High Concentrations of Plasma n3 Fatty Acids Are Associated with Decreased Risk for Late Age-Related Macular Degeneration

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Abstract

High dietary intakes of n3 (ω3) PUFA and fish have been consistently associated with a decreased risk for age-related macular degeneration (AMD). We assessed the associations of late AMD with plasma n3 PUFA, a nutritional biomarker of n3 PUFA status. The Antioxydants Lipides Essentiels Nutrition et Maladies Occulaires (Alienor) Study is a prospective, population-based study on nutrition and age-related eye diseases performed in 963 residents of Bordeaux (France) aged ≥73 y. Participants had a first eye examination in 2006–2008 and were followed for 31 mo on average. Plasma fatty acids were measured by GC from fasting blood samples collected in 1999–2001. AMD was graded from non-mydriatic color retinal photographs at all examinations and spectral domain optical coherence tomography at follow-up. After adjustment for age, gender, smoking, education, physical activity, plasma HDL-cholesterol, plasma TGs, apoE4, ARMS2 A69S polymorphisms, and follow-up time, high plasma total n3 PUFA was associated with a reduced risk for late AMD [OR = 0.62 for 1-SD increase (95% CI: 0.44–0.88); P = 0.008]. Associations were similar for plasma 18:3n3 [OR = 0.62 (95% CI: 0.43–0.88); P = 0.008] and n3 long-chain PUFA [OR = 0.65 (95% CI: 0.46–0.92); P = 0.01]. This study further supports the potential role of n3 PUFA in the prevention of late AMD and highlights the necessity of randomized clinical trials to determine more accurately the value of n3 PUFA as a means of reducing AMD incidence.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in industrialized countries (1). Its etiology is multifactorial, involving genetic and environmental factors (1). It comprises 2 late forms, both associated with severe visual impairment: neovascular and atrophic AMD. New treatments are restricted to the neovascular form and mainly stabilize vision (1). A preventive strategy would therefore be of great value. In particular, the role of nutritional factors has been put forward since the publication of a large, randomized clinical trial showing a 25% reduction in the incidence of late AMD with supplementation of antioxidants and zinc (2). Beside the role of antioxidants, current research suggests that n3 PUFA may also be of interest for the prevention of late AMD.

The n3 PUFA include a precursor (18:3n3, α-linolenic acid) and 3 long-chain derivatives [20:5n3 (EPA), 22:5n3 (DPA), and 22:6n3 (DHA)]. Synthesis of the long-chain derivatives from 18:3n3 is very limited in humans (3), who must therefore also rely

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3 Supplemental Tables 1–4, Supplemental Figure 1, and Supplemental Methods are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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5 Abbreviations used: Allenor, Antioxydants Lipides Essentiels Nutrition et Maladies Oculaires; AMD, age-related macular degeneration; GC, gas chromatography; 3C, Three-Cities Study; LC-PUFA, long-chain PUFA; SD-OCT, spectral domain optical coherence tomography.
on their dietary supply, mainly by fish and seafood. However, dietary intakes of n3 PUFA remain below the recommendations (1.0% of the total energy intake for 18:3n3 and 250 mg/d each for 22:6n3 and 20:5n3) (4) in the French elderly population (5,6), as in other industrialized countries (7,8). n3 Long-chain (LC)-PUFAs have important structural and protective functions in the retina (9). DHA reaches its highest concentration in the membranes of photoreceptors and is important in photoreceptor differentiation and survival as well as in retinal function (9). The anti-inflammatory properties of n3 LC PUFAs (9,10) are of particular interest in AMD, because inflammation appears to play a pivotal role in this affliction (11). Finally, n3 PUFAs may increase the retinal density of macular pigment, which filters blue light and has local antioxidant and anti-inflammatory activities (12).

In 2008, a meta-analysis (13) of 9 epidemiological studies (14–22) showed a significantly reduced risk for AMD in participants with high consumption of n3 PUFAs and fish. Since then, 9 additional studies have shown similar results (23–31). Because of the multiple difficulties of dietary assessment, nutritional biomarkers represent a more objective alternative for the assessment of nutritional status (32). Biomarkers of n3 PUFA, including plasma and erythrocyte n3 PUFA, have been evaluated in numerous studies, showing good correlation with dietary intake and sensitivity to change in supplementation studies (32). Such biomarkers have been widely used in studies on the associations of n3 PUFA with a variety of health outcomes (cardiovascular diseases, obesity and diabetes, neuro-psychiatric disorders, cancers) (33–37). However, to our knowledge, no published epidemiological study of AMD included measurements of biomarkers of n3 PUFA status.

