n–3 Fatty acid erythrocyte membrane content, *APOE* ε4, and cognitive variation: an observational follow-up study in late adulthood[1–3]

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ABSTRACT

Background: Evidence for an inverse relation between dietary intake of n–3 polyunsaturated fatty acids (PUFAs) and age-related cognitive decline is inconsistent. This inconsistency may arise because the relation is present only in the absence of the apolipoprotein E ε4 (*APOE* ε4) allele.

Objective: We aimed to determine the contribution of erythrocyte n–3 PUFA content to cognitive aging in the presence or absence of the *APOE* ε4 allele.

Design: We followed up 120 volunteers, born in 1936, at approximate ages of 64, 66, and 68 y. Their intelligence quotient at 11 y old was available. At first follow-up, we determined *APOE* genotype and measured the PUFA composition of erythrocyte membranes. Six cognitive tests were administered at all follow-ups. We related cognitive performance at ≈64 y old and cognitive changes from ≈64 to ≈68 y old to erythrocyte n–3 PUFA composition on recruitment and to *APOE* ε4 allele status.

Results: Total n–3 PUFA and docosohexaenoic acid concentrations were associated with benefits for cognition at ≈64 y old and from ≈64 to ≈68 y old. After adjustment for sex, *APOE* ε4 status, and intelligence quotient at 11 y old, the effects associated with total n–3 PUFA remained significant. Cognitive benefits were associated with higher erythrocyte n–3 PUFA content but were significant only in the absence of the *APOE* ε4 allele.

Conclusions: These data are evidence of a gene × environment interaction for cognitive aging. They are relevant to the analysis of trials of n–3 PUFA supplements in cognitive aging and dementia prevention, and they support heterogeneity in cognitive aging and, possibly, in Alzheimer disease. *Am J Clin Nutr* 2008;87:449–54.

KEY WORDS Polyunsaturated fatty acids, erythrocytes, cognitive decline, fish-oil consumption, apolipoprotein E, childhood intelligence, cognitive tests

INTRODUCTION

Progressive cognitive decline in later life is a major risk factor for Alzheimer disease (AD). Like AD (1), both genetic and environmental causal factors are significant in cognitive decline (2), but these factors vary in nature and extent, and some may interact. Among environmental influences, dietary variation may be important (3, 4). Many reports suggest inverse relations between the intake of some nutrients and the risk of AD, but, in the face of some contrary findings, no consensus exists as to their relative effects. The lack of consensus may result from the facts that the consumption of many foodstuffs is highly interrelated, that dietary self-reports are unreliable in the presence of cognitive impairment, or that genetic heterogeneity exists between samples. Among foodstuffs, a case can be made for the role of a low dietary intake of n–3 polyunsaturated fatty acids (PUFAs) in cognitive decline and AD (5–12), but not all reports support this possibility (13, 14).

The gene encoding for apolipoprotein E (*APOE*) has 3 alleles—ε2, ε3, and ε4; of these, only ε4 is associated with greater risk of cognitive decline (15) and AD (16). These risks may be greater when ε4 coexists with vascular risk factors for AD, but evidence for synergy between ε4 and vascular risk factors is not strong (17). We previously showed that erythrocyte membrane n−3 PUFA content was associated with cognitive performance at ≈64 y old (12). In a community-based sample, Huang et al (11) found that, in the absence of *APOE* ε4, self-reported oily fish intake was associated with a lower AD risk. To test the stability of the relation between erythrocyte n–3 PUFA content and cognitive function and to explore the role of *APOE* ε4 (11), we followed up the original sample to ≈68 y old. We hypothesized that the cognitive benefits of higher dietary n–3 PUFA would be seen in the absence of ε4.

SUBJECTS AND METHODS

Subjects

On 4 June 1947, the Scottish Council for Research in Education surveyed the mental ability of all children in Scottish schools who were born in 1936. These children took a version of the Moray House Test (MHT) of general intelligence. In 1998, the

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Scottish Council for Research in Education gave access to their its MHT records, which record, by birth name, date of birth, and school, a child's intelligence at age 10.5–11.5 y (18). A population-based study sample was ascertained by scrutiny of a local health register and by identifying by name and date of birth local residents for whom an intelligence score at ≈11 y old was available. Of the 660 persons invited to take part, 506 (76%) agreed to do so. Recruitment took place from November 1999 to February 2002; volunteers were 63–66 y old. Four volunteers were excluded because of dementia, and ascertainment was incomplete in 22 participants, which yielded an original sample of 478 subjects. All were living independently in the community and were in good general health. At the interview, demographic and dietary information, including the use of fish-oil supplements, was recorded (12).

