Dietary intake of α-linolenic acid and risk of age-related macular degeneration[1,2]

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ABSTRACT

Background: The relation between α-linolenic acid (ALA), a plant-derived omega-3 (n–3) fatty acid, and age-related macular degeneration (AMD) is unclear. European researchers reported that $\leq$40% of ALA can be present as *trans* forms.

Objective: We aimed to evaluate the associations between intake of ALA and intermediate and advanced AMD.

Design: Seventy-five thousand eight hundred eighty-nine women from the Nurses' Health Study and 38,961 men from Health Professionals Follow-Up Study were followed up from 1984 to 2012 and from 1986 to 2010, respectively. We assessed dietary intake by a validated food-frequency questionnaire at baseline and every 4 y thereafter. One thousand five hundred eighty-nine incident intermediate and 1356 advanced AMD cases (primarily neovascular AMD) were confirmed by medical record review.

Results: The multivariable-adjusted HR for intermediate AMD comparing ALA intake at the top quintile to the bottom quintile was 1.28 (95% CI: 1.05, 1.56; *P*-trend = 0.01) in the analyses combining 2 cohorts. The HR in each cohort was in the positive direction but reached statistical significance only in the women. However, the positive association was apparent only in the pre-2002 era in each cohort and not afterward (*P*-time interaction = 0.003). ALA intake was not associated with advanced AMD in either time period. Using gas-liquid chromatography, we identified both *cis* ALA (mean $\pm$ SD: 0.13% $\pm$ 0.04%) and *trans* ALA isomers (0.05% $\pm$ 0.01%) in 395 erythrocyte samples collected in 1989–1990. In stepwise regression models, mayonnaise was the leading predictor of erythrocyte concentrations of *cis* ALA and one isomer of *trans* ALA. We also found *trans* ALA in mayonnaise samples.

Conclusions: A high intake of ALA was associated with an increased risk of intermediate AMD before 2002 but not afterward. The period before 2002 coincides with the same time period when *trans* ALA was found in food and participants' blood; this finding deserves further study.   *Am J Clin Nutr* 2017;105:1483–92.

INTRODUCTION

Age-related macular degeneration (AMD)[12] is a chronic, progressive disease that can cause irreversible blindness (1). According to the 2005–2008 US NHANES, the estimated prevalence in persons aged $\geq$40 y was 5.7% for early or intermediate AMD compared with 0.8% for advanced AMD (2). Because the number of patients with AMD is expected to grow exponentially in the next few decades in the United States and worldwide as populations age (3–5), the identification of potential strategies for primary prevention would have marked public health significance.

Marine long-chain omega-3 fatty acids, including EPA and DHA, have been associated with a lower risk of AMD in multiple observational studies (6–10). However, epidemiologic research on the shorter-chain ω-3 fatty acid, α-linolenic acid (ALA), has yielded inconsistent findings. Some studies reported a positive association between the risk of AMD and intakes of ALA (6, 11) and vegetable-fat foods (12–14) that contain ALA whereas others did not (7, 8, 15, 16). Additional studies of ALA's effects are needed because this plant-derived fatty acid accounts for $\geq$85% of total ω-3 fatty acid intake in the United States (17) and presumably even more in parts of the world where access to fish is limited.

trans ALA is formed during partial hydrogenation, deep frying, and industrial deodorization (18, 19) and is hypothesized to interfere with the crucial biological functions of *cis* ALA. *trans* ALA has not received much attention in the United States. However, European researchers have reported the presence of *trans* isomers of ALA in food such as vegetable oils (18, 20), low-calorie spreads (21), and infant formulas (22, 23). Up to 40% of ALA can be present as *trans* isomers (19, 20). *trans* ALA also occurs in human serum (24) and maternal milk (25, 26).

We previously reported a positive association between the intake of ALA and AMD (6). With a 16-y-longer follow-up and 2500 more AMD cases than our previous study, our primary objective was to further evaluate this association. Our secondary aim was to examine *trans* ALA isomers in both foods and human bodies in the United States.

METHODS

Study population

The NHS (Nurses' Health Study), initiated in 1976, is an ongoing prospective cohort that includes 121,700 US female registered nurses aged between 30 and 55 y at baseline. The HPFS (Health Professionals Follow-up Study), initiated in 1986, includes 51,529 US male health professionals aged between 40 and 75 y at baseline. Both cohorts are predominantly white. Participants are mailed biennial questionnaires about lifestyle factors and disease outcomes and food frequency questionnaires (FFQs) every 4 y to assess diet in the preceding years. The study protocol was approved by the Institutional Review Boards at the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health.

We restricted the study population to those who were ≥50 y old at baseline and then added participants to the analysis once they reached age 50 y. Participants were censored at age 90 y to alleviate the concern of lower ascertainment of AMD cases (NHS, $n = 15$; HPFS, $n = 528$). At baseline, we excluded participants who did not return the initial FFQ, left >70 food items blank in the FFQ, reported implausible dietary intake (<600 or >3500 kcal/d for the NHS and <800 or >4200 kcal/d for the HPFS), or had prevalent AMD, cancer (except nonmelanoma skin cancer), diabetes, and cardiovascular disease. To minimize detection bias, we also excluded participants who never reported an eye exam over the entire follow-up period and skipped the person-time during any 2-y questionnaire interval in which they did not report an eye exam. After exclusions, a total of 75,889 women and 38,961 men contributed to the analysis. The flow-chart of the study participants can be found at the **Supplemental Figure 1**.

