Cognitive decline and fatty acid composition of erythrocyte membranes—The EVA Study¹–⁴

Barbara Heude, Pierre Ducimetière, and Claudine Berr

ABSTRACT

Background: Dietary factors might modify cognitive decline that results from aging. Fatty acids, which are limiting factors in brain development, are prime candidates.

Objective: We studied the relation between erythrocyte membrane fatty acid composition and cognitive decline in free-living volunteers.

Design: In 1995, erythrocyte membrane fatty acid composition was measured in 246 men and women (aged 63–74 y) from the Etude du Vieillissement Artériel (EVA) cohort. During a 4-y follow-up, cognitive abilities were assessed longitudinally with the Mini-Mental State Examination. Moderate cognitive decline was defined as a ≥2-point decrease over the 4 y. The predictive value of fatty acid proportions on cognitive decline was assessed with a multivariate logistic model that included age, sex, education level, and initial Mini-Mental State Examination score as covariates.

Results: Higher proportions of both stearic acid (saturated, 18:0) and total n–6 polyunsaturated fatty acids were associated with greater risk of cognitive decline; the odds ratios were 1.91 (95% CI: 1.16, 3.15) and 1.59 (95% CI: 1.04, 2.44), respectively, for 1-SD differences in fatty acid proportions. Conversely, a higher proportion of total n–3 fatty acids was associated with a lower risk of cognitive decline; the odds ratio was 0.59 (95% CI: 0.38, 0.93).

Conclusions: The inverse association between cognitive decline and the ratio of n–3 to n–6 fatty acids in erythrocyte membranes agrees with results obtained in some studies that assessed fatty acid intake by using dietary questionnaires. These results require confirmation but provide new rationale for studying how these modifiable risk factors might be implicated in the cognitive aging process. Am J Clin Nutr 2003;77:803–8.

KEY WORDS Aging, elderly, cognitive decline, cognitive impairment, fatty acids, erythrocyte membrane, longitudinal study, epidemiology, dementia, atherosclerosis, EVA Study, Mini-Mental State Examination

INTRODUCTION

Some studies have shown that cognitive deterioration in the elderly is associated with deficiencies of micronutrients and macronutrients (1–6). Various theories have led to the evaluation of nutritional factors as potential modifiers of the risk of cognitive impairment in the elderly.

Some vascular risk factors and cardiovascular diseases have been related to vascular dementia and Alzheimer disease (AD) (7), and less specifically to cognitive decline (8–10). Therefore, fatty acid intake might affect the development of cognitive impairment by way of the influence of fatty acids on atherosclerosis and thrombosis (11, 12).

Fatty acids, especially those in ester phospholipids, control the structure and function of biological membranes, including membranes in nervous tissues (13, 14). Thus, fatty acids strongly influence membrane fluidity. The central nervous system has the second highest concentration of lipids after adipose tissue. These brain lipids contain very high amounts of long-chain polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (AA, 20:4n–6) and docosahexaenoic acid (DHA, 22:6n–3). These 2 PUFAs, which are the major constituents of neural cell membrane phospholipids, belong to the n–6 and n–3 PUFA families and can only be obtained from the diet.

Intakes of linoleic acid (LA, 18:2n–6) and α-linolenic acid (ALA, 18:3n–3), both of which are especially important during periods of brain growth and development, have received considerable attention (15, 16). Lack of sufficient quantities of these fatty acids is a limiting factor in brain development. Much less is known about the role in the aging process of these essential fatty acids and their metabolites or other fatty acids, including saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). We examined the association between particular fatty acids in erythrocyte membranes and cognitive decline in a longitudinal study of elderly men and women, the Etude du Vieillissement Artériel (EVA) Study.

SUBJECTS AND METHODS

Study population

The study population consists of 1389 volunteers born from 1922 to 1932. These subjects were recruited during the years 1991–1993 from the electoral rolls of the city of Nantes in western France. The study protocol was approved by the Comité

¹ From the Institut National de la Santé et de la Recherche Médicale (INSERM) U258-IFR69, Hôpital Paul Brousse, Villejuif, France (BH and PD), and INSERM U360, Hôpital de la Salpêtrière, Paris (CB).
² The EVA Study was carried out under an agreement between INSERM and Merck, Sharp and Dhome, Chibret Laboratories (West Point, PA).
³ Supported by EISAI Laboratory (Paris).
⁴ Address reprint requests to B Heude, Unite INSERM 258, Hôpital Paul Brousse, 16, av Paul Vaillant Couturier, 94807 Villejuif Cedex France. E-mail: heude@vjf.insERM.fr.