When these persons were invited to the reassessment at ≈66 or 68 y old, 16 persons had died, 8 had moved away, and 84 were unavailable at age 66 y and an additional 64 were unavailable at age 68 y. Of the remaining persons, 354 returned at a mean ± SD age of 66.6 y ± 10 mo, and 308 subjects returned at a mean age of 68.8 y ± 7 mo; 9 of this latter group were not included because of poor health or frailty, and 29 other subjects did not complete all of the cognitive tests. All 3 assessments at ≈64, 66, and 68 y old were completed by 289 (60.5%) of the original sample of 478 subjects. For reasons of cost, erythrocyte membrane fatty acid composition was measured at wave 1 in a subsample of 120 (25%) of 480. This subsample was nonrandom, and it comprised equal numbers of fish-oil supplement users and nonusers matched by sex and childhood intelligence. All were 63.8–65.3 y old (mean ± SD age: 64.4 y ± 8 mo) on recruitment. Cognitive data were incomplete for 5 of these 120 subjects.

Written informed consent was obtained from all participants at the interview. The study was approved by the National Health Service Grampian Research Ethics Committee.

Methods

Cognition at ≈64, 66, and 68 y old

Cognitive tests (18) comprised the Mini-Mental State Examination, which was used as a brief screening tool for dementia; Raven's Standard Progressive Matrices (RPM) measured nonverbal reasoning; Rey's Auditory Verbal Learning Test (AVLT), which tests verbal declarative memory; the Uses of Common Objects Test, which tests executive function or purposive action; the Digit Symbol subtest of the Wechsler Adult Intelligence Scale–revised, which provided speed of information processing and a test of psychomotor performance; and the Block Design subtest of the Wechsler Adult Intelligence Scale, which measured constructional ability. All tests were administered again at ages 66 and 68 y.

APOE genotyping

Venous blood was drawn for DNA extraction. *APOE* genotypes were analyzed by polymerase chain reaction amplification of a 227-base pair fragment of the *APOE* gene, which contained 2 polymorphic sites that accounted for the 3 alleles—ε2, ε3, and ε4—according to Wenham et al (19).

Fatty acid measurements

The fatty acid content of erythrocyte membranes was measured within 12–24 mo of blood sampling; erythrocytes were separated from whole blood by centrifugation and stored at –70 °C (12). Data were available for total saturated fatty acids; total n–9 polyunsaturated fatty acids (PUFA); total n–6 PUFA; total n–3 PUFA; the specific PUFAs *cis*-linoleic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid (DHA); and total n–3 PUFA. Eicosapentaenoic acid, DHA, total n–3 PUFA, and the ratio of n–3 to n–6 PUFA were chosen to test the study hypotheses because, previously, these have been associated with cognitive function (12).

Statistical analysis

All erythrocyte membrane PUFA values were log transformed to achieve more normal distributions. *APOE* genotype was coded as the presence or absence of *APOE* ε4 allele; 2 *APOE* ε4ε2 genotypes were excluded from the analysis because of possible conflicting effects on cognitive decline (20). Analysis of variance was used to determine the distribution of erythrocyte membrane fatty acid and cognitive function at waves 1–3 with respect to *APOE* ε4 carrier status. All 5 cognitive tests (RPM, AVLT, Block Design, Digit, and Uses of Common Objects) were entered into a principal-components analysis that identified a single principal component that accounted for 44.0% of the cognitive test score variance at wave 1 (age 63–65 y), 49.6% of the variance at wave 2 (age 66 y), and 43.0% of the variance at wave 3 (age 68 y). Mixed linear models were then constructed to investigate associations between erythrocyte membrane PUFA values and cognitive test scores over 3 occasions—ie, the waves. An advantage of mixed linear models is that they are able to use all available data, which allows the inclusion of participants with missing data. Scores on the individual tests were considered as repeated measures of cognition performed on 3 separate occasions. The repeated-measures design could thus test hypotheses relating to differential effects between cognitive tests and between waves of testing. Hypotheses that tested for an association between an independent variable and cognition (taken as a repeated measure on the 6 cognitive tests on the 3 occasions) were tested as main effects in the models. Hypotheses that test associations between independent variables and cognition changing over time were tested by the interaction between cognition and wave. Akaike's Information Criterion and Schwarz's Bayesian Criterion both indicated that a diagonal repeated-measures covariance structure best fit the data. All analyses were performed with SPSS software (version 14.0; SPSS Inc, Chicago, IL).