AMD ascertainment

Our case validation and definition have been previously described (27). When a participant reported a diagnosis of AMD on a biennial questionnaire, we requested informed consent to review his or her medical record and then contacted the participant's eye doctor to either complete a standardized questionnaire or send us copies of ocular records to confirm the diagnosis. The questionnaire requested information from the medical record on the date of initial diagnosis, treatment received for AMD, clinical signs of AMD, best corrected visual acuity, and whether in the opinion of the treating doctor the visual acuity loss was primarily due to AMD. Photos and/or optical coherence tomography were also reviewed when available to confirm clinical AMD lesions. Cases with only small hard drusen (drusen size <63-μm diameter circle) were excluded. We defined intermediate AMD as having ≥1 of the following signs in ≥1 eye: intermediate drusen (≥63 and <125 μm), pigment abnormalities, large drusen (≥125 μm), or any noncentral geographic atrophy (GA). We defined neovascular AMD as having any of the following signs in ≥1 eye: retinal pigment epithelium detachment, subretinal neovascular membrane, disciform scar, or history of treatment with laser, photodynamic, or anti–vascular endothelial growth factor therapy for AMD. Central GA was defined as having a central GA lesion involving the center of the macula in ≥1 eye. Advanced AMD included both neovascular AMD and central GA. Additionally, all case definitions, except those recent neovascular AMD cases that had anti–vascular endothelial growth factor therapy, included a visual acuity of 20/30 or worse primarily due to AMD. This magnitude of vision loss is not only of clinical significance but also is severe enough to warrant medical attention so as to minimize potential detection bias arising from differential health consciousness. The person was used as the unit of analysis, and the worst eye was used for classification.

Dietary assessment

We began follow-up in 1986 for the HPFS and 1984 for the NHS, when the first comprehensive FFQ with 131 items was administered, and assessed dietary intake approximately every 4 y thereafter. Intake of ALA is from many foods, but the major sources among FFQ items include *1*) mayonnaise or other creamy salad dressing (1 tablespoon or 15 mL); *2*) oil and vinegar dressing (e.g., Italian; 1 tablespoon or 15 mL); *3*) beef, pork, or lamb as a main dish (4–6 ounces or 113–170 g); *4*) margarine (1 pat or 5 g); and *5*) other cheese (e.g., American, cheddar; 1 slice, 1 ounce or 28 g). These foods collectively accounted for 46% of ALA intake in the NHS and 38% in the HPFS at baseline. However, the total contribution from these foods decreased to 16% in 2010 in the NHS and 12% in 2006 in the HPFS. Walnut consumption (1 ounce or 28 g) was included in the 1998 FFQ and onward, which became the top contributor to ALA intake (18% in the NHS and 17% in the HPFS). Low-fat mayonnaise consumption (1 tablespoon or 15 mL) was included in the 1994 FFQ and onward. We started asking about the intake of flax seed oil and flax seed (yes or no) from the 2006 FFQ in the NHS and further inquired about the frequency of intake for flax seed (1 tablespoon or 14 g) in the 2010 FFQ in both the NHS and HPFS. Participants were asked to report how often, on average over the past year, they had consumed each food item (9 possible responses ranging from "≤1 time/mo" to "≥6 times/d"). The FFQs specifically inquired about the usual type of fat used for frying, sautéing, and baking. It also inquired about the usual brand and type of margarine by using an open-ended question. Such information was taken into account when calculating ALA intake. The daily nutrient intake was calculated by multiplying the consumption frequency of each food by its nutrient content and then summing across all foods. The fatty acid composition of food fried, baked, or sautéed at home was modified by the type of fat

reported. The nutrient composition data were primarily based on the USDA Nutrient Database supplemented with information from manufacturers and published reports. We adjusted all the nutrient intakes for total energy using the residual method to reflect the composition of the diet (28).

The reproducibility and validity of FFQs in measuring fat intake has recently been assessed in the Women's Lifestyle Validation Study (29), an extensive validation study that involved >700 women from the NHS and NHS II. After correcting for random within-person error in the diet records, the Spearman correlation between the intake of energy-adjusted ALA from the FFQ and from two 1-wk diet records was 0.57 (95% CI: 0.48, 0.65). Similarly, the de-attenuated correlation for ALA between the FFQ and four 24-h dietary recalls was 0.58.

Measurement of *trans* ALA and *cis* ALA

To confirm the presence of *trans* ALA in food supplies and in human bodies, we measured *trans* ALA among erythrocyte samples (n = 395) from the NHS participants by gas-liquid chromatography and reanalyzed some existing chromatography of mayonnaise samples collected before and after 2000. The methods were described elsewhere (30). Each *trans* ALA peak was identified by comparison with *trans* ALA standards (linolenic acid methyl ester isomer mix; Sigma-Aldrich), and the amount was expressed as the percentage of the total peak area. We identified 4 *trans* ALA isomers: *trans* ALA-A and -B have 2 *trans* double bonds whereas *trans* ALA-C and -D have 1 *trans* double bond. The concentration of erythrocyte *trans* ALA-A was extremely low and below the meaningful concentration (mean <0.01%) and was thus not included in the analysis. The overall CVs for *trans* ALA-B, -C, and -D were 23.6%, 33.4% and 40.8%, respectively. Higher CVs for *trans* ALA-C and -D were likely caused by the overlapping of those 2 peaks with 11t-Eicosenoic acid (20:1n-9t) and 8c-Eicosenoic acid (20:1n-12c), respectively. However, because the within-batch CV for each peak was <20% (an acceptable limit), we statistically accounted for the high overall CVs by recalibrating the concentration of each *trans* ALA isomer from all batches to its average batch according to a method outlined by Rosner et al. (31). Because *trans* ALA-C and -D peaks usually overlapped in chromatography, we combined those 2 in the analysis to reduce measurement error. We estimated the reproducibility of erythrocyte *trans* ALA by reanalyzing existing chromatography from an earlier within-person stability study in which 40 post-menopausal women gave 3 blood samples over 2–3 y (32). In that study, the intraclass correlation for *cis* ALA was 0.52. The intraclass correlation we calculated was 0.53 for *trans* ALA-B, 0.56 for *trans* ALA-C, and 0.43 for *trans* ALA-D, all indicating fair-to-good reproducibility.

To more accurately determine food sources of blood *cis* ALA, we also obtained previous measurements of erythrocyte and plasma *cis* ALA from participants of nested case-control studies of cardiovascular disease in the NHS and HPFS. The details on blood collection and measurements have been previously described (33, 34).

