See corresponding editorial on page 929.

Plasma n−3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study[1–3]

May A Beydoun, Jay S Kaufman, Jessie A Satia, Wayne Rosamond, and Aaron R Folsom

ABSTRACT

Background: Plasma fatty acids may affect the risk of cognitive decline in older adults.

Objectives: We prospectively studied the association between plasma fatty acids and cognitive decline in adults aged 50–65 y at baseline and conducted a subgroup analysis.

Design: From 1987 through 1989, the Atherosclerosis Risk in Communities (ARIC) Study analyzed plasma fatty acids in cholesteryl esters and phospholipids in whites residing in Minneapolis, MN. From 1990 through 1992 and from 1996 through 1998, 3 neuropsychological tests in the domains of delayed word recall, psychomotor speed, and verbal fluency were administered. We selected cutoffs for statistically reliable cognitive decline in each of these domains and a measure of global cognitive change computed by principal-components analysis. Multivariate logistic regression was conducted. Focusing on n−3 highly unsaturated fatty acids (HUFAs), a subgroup analysis assessed differential association across potential effect modifiers implicated in oxidative stress and increased risk of neurodegenerative disease.

Results: In the 2251 study subjects, the risk of global cognitive decline increased with elevated palmitic acid in both fractions and with high arachidonic acid and low linoleic acid in cholesteryl esters. Higher n−3 HUFAs reduced the risk of decline in verbal fluency, particularly in hypertensive and dyslipidemic subjects. No significant findings were shown for psychomotor speed or delayed word recall.

Conclusions: Promoting higher intakes of n−3 HUFAs in the diet of hypertensive and dyslipidemic persons may have substantial benefits in reducing their risk of cognitive decline in the area of verbal fluency. However, clinical trials are needed to confirm this finding. *Am J Clin Nutr* 2007;85:1103–11.

KEY WORDS Aging, cognitive decline, fatty acids, cholesteryl esters, phospholipids

INTRODUCTION

The effect of dietary intake on cognitive functioning has drawn interest over the past few years. Several epidemiologic studies have shown that n−3 fatty acids in blood differ significantly between persons with normal cognitive functioning and those with some form of cognitive impairment (1–3). These fatty acids in biomarkers of lipid intake have been historically associated with a lower risk of cardiovascular disease, including stroke (4) and coronary heart disease (5, 6). They were also linked with greater insulin sensitivity (7), a lower risk of dyslipidemia (8), a hypocoagulable profile (9), greater pulmonary function (10), and a lower risk of major depression (11), among other health benefits.

Many of these conditions were related to greater oxidative stress (12–17), which in turn causes neuronal loss and cognitive impairment in older adults (18). Consequently, it is essential to unveil any putative interaction between these conditions and the ability of this class of fatty acids to fulfill their beneficial effects. In addition, a genetic factor (*ApoE ε4* allele) has been consistently associated with greater risks of cognitive decline (19, 20) and progression from the preclinical stage to Alzheimer disease (21). It has also been associated with higher levels of oxidative stress (22).

The current study assessed the association of plasma cholesteryl ester and phospholipid concentrations of n−3 fatty acids with decline in 3 areas of cognition. We conducted a subgroup analysis of possible interaction between n−3 fatty acids and high levels of oxidative stress and the risk of neurodegenerative disease. We hypothesized that those subgroups with elevated physiologic oxidative stress would benefit most from higher intakes of dietary n−3 fatty acids as measured by plasma concentrations in cholesteryl esters and phospholipids. The main rationale behind this hypothesis is that oxidative stress triggers neurodegenerative processes by depleting the brain of vulnerable highly unsaturated fatty acids (HUFAs). Their replenishment, which can be achieved by increased dietary intake, can lead to the prevention of cognitive decline.