RESULTS

Description of sample

One hundred thirteen persons met inclusion criteria and contributed to the analyses (**Table 1**). Comparisons between subjects who dropped out after wave 1 or wave 2 with those who completed all 3 waves showed that drop-outs had significantly ($P < 0.005$) lower childhood intelligence and performed significantly less well at wave 1 on RPM ($P < 0.01$), Digit Symbol ($P < 0.05$), and Block Design $P < 0.05$) tests. There were no significant differences between drop-outs and completers in the use of fish-oil supplements, erythrocyte n–3 PUFA content, or *APOE* ε4 allele frequency. A greater-than-expected number of men carried the *APOE* ε4 allele ($P < 0.02$); full *APOE* genotype information indicated that there was no significant deviation from Hardy-Weinberg equilibrium (chi-square = 3.23; $P >$

TABLE 1
APOE carrier status, sex, and fish-oil supplement use in a follow-up study of cognitive aging in 113 volunteers living independently in the community[1]

	APOE ε4 carrier ($n = 38$)	*APOE* ε4 noncarrier ($n = 75$)	*P*
Age (y)	64.2 ± 0.63[2]	64.4 ± 0.68	NS[3]
Sex			0.017[4]
Male (*n*)	21	24	
Female (*n*)	17	51	
Fish-oil supplement			NS[4]
User (*n*)	20	36	
Nonuser (*n*)	18	39	

[1] *APOE,* apolipoprotein E.
[2] $\bar{x}$ ± SD (all such values).
[3] Difference based on ANOVA model.
[4] Differences between categorical variables by chi-square test.

TABLE 2
APOE carrier status and erythrocyte membrane fatty acid content in a follow-up study of cognitive aging of 113 volunteers living independently in the community[1]

Erythrocyte membrane fatty acid content	*APOE* ε4 carrier ($n = 38$)	*APOE* ε4 noncarrier ($n = 75$)	*P*[2]
Total n–3 PUFAs	8.1 ± 0.1.8[3]	8.6 ± 1.7	NS
EPA (20:5n–3)	0.89 ± 0.6	0.98 ± 0.9	NS
DHA (22:6n–3)	5.0 ± 1.2	5.0 ± 0.9	NS
n–6:n–3 PUFA ratio	2.8 ± 0.6	2.7 ± 0.9	NS

[1] *APOE,* apolipoprotein E; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Differences in log-transformed PUFA based on ANOVA model.
[3] $\bar{x}$ ± SD (all such values).

0.05). No significant differences between *APOE* ε4 carriers and noncarriers in the use of fish-oil supplements were detected.

At wave 1, principal components analysis provided a score on general mental ability at age 63–65 y to which all 5 cognitive tests contributed. This identified a single principal component (scree slope criterion >1) that explained 44.0% of the cognitive test score variance at wave 1 (age 63–65 y), 49.6% of the variance at wave 2 (age 66 y), and 43.0% of the variance at wave 3 (age 68 y). Relations between erythrocyte membrane total n–3 PUFA content and general intelligence at age 11 y or general mental ability at age 63–65 y by *APOE* ε4 carrier status are shown in **Figure 1**. In the absence of ε4, there were significant positive correlations between total n–3 content and general ability at both 11 y old ($n = 75$; $r = 0.25$, $P < 0.05$) and ≈63–65 y old ($n = 75$; $r = 0.35$, $P < 0.01$). Figure 1 also shows that, in the presence of ε4, there were no significant associations between total n–3 content and general mental ability at age 11 y. Although none of the differences between these correlations grouped by *APOE* ε4 carrier status were significant, we acknowledge that the sample size was inadequate to detect differences between correlations (type II error).