Statistical analysis

Participants contributed person-time to the analysis from the return of the baseline questionnaire if >50 y of age at baseline or

from reaching 50 y old to the confirmed diagnosis of AMD, death, loss to follow-up, or the end of follow-up (31 May 2012 for the NHS and 31 January 2010 for the HPFS), whichever occurred first. To best represent long-term intake and minimize measurement error (35), we calculated the cumulative average intake of ALA by averaging all available FFQs up to the start of each 2-y risk interval. The cumulative average value was categorized into quintiles based on the distribution in each cohort. Likewise, we calculated the cumulative average intakes of foods and categorize them into prespecified groups.

We used time-varying Cox proportional hazards models to estimate the HRs and 95% CIs associated with intermediate and advanced AMD, respectively. To control as finely as possible for confounding by age and calendar time and any possible 2-way interactions between these 2 time scales, we stratified the model jointly by age in months at the start of follow-up and calendar year of the current questionnaire cycle. We controlled for established and probable risk factors including race, BMI (in kg/m^2), pack-years of smoking, physical activity, aspirin use, history of hypertension, history of hypercholesterolemia, menopausal status and post-menopausal hormone use (in the NHS only), and dietary variables including intakes of total energy, linoleic acid (LA), and DHA.

Because of a change of major food sources to ALA intake around the early 2000s, which would potentially introduce a varying degree of confounding, we conducted a stratified analysis to evaluate the associations between intakes of ALA and AMD in the pre-2002 and post-2002 era. The early 2000s also marked a potential change in *trans* ALA content over time because there was an increasing public awareness of the adverse health effects by *trans* fatty acids and food industries started to reduce or eliminate them (36, 37). Several studies found a downward trend of total *trans* intake (38) and plasma concentrations (39) around that time. We calculated the cumulative average of ALA intake separately in each time period. We created product terms between the time-varying ALA variable and the binary indicator for time period and used a likelihood ratio test to test the significance of interaction by time comparing models with and without the product terms. A similar approach was used to test interactions between the intake of ALA and prespecified risk factors including age, smoking status, and menopausal hormone use.

We used stepwise linear regressions to identify foods that were significantly predictive of the blood measurements of *cis* and *trans* ALA ($P < 0.05$). This method has been described previously (27, 40). Briefly, we used the average of food intake from the 1986 and 1990 FFQs in the NHS and from the 1990 and 1994 FFQs in the HPFS to reduce the within-person variation of intake and to correspond with the time of blood draw. We developed the linear regression model by the biomarker type (erythrocyte and plasma) in each cohort.

We performed the statistical analyses separately in each cohort using SAS 9.3 (SAS Institute). To derive a pooled HR, we first combined the 2 cohorts and then applied a Cox proportional hazards model in the pooled data stratified by the cohort. Interpretation of the data was mainly based on pooled HRs unless otherwise specified. All hypothesis tests were 2-sided and used an α level of 0.05.

RESULTS

During 28 y of follow-up in the NHS and 24 y in the HPFS, we confirmed 2219 incident AMD cases (1209 intermediate and 1010 advanced cases) in women and 726 cases (380 intermediate

and 346 advanced cases) in men. The advanced AMD cases were predominantly neovascular AMD (96%).

In 1998 (the middle of the follow-up period), participants' demographic and lifestyle characteristics did not markedly vary across quintiles of ALA intake except that those in the higher-intake category were more likely to smoke cigarettes and have a higher BMI and were less likely to have hypercholesterolemia (**Table 1**). In terms of dietary intake, participants with higher intake of ALA were also more likely to have higher intakes of DHA, LA, *trans* fat, and saturated fat but overall a higher healthy-eating score.

ALA intake was moderately correlated (average Pearson $r = 0.62$) with LA intake, the analogous ω-6 fatty acid that shares many food sources with ALA, but weakly correlated with intake of food-sourced EPA ($r = 0.10$) or DHA ($r = 0.06$). During the follow-up, the age-adjusted intake of ALA increased ∼50% in the NHS from 1984 (median intake, 1.03 g/d) to 2010 (1.58 g/d) and 29% in the HPFS from 1986 (1.12 g/d) to 2006 (1.44 g/d). In contrast, the intake of food-sourced EPA and DHA did not appreciably change during the follow-up (data not shown).

In a pooled analysis between the NHS and HPFS comparing extreme quintiles, intakes of ALA had a statistically significant

TABLE 1
Age-standardized characteristics of participants in the NHS and HPFS according to cumulative average intake of ALA in 1998 (the middle of follow-up)[1]