In the present paper, we report the associations of the risk for AMD with plasma n3 fatty acids in a French elderly population.

Methods

Study aims. The Antioxydants, Lipides Essentiels Nutrition et maladies Oculaires (Alienor) Study is a prospective, population-based study aiming at assessing the associations of age-related eye diseases with nutritional factors (38). As stated in the protocol, the potential association of AMD with n3 PUFA status was one of the main hypotheses tested in this study (38).

This research followed the tenets of the Declaration of Helsinki. Participants gave written consent to participate in the study. The design of the Alienor study was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006.

Study sample. Participants of the Alienor Study were recruited from an ongoing population-based study on the vascular risk factors for dementia, the Three-City (3C) Study (39). The 3C Study included 9294 participants aged ≥65 y from 3 French cities (Bordeaux, Dijon, and Montpellier), among whom 2104 were recruited in Bordeaux. They were initially recruited in 1999–2001 and followed-up about every 2 y since (Supplemental Fig. 1). The Alienor Study consists of eye examinations, which have been offered to all participants of the 3C cohort in Bordeaux since the third follow-up (2006–2008). Among the 1450 participants reexamined between October 2006 and May 2008, 963 (66.4%) participated in the Alienor Study’s baseline eye examination (wave 1). Among participants included in the baseline eye examination, 395 were reexamined between May 2008 and June 2009 in the framework of an ancillary study on macular pigment. Between April 2009 and January 2011, 625 individuals participated to the first follow-up of the Alienor Study (wave 2). Overall, of the 904 survivors, 655 participants (72.4%) had at least one follow-up examination.

Classification of AMD. AMD was classified from nonmydriatic, 45° color retinal photographs performed using a nonmydriatic retinograph (TRC NW6S). Retinal photographs were interpreted according to the international classification (40). Late AMD was defined by the presence of neovascular AMD or geographic atrophy within the grid (3000 μm from the foveola). In addition, at wave 2, a Spectral-Domain Ocular Coherence Tomography (SD-OCT) examination was performed using Heidelberg Spectralis HRA+OCT (Heidelberg Engineering). More details are given in Supplemental Methods.

Measurement of plasma fatty acids. Plasma fatty acid composition was determined from the fasting blood samples collected at the baseline examination of the 3C Study (1999–2001) after storage at −80°C for 36 mo (33,34), which could guarantee the stability of the analytes. No participant declined using oral n3 fatty acid supplementation. Total lipids were extracted from plasma with 5 mL hexane/isopropanol (3:2, v: v). The plasma fatty acid composition was determined from 2 mL of the lipid extract after transformation into isopropyl esters (41). Separation of isopropyl esters was done on a gas chromatograph (Trace, Thermo-electron) using a 25-m Carbowax capillary column (i.d.: 0.32 mm) (41). The peaks were identified by comparison with reference fatty acid esters (Sigma Chemical) and peak areas were measured with an automatic integrator (DP700; Fisons Instruments). The results of each fatty acid were expressed as the percentage of total fatty acids. The ratio of total n6:n3 fatty acids was calculated as: % of total n6/% of total n3.

Covariates. Socio-demographic and lifestyle data were collected through face-to-face, standardized interviews. They included age, gender, educational level, BMI [weight (kg)/height2 (m2)], smoking in pack-years [pack-years = packs (20 cigarettes) smoked per day × years of smoking], and physical activity. For physical activity, a 3-level variable was computed to describe intensity of physical activity, as already published (6). Biological data were collected at the same time as the blood collection for plasma fatty acid measurements (1999–2001) and included plasma HDL-cholesterol, TGs, and genetic polymorphisms: Complement Factor H (CFH) Y402H, Age-Related Maculopathy Susceptibility 2 (ARMS2) A69S, and apolipoprotein E4 (APOE-4). The genetic polymorphisms were determined by the Lille Genopôle. On a candidate gene approach, CFH Y402H and APOE4 polymorphisms were genotyped from DNA extracted from leukocytes by using Taqman Genotyping Assays (Applied Biosystems). Afterwards, the ARMS2 A69S polymorphism was extracted from the genome-wide scan performed in the 3C Study (42). Plasma lipids were measured at the Biochemistry Laboratory of the University Hospital of Dijon, France from fasting blood samples by using routine enzymatic techniques.