There were no significant differences in erythrocyte PUFA composition by *APOE* ε4 carrier status (**Table 2**). At recruitment, *APOE* ε4 noncarriers had significantly ($P = 0.018$) higher scores than did *APOE* ε4 carriers on the AVLT, but not on other cognitive tests (**Table 3**). Data were available for 107 subjects at wave 2 (age 66 y) and for 70 subjects at wave 3 (age 68 y). *APOE* ε4 noncarriers had significantly ($P < 0.05$ for both) higher scores than did carriers on AVLT at both wave 2 (66.7 ± 14.1 and 60.4 ± 13.3, respectively) and wave 3 (64.9 ± 13.3 and 56.7 ± 14.3, respectively). There were no significant differences between the scores of *APOE* ε4 carriers and noncarriers on other cognitive tests at waves 2 and 3.

Repeated-measures mixed models of the effects of fatty acid erythrocyte membrane content on cognition

We used mixed linear models to explore the effects associated with *APOE* ε4 carrier status and erythrocyte PUFA composition on cognitive performance over time (ie, wave). The initial model tested the effect of total erythrocyte n–3 PUFA content on all repeated cognitive measures taken as a whole (ie, with no effects

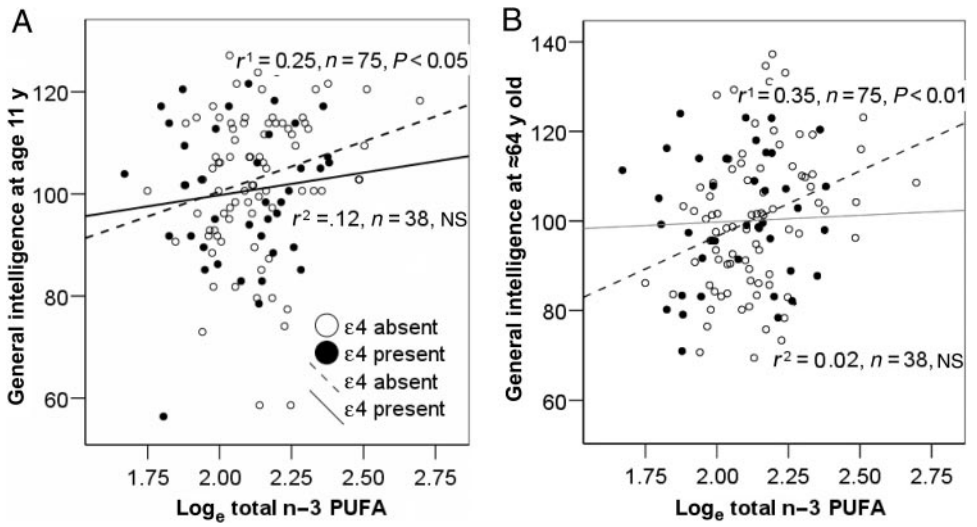

FIGURE 1. Correlations between the natural logarithm ($\log_e$) of total n–3 polyunsaturated fatty acids (PUFA) and general intelligence scores at age 10.5–11.5 y (A) and general intelligence at age 63–65 y (B) in the presence or absence of apolipoprotein ε4. Differences between correlations (r^1 and r^2) were not significant.

TABLE 3
APOE status, childhood intelligence, and cognitive test scores at ≈64 y old

Cognitive test	*APOE* ε4 carrier		*APOE* ε4 noncarrier		p^2
	n		*n*		
MHT at age 11 y	38	42.8 ± 12.2[3]	75	45.9 ± 12.2	NS
MMSE	38	29.1 ± 1.2	75	29.2 ± 0.9	NS
RPM	38	38.0 ± 8.1	75	36.6 ± 7.8	NS
AVLT	38	54.9 ± 1.2	72	60.7 ± 12.2	0.018
UOT	36	14.1 ± 5.6	71	14.0 ± 5.2	NS
DS	34	44.4 ± 11.2	70	45.9 ± 11.3	NS
BD	36	25.5 ± 8.7	70	24.7 ± 8.7	NS

[1] *APOE,* apolipoprotein E; MHT, Moray House Test of childhood general mental ability; MMSE, Mini-Mental State Examination; RPM, Raven's Progressive Matrices; AVLT, Auditory Verbal Learning Test; UOT, Uses of Common Objects Test; DS, Digit Symbol; BD, Block Design.
[2] Differences in cognitive function using ANOVA model.
[3] $\bar{x}$ ± SD (all such values).

on test type or on the wave being tested). Thus, this model assumes that there is a general cognitive trait common to the 6 cognitive tests that is repeatedly estimated with these tests on repeated occasions (waves of testing). There was a significant ($P = 0.031$) effect of total erythrocyte PUFA on this general cognitive trait. We next tested which components of total fatty acid content explained this association: significant components were total n–3 PUFA ($P = 0.008$) and DHA ($P = 0.008$). There was a statistical trend for eicosapentaenoic acid ($P = 0.069$), but this trend was abolished once total n–3 PUFA was entered into the model.