	ALA intake				
	Q1	Q2	Q3	Q4	Q5
NHS					
ALA, mg/d	738 ± 64	860 ± 25	943 ± 24	1036 ± 32	1242 ± 153
Participants, *n*	13,257	13,246	13,264	13,258	13,245
Age, y	64 ± 7	63 ± 7	63 ± 7	64 ± 7	64 ± 7
BMI, kg/m^2	26.1 ± 5.0	26.6 ± 5.1	26.8 ± 5.2	27.0 ± 5.4	27.1 ± 5.5
Caucasian, %	96	98	98	98	96
Current smoker, %	10	9	10	10	12
Never smoker, %	47	47	46	45	41
Physical activity, MET-h/wk	11 (4–24)	11 (4–24)	11 (4–23)	10 (4–23)	10 (3–22)
Hypertension, %	44	44	44	44	44
Hypercholesterolemia, %	59	58	57	56	54
Postmenopausal, %	94	94	94	94	94
Current menopausal hormone use,[2] %	44	45	46	45	45
Current aspirin use, %	47	49	49	49	47
Dietary intake					
DHA, mg/d	133 ± 83	137 ± 81	140 ± 82	139 ± 84	143 ± 91
LA, g/d	7.3 ± 1.5	8.1 ± 1.4	8.7 ± 1.3	9.3 ± 1.4	10.8 ± 1.9
trans Fat, g/d	2.5 ± 0.7	2.7 ± 0.7	2.9 ± 0.8	2.9 ± 0.8	3.0 ± 0.9
Saturated fat, g/d	17.7 ± 3.6	19.0 ± 3.5	19.7 ± 3.8	20.4 ± 4.0	21.3 ± 4.5
Monounsaturated fat, g/d	19.2 ± 3.6	20.8 ± 3.4	21.7 ± 3.6	22.5 ± 3.7	23.7 ± 4.0
Alternative healthy-eating index	50.6 ± 9.7	50.8 ± 9.3	51.0 ± 9.2	51.2 ± 9.3	52.1 ± 9.4
Total energy intake, kcal/d	1695 ± 434	1743 ± 436	1770 ± 433	1770 ± 433	1732 ± 441
HPFS					
ALA, mg/d	840 ± 79	987 ± 30	1085 ± 28	1196 ± 38	1445 ± 196
Participants, *n*	6148	6115	6161	6091	6180
Age, y	64 ± 9	64 ± 9	64 ± 9	64 ± 9	64 ± 9
BMI, kg/m^2	25.5 ± 3.2	26.0 ± 3.5	26.3 ± 3.6	26.4 ± 3.7	26.4 ± 3.7
Caucasian, %	94	95	96	96	95
Current smoker, %	5	5	4	5	6
Never smoker, %	52	51	51	49	48
Physical activity, MET-h/wk	24 (9–47)	24 (10–47)	24 (10–47)	23 (10–47)	23 (9–47)
Hypertension, %	38	38	37	36	37
Hypercholesterolemia, %	51	50	48	47	45
Current aspirin use, %	61	63	63	62	58
Dietary intake					
DHA, mg/d	206 ± 142	206 ± 127	203 ± 130	206 ± 133	213 ± 158
LA, g/d	9.3 ± 2.1	10.2 ± 1.9	10.8 ± 1.8	11.6 ± 1.8	13.2 ± 2.4
trans Fat, g/d	2.8 ± 1.1	3.1 ± 1.0	3.3 ± 1.0	3.4 ± 1.1	3.4 ± 1.1
Saturated fat, g/d	19.7 ± 4.8	21.7 ± 4.5	22.9 ± 4.7	23.8 ± 4.9	24.9 ± 5.5
Monounsaturated fat, g/d	23.3 ± 5.1	25.6 ± 4.6	26.9 ± 4.5	28.1 ± 4.6	29.5 ± 5.1
Alternative healthy-eating index	54.0 ± 10.4	53.9 ± 9.8	53.6 ± 9.9	53.8 ± 9.9	55.3 ± 10.1
Total energy intake, kcal/d	1934 ± 520	1985 ± 520	1997 ± 527	2009 ± 528	1968 ± 543

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated. ALA, α-linolenic acid; HPFS, Health Professionals Follow-up Study; LA, linoleic acid; MET-h, hours of metabolic equivalent tasks; NHS, Nurses' Health Study; Q, quintile.
[2] Current menopausal hormone use among postmenopausal women.

positive association with intermediate AMD (comparing extreme quintiles, HR: 1.31; 95% CI: 1.21, 1.53; *P*-trend <0.001), and this association was essentially unaltered after further adjusting for LA or saturated, monounsaturated, and *trans* fatty acids (**Table 2**). The multivariable-adjusted HR for ALA was in the positive direction in each cohort, although it was statistically significant only in the NHS. For the baseline 1986 FFQ in the HPFS, we further separated the intake of ALA by animal and plant sources (such information was not available in the NHS). The median baseline intake was 350 mg/d for animal-sourced ALA and 700 mg/d for plant-sourced ALA, and they were weakly negatively correlated (Pearson *r* = −0.22). By comparing extreme quintiles, plant-sourced ALA (adjusted HR: 1.54; 95% CI 1.04, 2.28; *P*-trend = 0.03) was more strongly associated with intermediate AMD than the animal-sourced counterpart was (adjusted HR: 1.17; 95% CI: 0.85, 1.62; *P*-trend = 0.22). When they were mutually adjusted for each other, the HR did

not materially change for plant-sourced ALA (adjusted HR: 1.60; 95% CI: 1.08, 2.38; *P*-trend = 0.02) or for animal-sourced ALA (adjusted HR: 1.23; 95% CI: 0.89, 1.70; *P*-trend = 0.13). By comparing extreme quintiles, the intake of LA also had a positive association with intermediate AMD (adjusted HR: 1.22; 95% CI: 1.05, 1.42; *P*-trend = 0.006); however, after further adjusting for ALA, the association was attenuated and no longer significant (adjusted HR: 1.06; 95% CI: 0.88, 1.27; *P*-trend = 0.50).

In contrast, we did not find any significant association between advanced AMD and the intake of ALA after multivariable adjustment (Table 2) or for LA (pooled HR for comparing extreme quintiles: 1.08; 95% CI: 0.91, 1.28; *P*-trend = 0.25).

To understand whether the association with intermediate AMD was specific to ALA, we examined the intakes of several other correlated fatty acids including *cis* 18:1, *trans* 18:1, *cis* 18:2, *trans* 18:2, saturated fat, and total *trans* fatty acids. By

TABLE 2
The HRs (95% CIs) of AMD according to quintiles of the cumulative average intake of α-linolenic acid[1]

