1.04 (0.82-1.31)	0.84 (0.65-1.08)	.03	.28

Abbreviations: ALS, amyotrophic lateral sclerosis; RR, relative risk.

[a] Calculated using median value for each quintile.

[b] Calculated from the Q statistic and is used to quantify differences between studies.

[c] Adjusted for age (years) and sex.

[d] Adjusted for age (years), sex, smoking status (never, past, or current), total vitamin E intake (quartiles), total major carotenoid intake (quartiles), body mass index (calculated as weight in kilograms divided by height in meters squared; <23, 23-<25, 25-<30, or ≥30), physical activity (low, average, or high), education level (<high school, high school, or >high school), and energy intake (quintiles).

CI, 0.58-0.92; *P* = .003 for trend) for ALA. Results were attenuated for marine ω-3 (RR, 0.93; 95% CI, 0.72-1.20; *P* = .06 for trend).

Additional analyses were conducted using the percentage of energy from ω-3 PUFA and from other sources as continuous variables. According to these analyses, adding 0.5% of energy from ω-3 PUFA while maintaining a constant intake of ω-6 fatty acids and reducing by an isocaloric amount the intake of other types of fat would reduce ALS risk by 34% (95% CI, 15-49). Total baseline energy intake, or percentage of energy from total fat or other types of dietary fat were not associated with ALS risk.

We observed no significant evidence of effect modification by age, smoking status, vitamin E supplement use, carotenoids, or BMI. Results were also unchanged when we adjusted for race/ethnicity or smoking status using continuous pack-years or adjusted using continuous BMI in multivariable models.

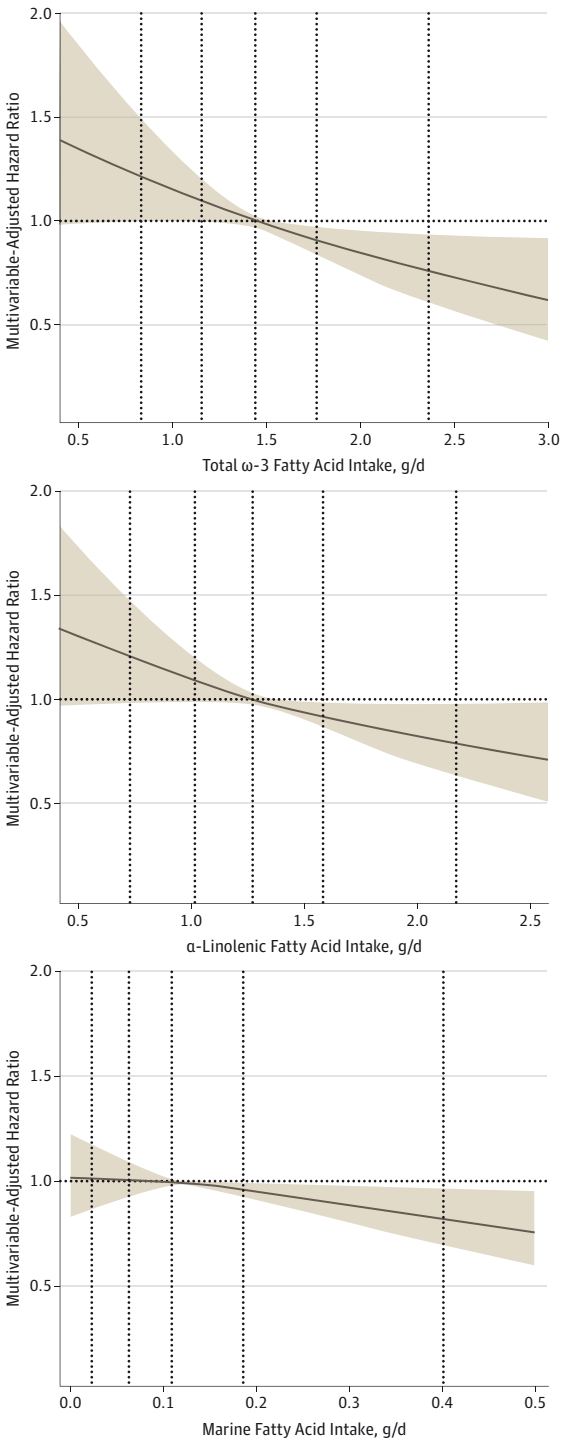
Discussion

In this pooled analysis of several large cohort studies with prospectively collected dietary information, we found that individuals with higher dietary intakes of ω-3 PUFA had a markedly reduced risk for ALS. Both ALA, the main ω-3 PUFA from vegetable sources, and marine ω-3 PUFA contributed to this association, which appeared to be independent of ω-6 intake.