Accepted February 1, 2002. Accepted for publication September 3, 2002.
Examinations

The EVA Study had 5 examination waves between 1991 and 2000. Subjects underwent complete cognitive testing and clinical examinations as described in previous publications (17, 18). In wave 3 of the study in 1995–1996, 1188 subjects (92% of the initial sample) were examined and blood samples were collected. Because of financial limitations, only 342 blood samples, selected in chronological order, were analyzed for erythrocyte fatty acids.

For the longitudinal analyses of cognitive decline, information on cognitive function collected in wave 3 (1995–96) and wave 5 (1999–2000) was used. Among the 342 subjects with biological determinations, complete cognitive information was available for 246 subjects. Baseline interviews and examinations were conducted at the study center. Data on demographic background, occupation, medical history, use of medications, and personal habits were obtained by using a standardized questionnaire during a face-to-face interview.

Cognitive examination

Global cognitive function was tested with the French version of the 30-point Mini-Mental State Examination (MMSE). The MMSE lasts 10 min and includes questions on orientation to time and place, attention and calculation, recall, language, and visual construction. We defined moderate cognitive decline as a decrease in ≥2 points on the MMSE over a 4-y period; this corresponds to the eleventh percentile of change. It represents a weak decline, but the sample size did not allow for the use of a more clinically relevant cutoff indicative of severe cognitive decline.

Fatty acid determination

The fatty acid composition of erythrocyte membranes was determined during the 2 y after the blood samples were obtained; samples were stored at −80 °C. The total lipids were extracted from the erythrocytes by using the procedure of Folch et al (19), and the total phospholipids were isolated with thin-layer chromatography and transesterified to the methyl esters. Fatty acid methyl esters were prepared by using the method of Morrison and Smith (20) and were analyzed with gas chromatography. The researchers who performed the analyses were blinded to the subjects’ characteristics, and the values were read twice; we used the mean of the 2 values for subsequent analyses. For the 41 fatty acids present in the erythrocyte membranes, the percentages ranged from <0.03% (for 20:1n−7, 18:3n−3, and 18:2tc) to >19% (for 16:0 and 18:0) of the total fatty acids.

Other measurements

The potential confounding variables that we considered were sex (dichotomous), age (continuous), and education (2 categories: <6 y or >6 y of school). Subjects were classified as smokers (current or past) or nonsmokers (never) at the time of blood sampling. Alcohol consumption was categorized as never or <2 drinks/d versus >2 drinks/d (1 drink was defined as 13 mL alcohol).

History of coronary artery disease was defined as self-reported history of myocardial infarction or angina pectoris. Measurements of intima-media thickness (IMT) obtained with echocarotid imaging were available, and the presence of carotid atherosclerotic plaques was also documented in these individuals (21). High blood pressure was defined as a systolic blood pressure ≥160 mm Hg or a diastolic blood pressure ≥95 mm Hg. Subjects with high blood pressure by this definition or those taking antihypertensive medication (β-blockers, calcium antagonists, angiotensin-converting enzyme inhibitors, or diuretics) were considered hypertensive.

BMI was computed as weight (kg)/height2 (m). Genomic DNA was extracted from white blood cells and apolipoprotein E polymorphism was determined as previously described (22).

RESULTS

Baseline characteristics of the sample

The change in the MMSE score over a 4-y period was studied in 246 subjects who participated in both examinations and had complete data, among the 342 subjects who were eligible. Seven deaths were reported, and 89 subjects refused to participate in the follow-up evaluation. Participants did not differ from nonparticipants in terms of erythrocyte membrane fatty acid composition, except that the mean LA content was higher in participants (P = 0.05). The baseline MMSE score was slightly but not significantly lower in nonparticipants than in participants (27.2 and 27.6, respectively; P = 0.11).

Baseline characteristics of the 246 subjects did not differ significantly from those of the 1188 subjects who had an examination at wave 3. These 2 groups did not differ significantly in terms of mean

Statistical analyses

In the fatty acid analyses, we identified a large number of fatty acids, so we restricted the analyses to a limited number of individual fatty acids and chemical families. We considered the 4 main families: SFAs, MUFAs, n-3 PUFAs, and n-6 PUFAs. For SFAs, we studied the 2 major components, stearic acid (18:0) and palmitic acid (16:0). For MUFAs, we studied oleic acid (18:1n−9). For each of the 2 types of PUFAs (n−3 and n−6), the 2 most abundant fatty acids were considered: DHA and eicosapentaenoic acid (EPA, 20:5n−3) for n−3 and AA and LA for n−6. Because they are expressed as percentages, fatty acid amounts are often highly correlated and thus their simultaneous introduction in statistical models needs to be done with caution. The equilibrium between n−3 and n−6 PUFAs is often studied extensively by using the ratio of n−3 to n−6 fatty acids. We also examined the ratio of DHA to AA, which is more interesting at a biological level because these 2 fatty acids result from concurrent metabolic pathways involving Δ6-desaturase (EC 1.14.99.25).