Statistical analyses. Of the 963 participants, 84 (8.7%) had no gradable eye examinations, 167 (17.3%) had missing data for plasma fatty acids, and 107 (11.1%) had missing data for confounders, leaving 605 participants (1170 gradable eyes) for the statistical analysis. Thirty-eight individuals with prevalent AMD were excluded from further incidence analyses. Of the 567 at-risk individuals, 26 had died and 140 were not included in the analyses due to refusal, lost to follow-up, or ungradable fundus photographs, resulting in 401 participants included in the incidence analyses. Among the 401 participants, 26 developed incident late AMD, among whom 13 were diagnosed from SD-OCT only.

The duration of follow-up (in months) was calculated from the date of fundus photography at baseline until diagnosis of late AMD or last gradable photograph.

Associations of n3 PUFA with socio-demographic factors, lifestyle, plasma lipids, and genetic polymorphisms were performed using ANOVA and Student’s t test. Correlations between quantitative variables (such as plasma fatty acids or plasma lipids) were assessed by Pearson correlation coefficient. Differences in prevalence according to age and gender were assessed by Pearson chi-square test and Poisson regression for incidence.

With regard to associations of late AMD with plasma n3 PUFA, we first performed statistical analyses combining prevalent and incident cases of late AMD, using similar methodology as that in a report from the Rotterdam Study (43). Associations of late (prevalent and incident) AMD with total and each of the plasma n3 PUFAs were estimated using logistic generalized estimating equations models (44), which allow
taking into account the data from both eyes and their correlation. For each n3 PUFA, ORs adjusted for age, gender, and follow-up time were estimated using late AMD as the dependent variable and the n3 PUFA variable, age, gender, and follow-up duration as the independent variables. After checking the linearity of the effect, n3 PUFA variables were used as continuous variables.

The ORs adjusted for all potential confounders were obtained by adding these confounders as independent variables to the models. Potential confounders retained in the final multivariate models were factors strongly associated with AMD in our cohort [smoking (45) and CFH (45) and ARMS2 (46) polymorphisms] and factors significantly associated with plasma n3 PUFAs (education, physical activity, plasma HDL-cholesterol, TGs, and ApoE4 polymorphism; \( P < 0.05 \)). Quantitative variables (age, plasma HDL-cholesterol, and TGs) were handled as continuous variables in the analyses. Collinearity was not detected between the variables included in the final models, nor was major confounding (variation by >10% of the estimates of the ORs when deleting one confounder from the models).

In sensitivity analyses, we restricted the statistical analysis to late AMD cases that had developed during follow-up (incident late AMD). In this model, participants’ eyes were considered at risk for incident late AMD if they had at least one follow-up examination and were free of late AMD at baseline. We used similar models as for the main statistical analysis. We also analyzed potential gene-environment interactions (n3 PUFA and genetic polymorphisms). Interactions were independently introduced in the fully adjusted model and retained if they were significant \( (P < 0.05) \). Finally, we studied separately late atrophic and neovascular AMD. In these analyses, participants without late AMD were the reference.

For all analyses, differences were considered significant at \( P < 0.05 \). All statistical analyses were performed using SAS version 9.2 (SAS Institute; procedure GENMOD for the generalized estimating equations analysis).

**Results**

Participants with and without missing data were very similar for all variables of interest in this study, except the proportion of participants with “no answer” for physical activity was significantly lower in those with complete data \( (P < 0.0001) \) ([Supplemental Tables 1 and 2](#SupplementalTables1and2)).

Among the 605 participants retained in the statistical analysis, the mean age was 80.0 ± 4.4 y at the baseline eye examination. The mean delay between blood sampling used for plasma n3 PUFA measurements and the baseline eye examination was 6.9 ± 0.6 y. As shown in [Supplemental Table 3](#SupplementalTable3), AMD prevalence increased with age (10.3% in participants older than 80 y vs. 3.0% in those younger than 80 y; \( P = 0.0003 \)) but did not differ between men and women (5.3 vs. 6.9%; \( P = 0.42 \)). In the 401 follow-up-participants, the mean ocular follow-up time was 30.7 ± 6.2 mo, resulting in 1011.3 person-years of follow-up. AMD incidence was 2.6/100 person-years, increasing from 1.8/100 person-years in participants younger than 80 y to 3.8/100 person-years in those older than 80 y \( (P = 0.05) \).