SUBJECTS AND METHODS

Subjects

The Atherosclerosis Risk in Communities (ARIC) Study is a cohort study established in 1987. It was designed to investigate the etiology of atherosclerosis and its clinical sequelae. In the

[1] From the Departments of Epidemiology (MAB, JSK, JAS, and WR) and Nutrition (JAS), School of Public Health, and the Carolina Population Center (MAB and JSK), University of North Carolina at Chapel Hill, Chapel Hill, NC; the Division of Epidemiology & Community Health, School of Public Health, University of Minnesota, Minneapolis, MN (ARF); and the Center for Human Nutrition, Department of International Health, Johns Hopkins School of Public Health, Baltimore, MD (MAB).

[2] Supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

[3] Reprints not available. Address correspondence to MA Beydoun, Center for Human Nutrition, Department of International Health, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Room E2610, Baltimore, MD 21205. E-mail: mbaydoun@jhsph.edu.

Received September 7, 2006.

Accepted for publication October 19, 2006.

original study, approximately 4000 adults aged 45–64 y were recruited in each of 4 US communities—Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD (for a total of 16 000 subjects)—and invited to 4 examinations 3 y apart (between 1987 and 89, between 1990 and 1992, between 1993 and 1995, and between 1996 and 1998), termed visits 1 through 4, respectively. Three of the 4 cohorts represented the ethnic mix of their communities, whereas in Jackson, MS, only African American residents were recruited (23).

In the current study, we focused on subjects with complete plasma fatty acid data, which were collected and analyzed at visit 1 (1987–1989) at the ARIC Study field center in Minneapolis ($n = 3570$). We excluded those aged <50 y because research clearly shows that the risk of cognitive decline in general, and of dementia in particular, is negligible before the age of 60 y (24), which is the age at which the youngest persons in the cohort were reexamined at visit 4. After exclusion of those without cognitive testing at visits 2 and 4, the remaining sample available for analysis was 2251 subjects.

Cognitive assessment

Three measurements of cognitive functioning were made during visits 2 and 4 of the ARIC Study. These measurements relied on the following instruments: the Delayed Word Recall Test (DWRT; 25); the Digit Symbol Substitution Test portion of the Wechsler Adult Intelligence Scale–Revised (DSST/WAIS-R; 26), and the Word Fluency Test (WFT) of the Multilingual Aphasia Examination, also known as the controlled oral-word association (27).

Delayed Word Recall Test

The DWRT screening tool assesses verbal learning and recent memory. It requires the respondent to recall 10 common words after a 5-min interval during which another test is administered. Test scores may range between 0 and 10 words recalled, and the time limit for recall is set at 60 s. The 6-mo test-retest reliability of the DWRT was previously shown to be high in 26 healthy elderly persons (Pearson correlation coefficient, $r = 0.75$) (25).

Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale–Revised

The DSST/WAIS-R is a paper-and-pencil test requiring timed translation of numbers 1 through 9 to symbols with the use of a key. The test measures psychomotor performance and, for most adults, is relatively unaffected by intellectual ability, memory, or learning (27). It appears to be a sensitive and reliable marker of brain damage (28). The test score can range from 0 to 93, and it reflects the correctly translated number of digit-symbol pairs within a time limit of 90 s. Short-term test-retest reliability over 2–5 wk has been found to be high ($r = 0.82$) in persons aged 45–54 y (26).

Word Fluency Test

The WFT requires subjects to think of as many words beginning with the letters F, A, and S as possible and to record these words in 60 s for each of the 3 groups of words. The total score corresponds to the total number of words generated during these 3 trials. The test is particularly sensitive to linguistic impairment (27, 29) and early mental decline in older persons (30). It is also a sensitive marker of damage in the left lateral frontal lobe (27, 29). The immediate test-retest correlation coefficient based on an alternative test form has been found to be high ($r = 0.82$; 31).