	Q1	Q2	Q3	Q4	Q5	*P*-trend
Intermediate AMD						
NHS						
Median, mg/d	760	875	963	1065	1252	
Cases/person-years	211/300,584	237/301,701	203/302,772	273/301,809	285/302,175	
Age-adjusted model	1 (Ref)	1.19 (0.99, 1.43)	1.02 (0.84, 1.24)	1.36 (1.14, 1.63)	1.38 (1.15, 1.64)	<0.001
Multivariate model 1	1 (Ref)	1.17 (0.97, 1.41)	1.01 (0.83, 1.22)	1.33 (1.11, 1.60)	1.34 (1.12, 1.61)	<0.001
Multivariate model 2	1 (Ref)	1.21 (1.00, 1.47)	1.05 (0.85, 1.29)	1.38 (1.12, 1.69)	1.31 (1.05, 1.64)	0.02
HPFS						
Median, mg/d	850	990	1093	1213	1425	
Cases/person-years	74/122,869	76/123,263	67/122,309	75/123,512	88/122,996	
Age-adjusted model	1 (Ref)	1.06 (0.77, 1.47)	0.95 (0.68, 1.32)	1.06 (0.77, 1.47)	1.22 (0.90, 1.67)	0.20
Multivariate model 1	1 (Ref)	1.04 (0.76, 1.44)	0.93 (0.67, 1.30)	1.03 (0.75, 1.43)	1.20 (0.88, 1.64)	0.24
Multivariate model 2	1 (Ref)	1.03 (0.75, 1.43)	0.92 (0.65, 1.30)	1.01 (0.72, 1.43)	1.18 (0.82, 1.69)	0.37
Pooled (NHS + HPFS)						
Multivariate model 1	1 (Ref)	1.15 (0.97, 1.34)	0.99 (0.84, 1.17)	1.26 (1.07, 1.47)	1.31 (1.12, 1.53)	<0.001
Multivariate model 2	1 (Ref)	1.16 (0.99, 1.37)	1.01 (0.85, 1.21)	1.28 (1.07, 1.52)	1.27 (1.05, 1.54)	0.01
Multivariate model 3	1 (Ref)	1.17 (0.99, 1.38)	1.02 (0.85, 1.22)	1.28 (1.07, 1.54)	1.28 (1.05, 1.56)	0.01
Advanced AMD						
NHS						
Median, mg/d	760	875	963	1065	1252	
Cases/person-years	189/300,571	196/301,736	212/302,750	195/301,877	218/302,246	
Age-adjusted model	1 (Ref)	1.10 (0.90, 1.35)	1.20 (0.99, 1.46)	1.09 (0.89, 1.33)	1.19 (0.98, 1.44)	0.14
Multivariate model 1	1 (Ref)	1.07 (0.88, 1.31)	1.15 (0.94, 1.40)	1.04 (0.85, 1.27)	1.12 (0.92, 1.36)	0.40
Multivariate model 2	1 (Ref)	1.06 (0.86, 1.30)	1.13 (0.91, 1.40)	1.01 (0.81, 1.27)	1.07 (0.84, 1.36)	0.77
HPFS						
Median, mg/d	850	990	1093	1213	1425	
Cases/person-years	66/122,877	73/123,272	67/122,316	66/123,518	74/123,014	
Age-adjusted model	1 (Ref)	1.15 (0.82, 1.60)	1.05 (0.75, 1.48)	1.02 (0.73, 1.44)	1.14 (0.81, 1.58)	0.66
Multivariate model 1	1 (Ref)	1.11 (0.80, 1.55)	1.02 (0.73, 1.44)	0.97 (0.69, 1.36)	1.08 (0.77, 1.51)	0.88
Multivariate model 2	1 (Ref)	1.09 (0.78, 1.53)	0.98 (0.69, 1.40)	0.90 (0.63, 1.31)	1.02 (0.69, 1.49)	0.81
Pooled (NHS + HPFS)						
Multivariate model 1	1 (Ref)	1.09 (0.92, 1.29)	1.12 (0.94, 1.32)	1.02 (0.86, 1.22)	1.11 (0.94, 1.32)	0.40
Multivariate model 2	1 (Ref)	1.07 (0.90, 1.28)	1.09 (0.91, 1.31)	0.98 (0.81, 1.19)	1.06 (0.86, 1.30)	0.88
Multivariate model 3	1 (Ref)	1.06 (0.89, 1.27)	1.08 (0.89, 1.30)	0.97 (0.80, 1.18)	1.05 (0.85, 1.30)	0.92

[1] Multivariate Cox regression model 1 included age (continuous), race (Caucasian or not), BMI (in kg/m^2: <18.5, 18.5–23, 23–25, 25–30, 30–35, or >35), pack-years of smoking (never, 1–9, 10–24, 25–44, 45–64, or ≥65 y), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, or ≥27 h of metabolic equivalent tasks/wk), current aspirin use (≥1 tablet/wk or none), history of hypertension and hypercholesterolemia, DHA (quintiles), and total energy intake (quintiles). In the NHS, models were additionally adjusted for postmenopausal status and menopausal hormone use (never, current, and past). Multivariate Cox regression model 2 = multivariate model 1 + linoleic acid (quintiles). Multivariate Cox regression model 3 = multivariate model 1 + linoleic acid + monounsaturated fat + saturated fat + trans fat (all in quintiles). *P*-trend was calculated by modeling the median value of each category as a continuous variable. AMD, age-related macular degeneration; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; Ref, reference.

comparing extreme quintiles, intakes of *cis* 18:1, *cis* 18:2, *trans* 18:2, and saturated fat each had a significant positive association with intermediate AMD (**Supplemental Figure 2**). However, all the associations were attenuated and no longer statistically significant after adjusting for ALA, whereas in all scenarios ALA persisted with a significant positive association (Supplemental Figure 2). On the other hand, intakes of *trans* 18:1 (*P*-trend = 0.05), *trans* 18:2 (*P*-trend = 0.04), and total *trans* fatty acids (*P*-trend = 0.03) had a suggestively significant association with advanced AMD after adjusting for ALA (**Supplemental Figure 3**). The association between the intake of ALA and intermediate AMD was apparent only among never smokers (pooled HR comparing extreme quintiles: 1.66; 95% CI: 1.23, 2.22; *P*-trend = 0.001) compared with ever smokers (pooled HR comparing extreme quintiles: 1.07; 95% CI: 0.83, 1.37; *P*-trend = 0.51) (*P*-interaction = 0.10) (**Supplemental Table 1**). In postmenopausal women, the HR for the association

between intake of ALA and intermediate AMD among current users of exogenous hormones was 1.84 (95% CI: 1.16, 2.92; *P*-trend = 0.04; *n* = 315 cases), whereas the HR among non–current users was 1.17 (95% CI: 0.89, 1.54; *P*-trend = 0.17; *n* = 789 cases) and *P*-interaction = 0.01. When we further restricted this analysis to the pre-2002 era, the HR among current users of exogenous hormones was 2.20 (95% CI: 1.27, 3.82; *P*-trend = 0.03; *n* = 235 cases) and non–current users was 1.12 (95% CI: 0.76, 1.63; *P*-trend = 0.35; *n* = 380 cases) and *P*-interaction = 0.005. There was no significant interaction by age (**Supplemental Table 2**).