We next tested for any differential effects of total n–3 PUFA and DHA over time (ie, change in cognitive outcome variable over waves 1, 2, and 3). In addition to its overall effect (ie, the effect of treating cognition as a single estimate by using repeated snapshots over time), total n–3 PUFA had a significant effect on change in cognition over time ($P = 0.002$): higher total n–3 PUFA concentrations were significantly associated with slightly higher scores at wave 1 ($P = 0.045$) and wave 2 ($P < 0.001$) than the scores explained by the overall effect of n–3 PUFA. Similarly, DHA had a significant ($P < 0.001$) effect on cognitive change over time, but the wave 2 and wave 3 scores were significantly ($P < 0.001$ for both) higher than the scores explained by the overall effect of DHA. After adjustment for the effects of total n–3 PUFA over time, there were no significant differential effects of total n–3 PUFA on any cognitive test. However, DHA had a marginally significant differential effect on cognitive tests ($P = 0.049$); it was associated with significantly ($P < 0.001$ for all) higher scores on the Mini-Mental State Examination, AVLT, and Block Design test than on the Uses of Common Objects Test. DHA also had a differential effect on cognitive tests over time ($P < 0.001$): Mini-Mental State Examination, AVLT, and Block Design test scores were significantly (all $P < 0.001$ for all) lower at wave 1 and wave 2 than at wave 3. There was a significant ($P < 0.005$) total n–3 PUFA × time × sex interaction, which indicated that the total n–3 × time interaction differs significantly between men and women.

Effects of interaction between fatty acid erythrocyte membrane content and *APOE* ε4 on cognition

To test our hypothesis concerning an interaction between *APOE* ε4 carrier status and erythrocyte PUFA composition on cognitive performance over time, we explored effects associated with sex, *APOE* ε4, and MHT score at age 11 y. We entered sex, *APOE* ε4, and MHT score at age 11 y into models testing the effects of total n–3 PUFA and DHA on all cognitive measures repeated over waves 1–3. Optimal models confirmed significant beneficial effects of sex, *APOE* ε4, and MHT score at age 11 y on cognitive performance, and significant effects remained for total n–3 PUFA but not for DHA (**Table 4**). The inclusion of sex, *APOE* ε4, and MHT score at age 11 y did not influence the pattern of effects: the results (Table 4) indicated that total n–3 PUFA was associated with a significantly ($P = 0.003$) greater positive effect on cognitive scores in *APOE* ε4 noncarriers than in carriers. There was also a significant ($P = 0.001$) effect associated with the waves: the effect on cognitive scores in *APOE* ε4 noncarriers was greater over waves 2 and 3 than over wave 1. The *APOE* ε4 genotype × $\log_e$ total n–3 PUFA interaction for the outcome variable of RPM at waves 1, 2, and 3 is shown in **Figure 2**. There was no significant effect of total n–3 PUFA in *APOE* ε4 carriers, but, in the noncarriers, an increase in RPM was significantly ($P < 0.01$) associated with increased total n–3 PUFA.

We recognized that attrition from the study was associated with lower performance on cognitive tests. To test whether this association had skewed the results, we entered a dummy variable indicating whether a participant was present at wave 3; we found no effect on the results. The main effect of being present at wave 3 was on overall cognition ($F = 0.001$, $P = 0.98$). There was no significant interaction of this variable with any specific cognitive test ($F = 0.76$, $P = 0.58$). Therefore, there was no evidence of attrition bias after adjustment for other significant effects.