Decline in the 3 separate areas of cognition was assessed. Cutoffs were determined for decline in each domain of cognition by using the Reliable Change Index (RCI) method to correct for measurement error and practice effects (32). RCI is defined as $[(X_2–X_1)–(M_2–M_1)]$/SD of the difference, where X_1 is the person's score at baseline, X_2 is the person's score at follow-up, M_1 and M_2 are the group mean pretest and follow-up scores, respectively, and SD of the difference is the observed SD of the difference scores. Scoring below an RCI of −1.645 was regarded as showing a "statistically reliable" deterioration in test scores. A composite measure of the 3 RCIs (obtained from DWRT, DSST, and WFT change scores) to assess global cognitive decline (GCD) was created by using principal-components analysis (PCA). Similarly, the cutoff chosen corresponded to a composite score of reliable decline <−1.645. Multivariate analysis included control for the baseline cognitive score in its continuous form (assessed at visit 2) on the particular instrument. Models with GCD as the main outcome controlled for the baseline measure of GCD. Similar approaches were previously used by others (33, 34).

Plasma fatty acid exposures

Twelve-hour fasting blood was collected according to the ARIC Study–wide protocol. The Minneapolis field center conducted fatty acid analysis in plasma phospholipid and cholesteryl ester fractions on visit 1 blood specimens. The procedure is described in detail elsewhere (35). The identity of 28 fatty acid peaks was shown by gas chromatography by comparing each peak's retention time to the retention times of fatty acids in synthetic standards of known compositions. The relative amount of each fatty acid (as a percentage of all fatty acids) was computed by integration of the area under the peak and division of the result by the total area for all fatty acids (×100). Data from the chromatogram were transferred electronically to a computer for analysis. Fatty acids are expressed as a percentage of the total in each fraction. Although all groups of fatty acids [saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), linoleic acid (LA), α-linolenate (LNA), and n−3 and n−6 HUFAs)] were assessed in relation to GCD, one main exposure of interest was considered for domain-specific and interaction analysis—ie, eicosahexaenoic acid (EPA) +docosahexaenoic acid (DHA) (20:5 + 22:6n−3, respectively), which were also the n−3 HUFAs. Test-retest reliability coefficients (subjects sampled 3 times at 2-wk intervals) for various plasma fatty acids ranged from 0.50 to 0.93 for cholesteryl esters and from 0.50 to 0.89 for phospholipids (36).

Covariates

Most covariates considered were measured at visits 1 or 2, although some were defined according to measurements that spanned all 4 visits. Age, sex, and education were all self-reported. Behavioral factors measured at visit 1 included smoking, alcohol and caffeine consumption, and physical activity (37). A validated index of physical activity, derived at visit 1, summed sports, work, and leisure indexes, which ranged from a score of 1 (low) to a score of 5 (high) (38). Body mass index (BMI; in kg/m^2) was computed at visit 1. Baseline dietary intakes

of antioxidants and other micronutrients (mainly vitamins B-6 and B-12 and folate) were also considered. The usual dietary intake of these nutrients was estimated from an interviewer-administered semiquantitative food-frequency questionnaire (FFQ) modified from a 61-item questionnaire developed and validated by Willett et al (39) against multiple food records among a subsample of the Nurses' Health Study cohort. Results of the validation study suggested that, for all nutrients considered, only ≤3% extreme quintile misclassification was seen, and that overlap between the upper 2 and lower 2 quintiles between the 2 methods was >70% (39). Daily intake of nutrients has been calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food was obtained from the Harvard Nutrient Database, for which the primary source was the Department of Agriculture handbook (40).