In the stratified analysis by time, by comparing extreme quintiles the intake of ALA was positively associated with intermediate AMD before 2002 but not after, and the interaction by time period was significant (*P* = 0.003) (**Table 3**). There was no significant association between the intake of ALA and advanced AMD in either time period. Mayonnaise and oil-and-vinegar salad dressing were both positively associated with intermediate AMD

TABLE 3
The HRs (95% CIs) of AMD according to quintiles of cumulative average intake of ALA before and after 2002[1]

	Q1	Q2	Q3	Q4	Q5	*P*-trend	*P*-interaction
Intermediate AMD							
Beginning to 2002							
NHS (*n* = 683 cases)							
Median, mg/d	748	865	957	1060	1242		
Multivariate model 2	1 (Ref)	1.18 (0.91, 1.53)	1.00 (0.75, 1.33)	1.36 (1.03, 1.80)	1.33 (0.98, 1.80)	0.05	
HPFS (*n* = 249 cases)							
Median, mg/d	830	970	1075	1197	1410		
Multivariate model 2	1 (Ref)	1.05 (0.69, 1.59)	0.90 (0.57, 1.40)	1.16 (0.75, 1.80)	1.50 (0.95, 2.38)	0.06	
2002 to end							
NHS (*n* = 499 cases)							
Median, mg/d	710	875	1010	1190	1620		
Multivariate model 2	1 (Ref)	0.94 (0.71, 1.24)	1.10 (0.83, 1.45)	0.89 (0.66, 1.20)	0.85 (0.62, 1.17)	0.25	
HPFS (*n* = 124 cases)							
Median, mg/d	870	1050	1200	1380	1790		
Multivariate model 2	1 (Ref)	1.05 (0.60, 1.83)	1.14 (0.66, 1.99)	1.23 (0.70, 2.17)	0.81 (0.43, 1.54)	0.57	
Pooled (NHS + HPFS)							
Beginning to 2002	1 (Ref)	1.13 (0.91, 1.41)	0.96 (0.76, 1.22)	1.30 (1.03, 1.64)	1.36 (1.06, 1.75)	0.008	0.003
2002 to end	1 (Ref)	0.96 (0.75, 1.23)	1.12 (0.87, 1.43)	0.95 (0.73, 1.24)	0.85 (0.64, 1.13)	0.21	
Advanced AMD							
Beginning to 2002							
NHS (*n* = 435 cases)							
Median, mg/d	748	865	957	1060	1242		
Multivariate model 2	1 (Ref)	1.17 (0.84, 1.64)	1.44 (1.03, 2.01)	1.10 (0.77, 1.59)	1.12 (0.76, 1.64)	0.91	
HPFS (*n* = 189 cases)							
Median, mg/d	830	970	1075	1197	1410		
Multivariate model 2	1 (Ref)	1.01 (0.64, 1.60)	1.01 (0.62, 1.63)	0.97 (0.58, 1.59)	1.03 (0.60, 1.79)	0.96	
2002 to end							
NHS (*n* = 546 cases)							
Median, mg/d	710	875	1010	1190	1620		
Multivariate model 2	1 (Ref)	0.78 (0.60, 1.02)	0.93 (0.71, 1.22)	1.07 (0.82, 1.40)	0.82 (0.60, 1.11)	0.54	
HPFS (*n* = 147 cases)							
Median, mg/d	870	1050	1200	1380	1790		
Multivariate model 2	1 (Ref)	0.69 (0.39, 1.21)	1.21 (0.73, 1.99)	0.83 (0.48, 1.44)	0.95 (0.55, 1.65)	0.93	
Pooled (NHS + HPFS)							
Beginning to 2002	1 (Ref)	1.11 (0.85, 1.46)	1.28 (0.97, 1.68)	1.05 (0.78, 1.41)	1.09 (0.79, 1.49)	0.96	0.14
2002 to end	1 (Ref)	0.76 (0.59, 0.96)	0.98 (0.78, 1.24)	1.01 (0.79, 1.29)	0.84 (0.65, 1.10)	0.59	

[1] Multivariate Cox regression model 2 was the same as in Table 2. The number of cases does not add up to the number in Table 2 because participants missing ALA intake in 2002 were excluded from the analysis of the post-2002 era. *P*-trend was calculated by modeling the median value of each category as a continuous variable. *P*-interaction was calculated by creating product terms between the time-varying ALA variable and the binary indicator for time period and testing the significance by a likelihood ratio test. ALA, α-linolenic acid; AMD, age-related macular degeneration; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; Q, quintile; Ref, reference.

before 2002, but neither was after 2002, nor did any other major ALA-bearing foods (**Figure 1**). In a sensitivity analysis, we used 1998 instead of 2002 as the cutoff for the 2 time periods. The association between the intake of ALA and intermediate AMD in each time period did not materially change; the HR comparing extreme quintiles was 1.36 (95% CI: 1.00, 1.83; *P*-trend = 0.02) before 1998 and 1.00 (95% CI: 0.78, 1.26; *P*-trend = 0.91) after 1998, but the *P*-interaction was slightly attenuated (*P* = 0.08).

We further assessed the interplay between DHA and ALA by examining their joint effects. Within each tertile of DHA, the HR increased as the intake of ALA increased from the bottom to the top tertile (**Supplemental Figure 4**). Compared with the intake amount at the top tertile of DHA but the bottom tertile of ALA, the intake at the bottom tertile of DHA and the top tertile of ALA had a 46% (HR: 1.46; 95% CI: 1.17, 1.83) increased risk of intermediate AMD, but the interaction between ALA and DHA was not statistically significant (*P* = 0.16). There was no apparent interaction between ALA and LA (*P*-interaction = 0.69).

We identified 4 *trans* ALA isomers in erythrocytes and mayonnaise: *trans* ALA-A, -B, -C, and -D (**Figure 2**). The amount of total *trans* ALA (mean ± SD: 0.05% ± 0.01%) was lower compared with *cis* ALA (0.13% ± 0.04%) in erythrocytes (**Supplemental Table 3**). Among individual isomers of *trans* ALA, the correlation with *cis* ALA was much stronger for *trans* ALA-B (Pearson *r* = 0.65) than for *trans* ALA-C/-D (*r* = 0.17) (**Supplemental Table 4**). Mayonnaise was the leading significant predictor for both *cis* ALA and *trans* ALA-B (Supplemental Table 3). Mayonnaise was also positively related to the plasma and erythrocyte concentrations of *cis* ALA among previously measured samples from our cohorts (**Supplemental Table 5**). When the 2 representative mayonnaise samples

collected before and after 2000 were further compared, the percentage of *trans* ALA among total ALA (*cis* and *trans*) decreased from 11.8% to 5.0%. Comparatively, the percentage of *trans* 18:2 among total 18:2 (*cis* and *trans*) decreased from 0.88% to 0.69%.