As shown in [Table 1](#Table1), women had a higher plasma concentration of 20:5n3 than men \( (P = 0.02) \). Higher education was associated with higher total n3 PUFAs \( (P = 0.01) \), n3 LC-PUFAs \( (P = 0.02) \), and 22:5n3 \( (P = 0.02) \). Higher physical activity was associated with higher plasma total n3 PUFAs \( (P = 0.002) \), n3 LC-PUFAs \( (P = 0.003) \), 20:5n3 \( (P = 0.01) \), and 22:5n3 \( (P = 0.0008) \). Participants bearing the CC genotype of the CFH Y402H polymorphism \( (P = 0.03) \), lower plasma total n3 LC-PUFAs \( (P = 0.005) \), 20:5n3 \( (P = 0.03) \), and 22:6n3 \( (P = 0.003) \). No association was found with age, smoking status, ARMS2 A69S, and ApoE4 polymorphisms.

Among plasma lipids, TGs were negatively correlated with total n3 PUFAs \( (r = -0.16; \; P = 0.0001) \), n3 LC-PUFAs \( (r = -0.17; \; P < 0.0001) \), 20:5n3 \( (r = -0.13; \; P = 0.001) \), and 22:6n3 \( (r = -0.16; \; P < 0.0001) \) ([Supplemental Table 4](#SupplementalTable4)). HDL-cholesterol was positively associated with 20:5n3 \( (r = 0.09; \; P = 0.03) \), whereas total cholesterol was not significantly correlated with any plasma n3 PUFA. No significant correlation was found between n3 PUFAs and BMI.

As shown in [Table 2](#Table2), after adjustment for age, gender, and follow-up time, all plasma n3 PUFAs except 20:5n3 and 22:5n3 were significantly associated with a lower risk for late AMD \( (OR = 0.64, \; P = 0.002) \). After adjustment for all potential confounders, the ORs remained virtually unchanged \( (OR = 0.65 \; \text{whatever the plasma fatty acid} \) and significantly associated with late AMD, except for 20:5n3 and 22:5n3. No interactions of plasma fatty acids with CFH, ARMS2, or ApoE4 polymorphisms were detected. The association between late AMD and the ratio of total n6:n3 fatty acids was not significant after multivariate adjustment \( (OR = 1.07; \; P = 0.29) \) (data not shown).

To limit potential reverse causality, we restricted the statistical analysis to incident cases of late AMD \( (n = 38 \; \text{eyes of 782 at-risk eyes}) \). High plasma total n3 PUFAs were associated with a reduced risk for late incident AMD \( (OR = 0.50 \; (95\% \; \text{CI:} \; 0.29–0.85); \; P = 0.01) \) and were not associated with late neovascular AMD \( (OR = 0.64 \; (95\% \; \text{CI:} \; 0.41–1.01); \; P = 0.06) \), although the association was in the same direction. Associations with 18:3n3 were significant for both atrophic \( (P = 0.04) \) and neovascular AMD \( (P = 0.04) \), whereas associations with n3 LC-PUFAs and 20:5n3 were significant only for atrophic AMD \( (P = 0.02 \) and \( P = 0.007 \), respectively).

**Discussion**

In accordance with our hypothesis, high plasma total n3 PUFA was significantly associated with a 38% reduction of the odds of late AMD.

The results of the present study, based on plasma measurements, are consistent with previous studies relying on dietary assessment. Indeed, in 2008, a meta-analysis estimated that the risk for late AMD (neovascular and/or atrophic) was reduced by 38% in participants with high dietary intakes of n3 LC-PUFAs \( (13) \). Our result for plasma total n3 PUFAs is very consistent with these findings. More recent prospective \( (26,27,30) \) and cross-sectional \( (24,25,29,31) \) dietary studies were also remarkably consistent with our results, although 2 studies found an increased risk for AMD in participants with high dietary n3 PUFAs \( (23,28) \).