First, we compared unadjusted mean fatty acid amounts in relation to moderate cognitive decline. Second, we used logistic regression with cognitive decline as the dependent variable and each fatty acid as the independent variable, controlling for age, sex, education, and baseline MMSE score (a continuous variable). The results are expressed as odds ratios (ORs) for 1-SD differences in fatty acid amounts. Additional terms included in the multivariate models were those that proved to be or were shown in the literature to be potential confounders: alcohol and tobacco use, history of coronary artery disease, hypertension, presence of carotid plaques and carotid IMT measurements, BMI, and apolipoprotein E4 status. We also determined correlations, using Pearson’s product-moment correlation coefficients, between the change in MMSE score and fatty acids. All statistical analyses were performed with SAS version 6.12 (SAS Institute Inc, Cary, NC).
Cognitive decline and fatty acid composition

There were significant differences in fatty acid composition between the decline and no-decline groups (Table 3). For SFAs, we measured a higher stearic acid content in the decline group than in the no-decline group (P = 0.009), but there was no significant association with palmitic acid content. There were no associations with total MUFA or oleic acid contents. Total n–3 PUFAs tended to be lower in the decline group (P = 0.055), and this difference was significant for the 2 major fatty acids, DHA and EPA (P = 0.042 and P = 0.045, respectively). Conversely, the mean total n–6 PUFA content was significantly higher in the decline group (P = 0.037). The ratios of n–3 to n–6 fatty acids and DHA to AA were significantly lower in the decline group (P = 0.043 and P = 0.047, respectively).

After adjusting the ORs for age, sex, education level and initial MMSE score, stearic acid content was consistently and significantly associated with an increased risk of cognitive decline (OR: 1.91; 95% CI: 1.16, 3.15 for a 1-SD difference) (Table 3). A similar significant association was observed for total n–6 PUFAs (OR: 1.59; 95% CI: 1.04, 2.44).

Content of n–3 PUFAs was inversely associated with cognitive decline (OR: 0.59; 95% CI: 0.38, 0.93 for a 1-SD difference). After adjustment, this association was significant for DHA but not for EPA. Inverse associations with cognitive decline were also found for the ratios of n–3 to n–6 fatty acids and DHA to AA, which might reflect equilibrium between PUFA contents (for 1-SD differences, OR: 0.55; 95% CI: 0.33, 0.91 for n–3:n–6 and OR: 0.57; 95% CI: 0.35, 0.92 for DHA:AA).

Stearic acid content was not significantly correlated with n–3 PUFA content (r = −0.10, P = 0.10), n–6 PUFA content (r = 0.12, P = 0.06), or n–3:n–6 (r = −0.12, P = 0.06). We performed further adjustments for other fatty acids and simultaneously introduced stearic acid and 1 of these 3 PUFA indicators into the previous multivariate models. The results remained significant for stearic acid when each PUFA indicator was introduced into the logistic model. Risk of cognitive decline remained significant for n–6 PUFA content (OR: 1.59; 95% CI: 1.03, 2.43) and n–3:n–6 (OR: 0.60; 95% CI: 0.37, 0.98), and a similar trend was observed for n–3 PUFA content (OR: 0.66; 95% CI: 0.42, 1.03).

We also performed analyses without any cutoff value for change in MMSE score (data not shown). We studied the correlation between change in MMSE score and fatty acid contents

---

**Table 1**
Baseline characteristics of participants who had no cognitive decline compared with those who had a moderate cognitive decline in their Mini-Mental State Examination (MMSE) scores over a 4-y period