DISCUSSION

In this large, prospective study with 24–28 y of follow-up, a higher intake of ALA was associated with a modest, increased risk of visually significant intermediate AMD but not with advanced AMD. This association was independent of intakes of other correlated fatty acids and could not be attributed to obvious sources of confounding. However, when further stratified by the time period, the ALA intake only in the pre-2002 era seemed harmful. No increase in risk of AMD was seen after that time. In accordance with European studies, we confirmed the presence of a small amount of *trans* ALA isomers in both food and human blood samples in the United States that were collected in the pre-2002 era when partially hydrogenated oils were more prevalent.

The intake of ALA was positively associated only with the risk of intermediate AMD but not with advanced AMD. Correspondingly, intakes of EPA and DHA were also associated only with intermediate AMD but in the opposite direction (41). The reasons for the differential associations have been described (41). Briefly, we speculate that advanced AMD and a subset of intermediate AMD are 2 different disease processes, not just a continuum of progression, given the diverse clinical manifestations and underlying genetic profiles of AMD. Our AMD ascertainment scheme may have accrued mostly those intermediate AMD cases that were slow progressing or not destined to progress further.
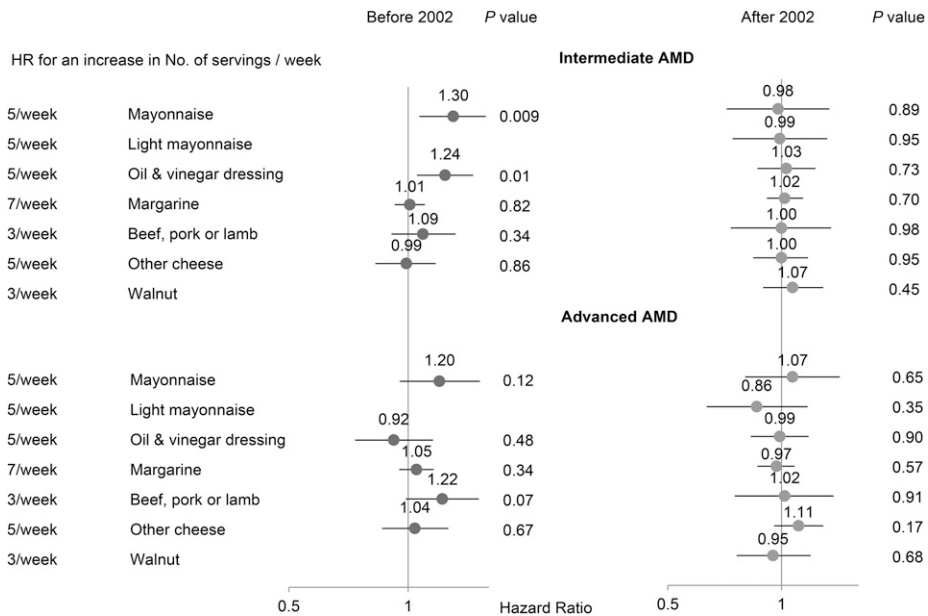

FIGURE 1 The associations of primary α-linolenic acid–containing foods with intermediate and advanced AMD. Each food was modeled as a continuous variable in a multivariate Cox regression model that included age (continuous), race (Caucasian or not), BMI (in kg/m²: <18.5, 18.5–23, 23–25, 25–30, 30–35, or >35), pack-years of smoking (never, 1–9, 10–24, 25–44, 45–64, or ≥65 y), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, or ≥27 h metabolic equivalent tasks/wk), current aspirin use (≥1 tablet/wk or none), history of hypertension, history of hypercholesterolemia, and total calories (quintiles). In the Nurses' Health Study, models were additionally adjusted for postmenopausal status and menopausal hormone use (never, current, and past). AMD, age-related macular degeneration.
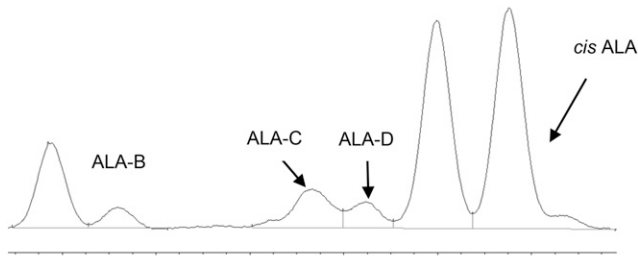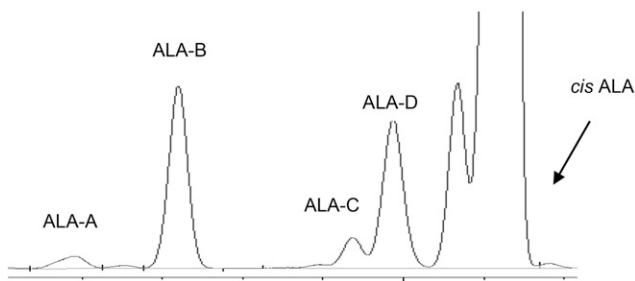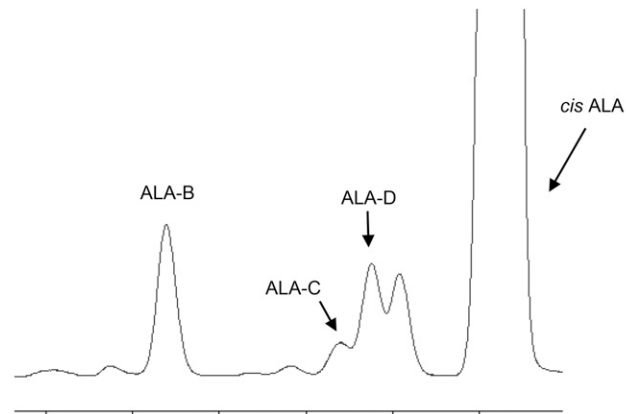
A Erythrocyte (collected between 1989 and 1990)

B Mayonnaise (collected before 2000)

C Mayonnaise (collected after 2000)

FIGURE 2 Partial gas-liquid chromatography of *trans* and *cis* ALA isomers. ALA-A and ALA-B are *trans* ALAs with 2 *trans* bonds; ALA-C and ALA-D are *trans* ALAs with 1 *trans* bond. In all erythrocyte (A) and mayonnaise (B and C) samples, the amount of *trans* ALA-A was extremely low and could not always be identified. ALA, α-linolenic acid.