Few studies have differentiated atrophic from neovascular AMD. A significantly reduced risk of neovascular AMD in individuals with high consumption of fish or n3 PUFAs was found in 4 studies \( (19,24,27,29) \) but not in 2 others \( (17,25) \). In the Age-Related Eye Diseases Study, no association of atrophic AMD with n3 PUFAs was found in the initial case-control study \( (19) \), but a significant association with incident atrophic AMD was evidenced at both the 6- and 12-y follow-ups \( (25,27) \). In the present study, plasma total n3 PUFAs were associated with a reduced risk for atrophic and neovascular AMD \( (OR = 0.50, \; P = 0.01 \) and \( OR = 0.64, \; P = 0.06, \) respectively) but achieved significance only for atrophic AMD.

Although there is a strong rationale for the protective effect of n3 PUFAs in AMD, the assessment of the n3 PUFA status in humans is difficult. Dietary assessment methods rely on the
TABLE 1 Variations of plasma n3 PUFAs according to socio-demographic factors, lifestyle, and AMD-related genetic polymorphisms (Alienor Study, baseline 1999–2000, Bordeaux, France)¹

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n3</th>
<th>n3</th>
<th>LC-PUFA²</th>
<th>20:5n3</th>
<th>22:5n3</th>
<th>22:6n3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>PUFA</td>
<td>18:3n3</td>
<td>18:2n6</td>
<td>18:4n3</td>
<td>18:5n3</td>
</tr>
<tr>
<td>% of total plasma fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Socio-demographic factors

Age, y

| <80  | 332 | 4.5 ± 1.2 | 0.40 ± 0.14 | 4.1 ± 1.2 | 1.1 ± 0.6 | 0.48 ± 0.22 | 2.5 ± 0.7 |
| ≥80  | 273 | 4.4 ± 1.3 | 0.43 ± 0.22 | 4.0 ± 1.3 | 1.1 ± 0.6 | 0.46 ± 0.12 | 2.5 ± 0.8 |
| P value³  | 0.74 | 0.09 | 0.56 | 0.94 | 0.16 | 0.47 |

Gender

| Men  | 228 | 4.4 ± 1.2 | 0.41 ± 0.14 | 3.9 ± 1.2 | 1.0 ± 0.5 | 0.49 ± 0.26 | 2.5 ± 0.7 |
| Women | 377 | 4.5 ± 1.4 | 0.42 ± 0.20 | 4.1 ± 1.3 | 1.1 ± 0.7 | 0.47 ± 0.12 | 2.5 ± 0.8 |
| P value³  | 0.13 | 0.58 | 0.15 | 0.02 | 0.39 | 0.48 |

Education

| None or primary school | 176 | 4.2 ± 1.2 | 0.40 ± 0.23 | 3.8 ± 1.2 | 1.0 ± 0.6 | 0.44 ± 0.11 | 2.4 ± 0.7 |
| Secondary school      | 171 | 4.5 ± 1.4 | 0.40 ± 0.14 | 4.1 ± 1.4 | 1.1 ± 0.7 | 0.50 ± 0.29 | 2.5 ± 0.8 |
| High school           | 61  | 4.6 ± 1.3 | 0.43 ± 0.14 | 4.2 ± 1.3 | 1.1 ± 0.6 | 0.49 ± 0.12 | 2.6 ± 0.8 |
| University            | 197 | 4.6 ± 1.2 | 0.43 ± 0.16 | 4.2 ± 1.2 | 1.1 ± 0.6 | 0.48 ± 0.11 | 2.6 ± 0.7 |
| P value³  | 0.01 | 0.19 | 0.02 | 0.10 | 0.02 | 0.08 |

Smoking status

| Nonsmoker | 404 | 4.5 ± 1.3 | 0.41 ± 0.20 | 4.1 ± 1.3 | 1.1 ± 0.6 | 0.47 ± 0.11 | 2.5 ± 0.8 |
| <20 pack-years | 100 | 4.3 ± 1.2 | 0.42 ± 0.14 | 3.9 ± 1.2 | 1.0 ± 0.5 | 0.46 ± 0.12 | 2.5 ± 0.7 |
| ≥20 pack-years  | 101 | 4.4 ± 1.2 | 0.40 ± 0.13 | 4.0 ± 1.2 | 1.0 ± 0.6 | 0.51 ± 0.37 | 2.5 ± 0.8 |
| P value³  | 0.54 | 0.66 | 0.50 | 0.22 | 0.07 | 0.71 |