<table>
<thead>
<tr>
<th></th>
<th>No decline (n = 219)</th>
<th>Decline (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>40.2</td>
<td>56.6</td>
</tr>
<tr>
<td>Age (y)</td>
<td>68.6 ± 3.1</td>
<td>68.8 ± 3.3</td>
</tr>
<tr>
<td>Primary education only (%)</td>
<td>29.7</td>
<td>27.9</td>
</tr>
<tr>
<td>Alcohol consumption &gt;2 drinks/d (%)</td>
<td>18.7</td>
<td>25.9</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>7.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Former smoker (%)</td>
<td>34.2</td>
<td>44.4</td>
</tr>
<tr>
<td>Current hypertension (%)</td>
<td>38.4</td>
<td>40.7</td>
</tr>
<tr>
<td>History of coronary artery disease (%)</td>
<td>10.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Presence of carotid atherosclerotic plaques (%)</td>
<td>32.4</td>
<td>25.9</td>
</tr>
<tr>
<td>Intima-media thickness (mm)</td>
<td>6.8 ± 1.2</td>
<td>7.1 ± 1.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 4.0</td>
<td>26.4 ± 2.2</td>
</tr>
<tr>
<td>Apolipoprotein E4 genotype (%)</td>
<td>24.5</td>
<td>14.8</td>
</tr>
</tbody>
</table>

1 Moderate cognitive decline was defined as a decrease of ≥2 points. None of the comparisons between the no-decline and decline groups were significant on the basis of Student’s t tests.

2 x ± SD.

3 One drink = 13 mL alcohol.


---

**Table 2**
Erythrocyte membrane fatty acid contents in 246 participants

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Value</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SFAs</td>
<td>43.5 ± 1.3 (40.6–52.0)</td>
<td></td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>13.1 ± 0.9 (10.0–15.6)</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>19.1 ± 1.2 (16.4–26.9)</td>
<td></td>
</tr>
<tr>
<td>Total MUFAs</td>
<td>16.8 ± 1.2 (13.7–20.0)</td>
<td></td>
</tr>
<tr>
<td>Oleic acid (18:1n–9)</td>
<td>11.1 ± 0.9 (8.8–13.9)</td>
<td></td>
</tr>
<tr>
<td>Total PUFAs</td>
<td>38.1 ± 1.8 (25.0–43.5)</td>
<td></td>
</tr>
<tr>
<td>Total n–6 PUFAs</td>
<td>27.8 ± 2.0 (21.0–35.2)</td>
<td></td>
</tr>
<tr>
<td>AA (20:4n–6)</td>
<td>13.6 ± 1.3 (8.2–17.8)</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (18:2n–6)</td>
<td>9.2 ± 1.4 (5.9–13.2)</td>
<td></td>
</tr>
<tr>
<td>Total n–3 PUFAs</td>
<td>9.9 ± 1.7 (2.8–18.9)</td>
<td></td>
</tr>
<tr>
<td>DHA (22:6n–3)</td>
<td>6.3 ± 1.1 (1.5–9.9)</td>
<td></td>
</tr>
<tr>
<td>EPA (20:5n–3)</td>
<td>1.1 ± 0.5 (0.2–5.8)</td>
<td></td>
</tr>
<tr>
<td>n–3:n–6 fatty acids</td>
<td>0.4 ± 0.1 (0.1–0.9)</td>
<td></td>
</tr>
<tr>
<td>DHA:AA</td>
<td>0.5 ± 0.1 (0.2–1.2)</td>
<td></td>
</tr>
</tbody>
</table>

1 ± SD; range in parentheses. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
and obtained associations similar to those obtained with logistic regression: n–3 PUFAs ($r = 0.09, P = 0.14$), n–6 PUFAs ($r = -0.12, P = 0.05$), n–3:n–6 ($r = 0.13, P = 0.04$), stearic acid ($r = -0.13, P = 0.05$). The results were quite similar when the analyses were restricted to the 150 subjects with high initial MMSE scores (defined as >27). However, the results were not modified when other potential risk factors (listed in Table 1) were introduced (data not shown).

### DISCUSSION

To our knowledge, this is the first report relating the fatty acid composition of erythrocyte membranes to cognitive decline in the elderly. We observed that lower contents of n–3 PUFAs and higher contents of stearic acid and n–6 PUFAs were associated with a higher risk of cognitive decline.

This study included volunteers with high cognitive function and a very good overall level of education, which is a known protective factor against cognitive impairment; in fact, very few subjects experienced a substantial decrease in MMSE score. The changes in MMSE scores observed during the study suggest that the moderate decline considered in this analysis corresponds to a progressive mean decrease over time. The results of analyses that correlated change in MMSE with fatty acid contents support the existence of some linear relation, and as a consequence, the importance of the choice of the cutoff value is reduced. Choosing a 3-point decrease in MMSE score, rather than a 2-point decrease, as a threshold would have yielded similar patterns of association but with very low statistical power. Nevertheless, individual MMSE changes over time should be interpreted with caution because of the phenomenon of regression to the mean, which is complicated by a general learning effect and a ceiling effect resulting from the score range.