All of these covariates were considered as potential confounders in the multivariate analysis. Potential effect modifiers were grouped as genetic (presence of ApoE ε4 allele) and comorbid conditions. To measure hypertensive status, blood pressures were calculated as the average of the second and third of 3 consecutive measurements taken with a random-zero sphygmomanometer (DINAMAP* 1846 SX automated oscillometer; Critikon, Inc, Tampa, FL). The cutoff often used for hypertension is ≥140 mm Hg for systolic (SBP) and ≥90 mm Hg for diastolic (DBP) blood pressure. Hypertensive subjects were defined as those who screened positive for measured hypertension at any visit of visits 1 through 4 or who were taking antihypertensive medication during the 2 wk immediately before any of the visits. Other conditions included stroke or transient ischemic attacks (TIAs), type 2 diabetes mellitus (defined as fasting blood glucose ≥ 140 mg/dL, self-reported diabetes, or the use of glucose-lowering medication), and dyslipidemia [fasting blood HDL cholesterol < 40 (men) or < 50 (women) and triacylglycerol level > 150 mg/dL as recommended by the National Cholesterol Education Program (41)] at any visit, hypercoagulable profile (upper quintile of ≥2 of fibrinogen, van Willebrand factor, and factor VIII), poor pulmonary function [forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) ≤ 0.70 as measured by a spirometer at visits 1 or 2], and depressive symptoms at visit 2 using 21-item vital exhaustion scale (42, 43). A binary cutoff for depression was chosen on the basis of the 80th percentile of the scale scores.

Statistical analysis

We carried out univariate analyses of predictor and outcome variables as well as covariates. For bivariate analyses of exposure and outcome, we computed means of predictor variables across outcome groups (0: no decline; 1: declined) and assessed statistical significance of differences by using an independent-sample *t* test for continuous predictors and the chi-square test for categorical predictors at α of 0.05. We computed the ORs of decline by each 1-SD change in exposure by conducting multivariate logistic regression analysis. Control for confounding was done using backward elimination, retaining in the model those variables that changed the estimated effect (odds ratio) of the exposure by >5%. This level is more suitable than 10% because the sensitivity of odds ratios to confounding effects tends to increase with increase in sample size (44). After obtaining a parsimonious model, we first presented odds ratios for global cognitive decline and plasma concentrations of various groups of fatty acids. Subsequently, and focusing on n−3 HUFAs, we presented stratum-specific odds ratios for each subgroup of interest (eg, hypertensives versus normotensives). We also carried out multivariate logistic regression models adding the interaction term (n−3 HUFA × binary subgroup variable) into a model with the main effects included as well (ie, n−3 HUFA and binary subgroup variable). We compared this model with the one without the interaction term and used likelihood ratio tests to assess statistical significance of interaction between exposure and potential effect modifiers in determining the outcome. For this purpose, a type I error level of 0.10 was considered valid (45, 46). Statistical analyses were conducted with STATA software (version 8.2; Stata Corp, College Station, TX; 47).

RESULTS

Baseline characteristics

A total of 2251 study subjects had complete plasma analysis at visit 1 and cognitive decline data between visits 2 and 4. All subjects were white men and women residing in the suburbs of Minneapolis (one of the ARIC Study centers). Looking at distribution of characteristics of this sample of subjects by cognitive status, those who did not decline differed from those who did on several demographic, behavioral, and health-related factors. Specifically, they were on average younger and more physically active, reported less depressive symptoms, and had a relatively hypocoagulable profile. Those who declined compared with those who did not, also had higher baseline cognitive score and as expected, greater decline in cognitive function between the 2 visits (**Table 1**).

Plasma fatty acids and global cognitive decline

The mean plasma concentration of individual as well as families of fatty acids across global cognitive decline categories is shown in **Table 2**. Within the cholesteryl ester fraction, those who declined had higher concentrations of palmitic and arachidonic acids and lower concentration of linoleic acid. After control for other potentially confounding factors, including other fatty acids, total PUFAs, total n−6 PUFAs, and linoleic acid, were all inversely related to decline, whereas a higher concentrations of arachidonic acid remained a significant risk factor for decline. In the plasma phospholipid fraction, the unadjusted means revealed the same pattern across cognitive status. However, adjusted odds ratios of decline with each SD increment in these fatty acids did not differ significantly from the null value of 1, with the exception of palmitic acid (an SFA), which was consistently associated positively with the risk of cognitive decline. Hence, n−3 PUFAs in general and DHA+EPA in particular had no significant effect on global cognitive decline.