Physical activity

| Low    | 332 | 4.4 ± 1.2 | 0.40 ± 0.14 | 4.0 ± 1.2 | 1.0 ± 0.6 | 0.47 ± 0.11 | 2.5 ± 0.7 |
| Medium | 124 | 4.8 ± 1.5 | 0.41 ± 0.15 | 4.4 ± 1.5 | 1.2 ± 0.8 | 0.53 ± 0.33 | 2.6 ± 0.8 |
| High   | 63  | 4.6 ± 1.3 | 0.47 ± 0.36 | 4.1 ± 1.3 | 1.1 ± 0.7 | 0.47 ± 0.12 | 2.5 ± 0.7 |
| No answer | 86  | 4.2 ± 1.0 | 0.42 ± 0.16 | 3.8 ± 1.0 | 1.0 ± 0.4 | 0.43 ± 0.10 | 2.4 ± 0.7 |
| P value³ | 0.002 | 0.06 | 0.003 | 0.01 | 0.0008 | 0.07 |

Genetic polymorphisms

CFH Y402H

| TT     | 272 | 4.6 ± 1.3 | 0.41 ± 0.21 | 4.2 ± 1.2 | 1.1 ± 0.6 | 0.48 ± 0.12 | 2.6 ± 0.8 |
| TC     | 261 | 4.5 ± 1.3 | 0.42 ± 0.15 | 4.0 ± 1.3 | 1.1 ± 0.7 | 0.47 ± 0.11 | 2.5 ± 0.8 |
| CC     | 72  | 4.0 ± 1.2 | 0.39 ± 0.15 | 3.6 ± 1.1 | 0.9 ± 0.5 | 0.50 ± 0.43 | 2.2 ± 0.8 |
| P value³  | 0.004 | 0.47 | 0.005 | 0.03 | 0.44 | 0.003 |

ARMS2 A85S

| GG     | 383 | 4.5 ± 1.3 | 0.42 ± 0.19 | 4.0 ± 1.2 | 1.1 ± 0.6 | 0.48 ± 0.21 | 2.5 ± 0.7 |
| GT     | 197 | 4.4 ± 1.3 | 0.40 ± 0.16 | 4.0 ± 1.3 | 1.1 ± 0.6 | 0.46 ± 0.11 | 2.5 ± 0.8 |
| TT     | 25  | 4.7 ± 1.4 | 0.39 ± 0.14 | 4.3 ± 1.3 | 1.1 ± 0.5 | 0.47 ± 0.11 | 2.7 ± 0.9 |
| P value³  | 0.53 | 0.55 | 0.49 | 0.86 | 0.47 | 0.35 |

ApoE4

| No allele E4 | 493 | 4.4 ± 1.3 | 0.41 ± 0.19 | 4.0 ± 1.3 | 1.1 ± 0.6 | 0.48 ± 0.20 | 2.5 ± 0.8 |
| At least 1 allele E4 | 112 | 4.5 ± 1.2 | 0.42 ± 0.15 | 4.1 ± 1.2 | 1.0 ± 0.6 | 0.47 ± 0.11 | 2.6 ± 0.8 |
| P value³  | 0.43 | 0.44 | 0.48 | 0.38 | 0.73 | 0.05 |

¹ Values are mean ± SD. AMD, age-related macular degeneration; LC-PUFA, long-chain PUFA.
² n3 LC-PUFA = 20:5n3 + 22:5n3 + 22:6n3.
³ P value for Student’s t test or ANOVA among categories of variable.