No dietary intake data were collected in the EVA Study, but the erythrocyte membrane fatty acid composition can reflect dietary fat intake. Some fatty acid contents, especially the amounts of total n–3 and n–6 PUFAs, can be used as biochemical indicators of their corresponding midterm (60–90 d) dietary intakes (23–25). This contrasts with plasma fatty acid concentrations, which fluctuate according to shorter-term intake. Thus, some comparison between our results regarding erythrocyte fatty acids and those obtained from dietary surveys in other populations seems warranted.

In the Zutphen Study of 476 men aged 69–89 y, Kalmijn et al (26) found that high LA intake was associated with cognitive impairment after adjustment for age, education, cigarette smoking, alcohol consumption, and energy intake. Consumption of fish, an important source of long-chain n–3 PUFAs, was inversely but not significantly associated with cognitive impairment and cognitive decline. In the Rotterdam Study of 5386 participants >55 y of age, fish consumption was inversely related to incident dementia and, in particular, to AD (27). High intake of saturated fat was associated, but not significantly, with a higher risk of dementia. In a cross-sectional study of 260 individuals aged 65–90 y, Ortega et al (28) showed that lower intakes of SFAs or MUFAs were associated with better cognitive performance, but they found no association with total PUFAs and no data were reported on the n–3 and n–6 families. Thus, 3 studies showed an inverse association between fish intake and cognitive impairment or cognitive decline, in agreement with our results on n–3 PUFAs. Other comparisons are difficult to make because the SFA and MUFA contents of erythrocyte membranes did not reflect the dietary intake of these fatty acids.

Only one cross-sectional study reported data on fatty acid analysis of blood plasma in patients with AD, other types of dementia, and cognitive impairment compared with control subjects (29). Low concentrations of n–3 PUFAs (total n–3 and n–3:n–6) appeared to be risk factors for cognitive impairment and dementia. A striking analogy can be made with our results, even though the latter apply to moderate cognitive decline in otherwise normal subjects.

The present data, collected with a longitudinal design, provide new epidemiologic arguments for the potential influence of PUF intake on cognitive deterioration and possibly on the early...
development of AD. Relevant experimental results in aged animals are lacking, but supplementation with long chain n–3 PUFAs was shown to improve environmental adaptation ability in young mice (30). The possible mechanisms are multiple and nonexclusive; they may involve vascular abnormalities per se (31), more general pathologic conditions such as inflammation (32), or both.

Vascular abnormalities may impair nutrient and oxygen supplies that sustain normal cerebral function, and n–3 PUFAs intake was shown to be beneficial in many instances. EPA and DHA intakes may lower the risk of thrombosis (33) and reduce blood pressure, which is a strong determinant of alterations of the arterial wall (34). These fatty acids also reduce triacylglycerol concentrations and improve glycemic control in diabetics (35). However, a specific relation of n–3 PUFA intake (or n–3:n–6) with atherosclerosis of the large vessels has not been established. Both n–3 and n–6 PUFA intakes are generally considered to be antiatherogenic via the mechanism of reducing circulating LDL cholesterol (36). In the present study, history of coronary artery disease, echographic evidence of carotid atherosclerosis (plaques and IMT measurements), and hypertension were not specifically associated with change in MMSE. Actually, our data gave no indication of the possible involvement of vascular abnormalities in the relation between cognitive deterioration and erythrocyte membrane fatty acid composition, but the size of the population was clearly insufficient for that purpose.

Metabolism of AA, the most abundant n–6 PUFA in erythrocytes, gives rise to inflammatory mediators, whereas n–3 PUFAs found in fish as AA antagonists. Thus, n–3 PUFAs have anti-inflammatory properties and may decrease the production of proinflammatory cytokines in humans (37, 38). In fact, neuroinflammation seems to be a central feature of AD, and many compounds, such as various cytokines and prostaglandins, have been identified in brain tissue from AD patients; these compounds are known to promote and sustain inflammatory responses (32). Moreover, some epidemiologic studies suggest that the use of nonsteroidal antiinflammatory drugs might reduce the risk of AD (39). The relevance of these findings to the normal cognitive decline of aging has not been documented yet, however.

The influence of fatty acids on the biological mechanisms that lead to cognitive impairment with age should be explored further. If our preliminary results are confirmed, evaluation of the potential effect of dietary recommendations or specific dietary supplements on the cognitive decline of aging might be warranted.

We thank N Combes (ISTAB, Bordeaux) for participating in interesting discussions and for performing the biological analysis of the fatty acids.

REFERENCES