DISCUSSION

Our data suggest that cognitive benefits at wave 1 and from wave 1 to wave 3 were attributable to total n–3 PUFA and DHA. After adjustment for the effect of sex, *APOE* ε4 status, and MHT score at age 11 y, the effects associated with DHA were no longer significant; however, those associated with total n–3 PUFA persisted. Cognitive benefits were associated with higher total erythrocyte n–3 PUFA content but were significant only in the absence of the *APOE* ε4 allele. Attrition from the study was associated with lower performance on cognitive tests at recruitment. When the effects on cognitive performance at follow-up were tested, we concluded that attrition had censored these results so that the more able subjects were more likely to remain in the study. However, we do not conclude that this censoring made more likely the detection of significant relations between total

TABLE 4
Optimal model of effects of total n–3 polyunsaturated fatty acids (PUFAs) on 6 cognitive test scores repeated over 3 waves (time)[1]

	df			
Source	Numerator	Denominator	*F* ratio	P^2
Intercept	1	730.1	81.9	<0.001
Test type	5	422.9	35.7	<0.001
Sex × time	3	338.2	5.40	0.001
Test type × time	10	301.7	421.5	<0.001
Total n–3 × sex × time	3	336.1	4.46	0.004
APOE ε4	2	758.2	6.12	0.002
Time × *APOE* ε4	4	634.6	4.86	0.001
Total n–3 × *APOE* ε4	2	755.7	5.81	0.003
Total n–3 × time × *APOE* ε4	4	632.3	4.46	0.001
MHT score at age 11 y	1	719.0	272.4	<0.001
MHT score at age 11 y × test type	5	283.3	3.08	0.010
Total n–3 × time	2	607.2	3.07	0.047
MHT score at age 11 y × time	2	675.0	69.8	<0.001

[1] *APOE,* apolipoprotein E; MHT, Moray House Test. Nonsignificant effects, including main effects, are not included in the table.
[2] Probability.

n3- PUFA and cognition; participation in all 3 waves of the study was not associated with cognitive benefits. The lack of measurement of n–3 PUFA at waves 2 and 3 of cognitive testing is an important limitation. The use of a multivariate approach with a repeated-measures design reduces the type 1 statistical error associated with multiple univariate models. The study would have been strengthened by the inclusion of repeated (waves 1, 2, and 3) measures of erythrocyte membrane – and brain-specific n–3 PUFA metabolism (21).

In total, these data support the proposal that, in late midlife (at ≈64–68 y old), there are enduring cognitive benefits associated with higher erythrocyte n–3 PUFA content. Cognitive benefits were significant only in the absence of the *APOE* ε4 allele. However, a reverse explanation seems reasonable: constituents of dietary fish oil could impair cognitive function but only in the presence of the *APOE* ε4 allele. That explanation would imply that the cognitive benefits associated here with the absence of *APOE* ε4 are attributable to improvement on practice and are unrelated to *APOE* ε4 or n–3 PUFA. This positive practice effect would be opposed in the presence of *APOE* ε4 by adverse effects of n–3 PUFA. This proposal is testable in trials of n–3 PUFA supplementation. An inspection of Figures 1 and 2 raised a third possibility—that, in the presence of *APOE* ε4, greater variability of n–3 PUFAs is associated with lower cognitive performance. This interpretation could be associated with the antioxidant status of the participants and could be tested in a future study by the inclusion of measurements of antioxidant defenses.

Dietary n–3 PUFAs are mostly derived from the consumption of nonprocessed oily fish (eg, salmon, tuna, mackerel, and sardines). In ≈30% of the local Scottish population, oily fish meals are supplemented by daily doses of n–3 PUFAs taken as marine oil or fish-oil capsules. These preparations are often reinforced with other micronutrients, such as antioxidant vitamins. We previously showed that those who take fish-oil supplements have significantly greater erythrocyte n–3 PUFA content and also had higher scores at age 11 y on a test of general intelligence (12). Potentially better cognitive performance in late midlife, found in this sample to be associated with higher erythrocyte n–3 PUFA