A potential limitation to our results is the questionable representativeness of the sample. First, two-thirds of the participants in the 3C Study did not differ from those who did not participate for most variables of interest in our study, in particular plasma n3 PUFAs, 20:5n3, and 22:6n3 (38). Second, about one-third of the sample could not be included in the present statistical analysis, mainly because of missing data on AMD status, plasma n3 PUFAs, or genetic polymorphisms. Again, participants with complete data were very similar to those with missing data (Supplemental Tables 1 and 2). Moreover, the prevalence of AMD in our study was
Plasma n3 fatty acids and risk for AMD

TABLE 2  Associations of plasma n3 PUFA with the risk for late AMD (Alienor Study 2006–2011, Bordeaux, France)1

<table>
<thead>
<tr>
<th>Plasma n3 PUFA</th>
<th>Without late AMD2</th>
<th>With late AMD3</th>
<th>OR (95% CI)4,5</th>
<th>P value</th>
<th>Fully adjusted OR (95% CI)5,6 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n3 PUFA</td>
<td>4.0 ± 1.2</td>
<td>4.0 ± 1.2</td>
<td>0.64 (0.45, 0.90)</td>
<td>0.01</td>
<td>0.62 (0.44, 0.88)</td>
</tr>
<tr>
<td>18:3n</td>
<td>0.42 ± 0.19</td>
<td>0.38 ± 0.11</td>
<td>0.65 (0.47, 0.89)</td>
<td>0.007</td>
<td>0.62 (0.43, 0.88)</td>
</tr>
<tr>
<td>n3 LC-PUFA6</td>
<td>4.1 ± 1.3</td>
<td>3.7 ± 1.1</td>
<td>0.66 (0.47, 0.94)</td>
<td>0.02</td>
<td>0.65 (0.46, 0.92)</td>
</tr>
<tr>
<td>20:5n</td>
<td>1.1 ± 0.6</td>
<td>0.95 ± 0.49</td>
<td>0.71 (0.49, 1.03)</td>
<td>0.07</td>
<td>0.66 (0.44, 1.03)</td>
</tr>
<tr>
<td>22:5n</td>
<td>0.48 ± 1.19</td>
<td>0.44 ± 0.11</td>
<td>0.67 (0.45, 1.00)</td>
<td>0.05</td>
<td>0.65 (0.41, 1.02)</td>
</tr>
<tr>
<td>22:6n</td>
<td>2.5 ± 0.8</td>
<td>2.3 ± 0.8</td>
<td>0.70 (0.49, 0.99)</td>
<td>0.046</td>
<td>0.73 (0.53, 0.99)</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD. AMD, age-related macular degeneration; LC-PUFA, long-chain PUFA.
2 n = 1074 eyes/541 participants.
3 n = 96 eyes/64 participants.
4 OR for 1-SD increase in plasma n3 PUFA, estimated using generalized estimating equations logistic regression adjusted for age, gender, and follow-up time.
5 Fully adjusted OR for 1-SD increase in plasma n3 PUFA, estimated using generalized estimating equations logistic regression adjusted for age, gender, smoking, educational level, physical activity, plasma HDL-cholesterol, plasma TG, CFH Y402H, ApoE4, and ARMS2 A69S polymorphisms, and follow-up time.
6 n3 LC-PUFA = 20:5n3+22:5n3+22:6n3.

Another limitation may be that our results rely on a single measurement of plasma fatty acids that was performed several years before the first eye examination. This represents only a crude estimate of long-term fatty acid status, in particular because of day-to-day variability in dietary habits and/or change of diet over time. However, this measurement error is likely to be independent of AMD status and most probably induces a bias toward null in the estimation of associations of AMD with plasma fatty acids. Another limitation may be that our results rely on a single measurement of plasma fatty acids that was performed several years before the first eye examination. This represents only a crude estimate of long-term fatty acid status, in particular because of day-to-day variability in dietary habits and/or change of diet over time. However, this measurement error is likely to be independent of AMD status and most probably induces a bias toward null in the estimation of associations of AMD with plasma fatty acids. Measurement of n3 PUFA in RBC membranes may represent a biomarker for longer term status, with a half-life of 120 d (47). However, both plasma and RBC membranes n3 PUFAs appear as robust biomarkers of n3 PUFA status (32,33).

The relatively small number of late AMD cases (n = 96 eyes in 64 participants) limited the statistical power for the analysis of subcategories (atrophic/neovascular). In particular, although the association of total n3 PUFAs with neovascular AMD was not significant (P = 0.06), the estimated OR was in the expected range (OR = 0.64). Statistical power was also very low for detecting interactions.