Among the previous 6 epidemiologic studies (6–8, 11, 14, 16) that had specific intake information for ALA, 2 studies with dietary data collected before 2002 (6, 11) showed a significant positive association between the intake of ALA and intermediate AMD. Measurement error arising from a single assessment of diet and/or a small number of cases may have obscured a modest association for ALA in the other studies. No prior studies (7, 14, 16) have showed any significant association with advanced AMD, and the numbers of cases were very small (<80 cases). In 2 studies (12, 13), a higher intake of vegetable fat was related to an elevated risk of advanced AMD, but information on ALA was not available.

The lack of association between ALA intake and AMD after ∼2002 strongly suggests that ALA is not the primary causal factor. We considered the reduction in *trans* ALA as a possible explanation. In accordance with European literature, we were able to confirm *trans* ALA in foods and blood samples of US participants. Furthermore, one isomer of *trans* ALA shared the

same food, mayonnaise, with the *cis* ALA. Information on the *trans* ALA intake in the United States is virtually nonexistent likely because the intake of its precursor *cis* ALA is already much lower compared with oleic acid and LA, the precursors to major *trans* fatty acids in the US diet. For example, according to NHANES 2009–2010 data, the mean intakes are 1.50 g ALA/d, 26 g oleic acid/d, and 15 g LA/d (42). However, studies suggested that ALA is 12–15 times more easily isomerized into *trans* forms than LA is because of the more unsaturated nature of ALA and ≤40% of ALA can be present as *trans* isomers in edible oils (19, 20). Based on a few US food samples (e.g., mayonnaise, white bread, muffins), we crudely estimated the ratio of *trans* ALA to *cis* ALA was 1:7.5. By assuming that this ratio is generalizable to other *trans* ALA-containing foods, this translates into an intake of 0.2 g *trans* ALA/d on the basis of a mean intake of 1.5 g ALA/d in the US population (17), which is slightly lower but in the same magnitude as the estimated intake among Dutch and Scottish (0.5–0.7 g/d) and French participants (0.2–0.4 g/d) (24). Our study and the European study (24) both suggested a low concentration of erythrocyte *trans* ALA (∼0.05% of total fatty acids) under a habitual diet. However, a study among 50 Indian participants with a high use (86%) of ALA-rich cooking oils, canola and mustard, and a high consumption of fried snacks and sweets (>3 d/wk) showed a markedly high concentration of *trans* ALA in serum, adipose, and cheek epithelium tissues (content ranging from 1.2% to 1.8%) (43).

trans ALA seems to be a plausible explanation for our findings. Long-term feeding rats of a diet high in *trans* ALA severely disturbed visual function and resulted in a significant linear increase in *trans* DHA and a decrease in *cis* DHA (44, 45). In humans, *trans* ALA is absorbed and incorporated into tissue lipids (24). Small amounts of *trans* EPA and DHA are present in human platelets (46). We speculate that the intake of ALA may mostly reflect the intake of *trans* ALA, particularly in the pre-2002 era when partially hydrogenated oils were more prevalent. The positive association between the intake of ALA and intermediate AMD in the pre-2002 era may be mediated by *trans* ALA that was converted to *trans* DHA in vivo, which interfered with the function of natural *cis* DHA in the retina. This hypothesized mechanism is consistent with our finding that a higher intake of ALA was related to a much greater risk of intermediate AMD among postmenopausal women taking exogenous hormones. Exogenous hormones are known to enhance Δ6 desaturase activities and were found to increase the conversion from ALA to DHA by 62% in women (47, 48). One recent study also showed an increased erythrocyte concentration of DHA in women using hormone replacement therapy compared with women who were not (49). On the other hand, *trans* ALA may affect the risk of AMD by its own metabolic effects, rather than through conversion to *trans* DHA. In a European study where 88 healthy men were randomly assigned to receive either a diet high (1.41 g/d) or low (0.06 g/d) in *trans* ALA for 6 wk, the concentration of *trans* ALA in plasma lipids was significantly increased compared with that of the control group (24). This study further showed a statistically significant increase in ratio of HDL to LDL in the high–*trans* ALA group (50).

Our study has several limitations. As with other observational studies, we cannot exclude confounding or chance in our findings. Measurements of some *trans* ALA isomers in the food and blood

may lack precision given that they co-eluted with other fatty acids. The diagnosis of AMD, especially intermediate AMD, may be susceptible to misclassification because the initial detection was done by the participant's eye physician in a clinical examination. We tried to reduce misclassification by requesting and reviewing photos and/or optical coherence tomography when available to confirm clinical AMD lesions. However, we expect the misclassification to be nondifferential with respect to the intake of ALA, which would lead to an underestimation of the true association.

In summary, the higher intake of the shorter-chain ω-3 fatty acid, ALA, was associated with an increased risk of intermediate AMD before 2002; the intake of ALA after 2002 does not appear to increase the risk of AMD. This finding is important because of other health benefits of this essential fatty acid. We found a small amount of *trans* ALA in US food and participant's blood, particularly before 2002 when partially hydrogenated oils were prevalent, and we hypothesize that this may account for the different relations between the intake of ALA and the risk of AMD over time. This possible mechanism deserves further investigation because in many countries partially hydrogenated oils are widely used or the quality of industrial deodorization of vegetable oils is suboptimal.

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