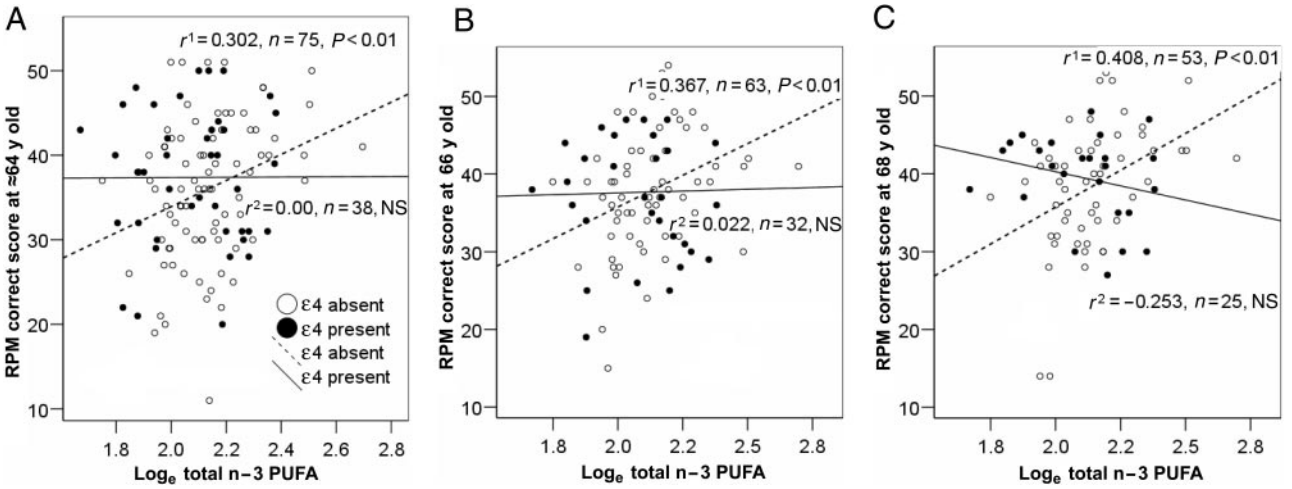

FIGURE 2. Correlations between the natural logarithm ($\log_e$) of total n–3 polyunsaturated fatty acids (PUFAs) and correct scores on Raven's Progressive Matrices (RPM) at ≈63–65 y old (A), 66 y old (B), and 68 y old (C) in the presence or absence of apolipoprotein ε4. - - -, r^1; —, r^2. In A and B, these correlations do not differ significantly, but the difference between them in C is significant ($P < 0.01$).

content, may be explained by a life-long healthier lifestyle that includes a diet rich in marine oils or supplementation with n–3 PUFAs and many other micronutrients, or both. This seems an unlikely explanation of the results reported here, however, because the effect was confined to subjects without the *APOE* ε4 allele, and that allele is not associated with childhood intelligence (15).

Earlier studies examined the possibility that the greater risk of AD associated with the *APOE* ε4 allele is mediated through a greater risk of vascular disease (22), which may trigger a cascade of neurochemical events leading to AD. Prince et al (17) showed that the presence of *APOE* ε4 increased AD risk independent of vascular risk. Similar findings were also reported by Kivipelto et al (23). In light of these and related studies, it is reasonable to postulate ≥2 pathways that lead to AD, one followed in the presence of *APOE* ε4and the other followed in its absence. Previous postulations about the possible neuroprotective contributions of n–3 PUFAs emphasized their antiinflammatory properties (24), their roles in neurodevelopment and neural repair, and their potential to modify gene expression (25). However, other pathways to AD that involve *APOE* ε4 and n–3 PUFA metabolism are conceivable. The predisposing role of A*POE* ε4 in the pathogenesis of atherosclerosis and cardiovascular disease may be related to A*POE* ε4 influences on lipid responsiveness to dietary n–3 PUFA intake (26). Current trials of the potential benefits of n–3 PUFA supplements in the prevention of age-related cognitive decline (27) may, therefore, allow analysis by *APOE* ε4 status together with measurement of n–3 PUFAs obtained from biological membranes. There also may be differences between men and women in the cognitive benefits of n–3 PUFA supplementation, and possible sex differences should be tested in the analysis of trial data.

The authors' responsibilities were as follows: LJW: guarantor of the data reported here; LJW, IJD, and JMS: study design and joint responsibility for and primary authorship of the manuscript; HCF: supervision of data collection; HCF and VJB: collection of data and management of the database; KAR, VJB, and JMS: statistical analyses; KWW: supervision of erythrocyte PUFA measurements and contributions to study conduct and analysis of results; all authors: contributions to the writing and revision of the manuscript. None of the authors had a personal or financial conflict of interest.

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