In observational studies, confounding is always a concern. We therefore adjusted for potential confounders, including socio-demographic status, smoking, physical activity, plasma lipids, and AMD-related genetic polymorphisms. However, we cannot totally exclude residual confounding. Furthermore, confounding is of particular concern in nutritional epidemiology, because nutrients are inter-correlated, in particular because of common food sources. For instance, fish is a major source of n3 LC-PUFAs but also of vitamin D, which was associated with the risk for AMD in 2 studies (54,55). Associations of AMD with plasma n3 PUFAs may therefore be a marker for a wider set of nutrients.

Surprisingly, plasma n3 PUFAs were also significantly associated with the CFH Y402H polymorphism, with lower amounts in the CC polymorphism. Low plasma n3 PUFAs may therefore contribute to the increased risk for AMD in individuals bearing this genotype. Moreover, in the Blue Mountains Eye Study, fish consumption was associated with a reduced risk for AMD only in those participants bearing the CC genotypes (56). We could not confirm this interaction between CFH Y402H and plasma n3 PUFA in the present study. Further data on the inter-relations among CFH, n3 PUFA, and AMD are needed to better characterize them and understand the underlying mechanisms.

Reverse causation (modification of n3 PUFA status due to disease) is also a concern in epidemiology. However, in the present prospective study, plasma n3 PUFA measurements preceded by 6.9 y the first eye examination. Moreover, when restricting statistical analysis to incident cases, which were free of late AMD at blood sampling, the associations of plasma n3 PUFA with AMD were similar to that observed in the same age group in other studies performed in Europe (48,49) and other industrialized countries (50). By contrast, incidence rates were higher than in similar studies (51,52). However, these studies were based on retinal photographs only, whereas the present study also included an SD-OCT examination, which has been shown to be much more sensitive for the diagnosis of late AMD (53). In the present study, one-half of the incident cases (13 of 26) were detected only by SD-OCT. Finally, although the distribution of plasma fatty acids may be different from the general population, this is unlikely to bias the estimation of the associations of AMD with plasma fatty acids.

<table>
<thead>
<tr>
<th>Plasma n3 PUFA</th>
<th>Late atrophic AMD2</th>
<th>Late neovascular AMD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n3 PUFA</td>
<td>0.50 (0.29, 0.85)</td>
<td>0.64 (0.41, 1.01)</td>
</tr>
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<td>18:3n</td>
<td>0.59 (0.36, 0.97)</td>
<td>0.63 (0.40, 0.97)</td>
</tr>
<tr>
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<td>0.67 (0.43, 1.05)</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>22:6n</td>
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<td>0.68 (0.46, 1.02)</td>
</tr>
</tbody>
</table>

1 AMD, age-related macular degeneration; LC-PUFA, long-chain PUFA.
2 OR were calculated for total (n = 1119 eyes, 565 participants)/participants with late atrophic AMD (n = 45 eyes, 30 participants).
3 OR were calculated for total (n = 1126 eyes, 568 participants)/participants with late neovascular AMD (n = 51 eyes, 34 participants).
4 OR for 1-SD increase in plasma n3 PUFA, estimated using generalized estimating equations logistic regression adjusted for age, gender, smoking, educational level, physical activity, plasma HDL-cholesterol, plasma TGs, CFH Y402H, ApoE4 and ARMS2 A69S polymorphisms, and follow-up time.
5 n3 LC-PUFA = 20:5n3+22:5n3+22:6n3.

TABLE 3  Associations of plasma n3 PUFA with the risk of late atrophic and neovascular AMD (Alienor Study, 2006–2011, Bordeaux, France)1

Plasma n3 PUFA | Late atrophic AMD2 | Late neovascular AMD3 |
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PUFA with late AMD remained significant. However, we cannot exclude that some participants were aware of or affected by early AMD lesions at the moment of blood sampling.

In conclusion, this study gives further support to the potential role of n3 PUFAs in the prevention of AMD. Epidemiological studies are strikingly consistent in this field. However, the causal nature of these relationships will only be determined by clinical trials. In particular, the effect of supplementation with n3 PUFAs on incident late AMD is currently being tested in the Age-Related Eye Diseases Study 2, an ongoing, randomized, clinical trial conducted in >4000 participants (57). Further research is also needed to better understand the differences between the precursor and its long-chain derivatives in their relationships to AMD and the potential interactions with genetic risk factors of AMD.

Acknowledgments


Literature Cited


Plasma n3 fatty acids and risk for AMD