Previous research of fat intake and ALS risk is sparse and results are inconsistent across studies. In a case-control study in Japan, ALS risk was reported to decrease with increasing intakes of total fat, saturated fat, monounsaturated fat, and total PUFA; however, no association was found with ω-3 PUFA.[10] Also, an inverse association between intake of total PUFA (separate results for ω-6 and ω-3 were not reported) was reported in a case-control study conducted in the Netherlands.[9] In contrast, an increased ALS risk across quartiles of intakes for total fat, saturated fat, cholesterol, and PUFA was reported in a case-control study in Washington state.[35] However, these studies may be vulnerable to combinations of recall and selection biases (where individuals without ALS did not represent the source population of individuals with ALS) when examining dietary exposures. Furthermore, the case-control design cannot adequately account for the effects of disease status itself on diet.

Our current study also had limitations. We used death rather than incidence in the CPS-II Nutrition, MEC, and NIH-AARP

Figure 2. Relationship of ω-3 Fatty Acids With Risk For Amyotrophic Lateral Sclerosis (ALS) Using Restricted Cubic Splines



The solid lines and shaded regions represent the hazard ratios of ALS for changes in intake relative to the median of specified polyunsaturated fatty acid (PUFA) and corresponding 95% CIs, respectively. The dotted vertical lines correspond to the fifth, 25th, 50th, 75th, and 95th percentiles for each fatty acid. To create these estimates, studies were pooled into a single data set and stratified by study, sex, age, and year of questionnaire. Each curve is also adjusted for smoking status, total vitamin E intake, total major carotenoid intake, body mass index, physical activity, education level, and energy intake.

studies. Death is an imperfect proxy for incidence because it may bias results in favor of shorter-term survivors. However, most patients with ALS rapidly progress (median survival, 1.5-3 years) and the observed consistent results in the lagged analysis suggests such bias minimally impacted our results.[36-39] Previous research also indicated that 70% to 90% of ALS cases were identified using death certificates listing motor neuron disease as the cause of death, allowing for potential misclassification of the outcome.[40-42] Nevertheless, it is possible that the lower risk for ALS deaths among individuals with higher ω-3 PUFA intake in our cohort was in part owing to a beneficial effect of high ω-3 PUFA intake on the survival of patients with ALS. However, this possibility did not affect the main conclusion that high ω-3 PUFA intake could be beneficial in delaying the onset or progression of ALS. Additionally, we did not have genetic or family history information and could not assess whether PUFA intake affected differently the risk for sporadic or familial ALS. Some error in estimating nutrient intakes was also inevitable, either because of inaccurate reporting or because of changes in intake during the follow-up. Given the longitudinal design, error in measuring ω-3 PUFA is most likely independent of future ALS risk and will thus tend to weaken the evidence of a protective effect. On the other hand, some residual confounding due to errors in measuring other nutrients or confounding by unknown factors related to both ALS and reported ω-3 PUFA intake cannot be excluded; however, confounding is unlikely to fully explain independent associations observed for both ALA and marine ω-3 PUFA (which have different food sources and are only weakly correlated with each other).

Despite these limitations, our study had several strengths including the large number of participants and documented cases, validated dietary assessment methods, and extended follow-up in each of the cohorts. Another notable strength was each study's prospective design, which is particularly important as most previous case-control studies used prevalent cases of ALS and may have been vulnerable to recall bias, which is common in studies of dietary exposures. Additionally, our study was also likely to include a wide spectrum of patients with ALS, thus minimizing the selection bias that may occur in studies that recruit patients with ALS from tertiary care centers.[39]

Biologic activity of PUFAs within the brain depends on the chain length as well as the number and position of the double bonds of the specific PUFA in question. Previous animal studies suggest ALA slows peroxidation activation of the binding of neural nuclear transcription factor κB and reduces glutamate-mediated excitotoxic damage and oxidative stress, possibly preventing neuronal cell death.[43-46] Neuroprotective properties of ALA have also been noted in prolonging neuronal survival through reduction in immunoreactivity of proapoptotic proteins.[47]

In addition to individual biologic effects, ALA can be converted to EPA and eventually DHA via various fatty acid elongation enzymes (although the extent to which this occurs likely depends on an individual's underlying direct DHA intake).[5,48] Therefore, circulating levels of DHA and EPA available for uptake by the brain represent a combination of those derived from the diet and those biosynthesized in the liver from ALA. Both EPA and DHA themselves may have additional neuroprotec-

tive properties. In vivo studies have suggested that DHA potentially reduces levels of neural inflammation through prevention of microglial activation by proinflammatory cytokines or through production of anti-inflammatory and antioxidative neuroprotectin-D1.[49,50] Both ALA and DHA are essential fatty acids derived from the diet, and endogenous synthesis of neuroprotectin-D1 is heavily dependent on sufficient levels of DHA. Therefore, moderate dietary intake of such ω-3 PUFAs may present a modifiable means of promoting neuroprotection.

Conclusions

Overall, the results of our large prospective cohort study suggest that individuals with higher dietary intakes of total ω-3 PUFA and ALA have a reduced risk for ALS. Further research, possibly including biomarkers of PUFA intake, should be pursued to confirm these findings and to determine whether high ω-3 PUFA intake could be beneficial in individuals with ALS.

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