n−3 Fatty acids and cognition: a subgroup analysis

A set of multivariate logistic models of plasma n−3 HUFAs (DHA+EPA) on cognitive decline in the 3 areas of interest as well as globally for both the cholesteryl ester and phospholipid fractions is shown in **Table 3**. Covariates considered as potential confounders included baseline cognitive score and demographic, behavioral, and nutritional factors (including other groups of

TABLE 1
Characteristics of study subjects with complete cognitive and plasma data by global cognitive decline (GCD) status: Atherosclerosis Risk in Communities Study, 1987–1998

Characteristic	GCD (Reliable Change Index < − 1.645)	
	No decline (n = 2111)	Decline (n = 140)
Women (%)	50.69	50.70
Age (y)[2]	56.21 ± 4.22[3,4]	57.74 ± 4.22
Education (%)[2]		
Incomplete high school	6.78	5.00
High school	36.40	32.86
> High school	56.82	62.14
ApoE ε4 allele[2]	28.74	30.37
Smoking status (%)[2]		
Never smoker	40.38	40.71
Former smoker	41.94	40.0
Current smoker	17.68	19.28
Alcohol (g/d)[2]	8.01 ± 13.48	9.04 ± 13.47
Caffeine (mg/d)[2]	350.59 ± 326.82	310 ± 311
Physical activity scale[2]	7.34 ± 1.32[4]	7.09 ± 1.39
BMI (kg/m^2)[2]	27.21 ± 4.41	26.60 ± 4.33
Total energy intake (kcal/d)[2]	1583 ± 559	1546 ± 568
Vitamin A (in 1000 IU/d)[2]	8.65 ± 6.90	8.63 ± 5.82
Vitamin B-6 (mg/d)[2]	1.75 ± 0.67	1.74 ± 0.64
Vitamin B-12 (μg/d)[2]	7.06 ± 3.47	7.01 ± 3.97
Vitamin C (mg/d)[2]	112.80 ± 70.78	110.66 ± 56.16
Vitamin E (mg/d)[2]	4.66 ± 3.02	4.66 ± 2.99
Folate (μg/d)[2]	218.80 ± 95.21	213.80 ± 91.48
Stroke/TIAs (%)[5]	9.00	13.6
Hypertensive (%)[5]	48.93	54.58
Dyslipidemia (%)[5]	37.38	32.14
Type 2 diabetes mellitus (%)[5]	13.64	17.14
Depression scale[6]	8.08 ± 7.03[4]	9.73 ± 8.25
Poor pulmonary function (FEV_1/FVC <70) (%)[6]	18.82	20.71
Hypercoagulable profile (%)[2]	10.75[4]	16.43
Fibrinogen value	293.87 ± 59.95	302.91 ± 74.32
vWF	110.71 ± 39.50[4]	117.75 ± 41.77
Factor VIII	124.00 ± 32.33	128.29 ± 37.63
Baseline cognitive scores (visit 2)[6]		
DWRT	6.75 ± 1.43[4]	7.29 ± 1.32
DSST/WAIS-R	51.58 ± 10.10	52.76 ± 0.91
WFT	37.05 ± 11.28[4]	45.66 ± 12.09
Cognitive decline (visit 4 − visit 2)[6]		
DWRT	−0.10 ± 1.42[4]	−1.94 ± 1.62
DSST/WAIS-R	−3.88 ± 5.79[4]	−13.1 ± 8.06
WFT	−1.12 ± 7.13[4]	−12.77 ± 8.91

[1] n = 2251. TIAs, transient ischemic attacks; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; vWF, von Willebrand factor; DWRT, Delayed Word Recall Test; DSST/WAIS-R, Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale–Revised; WFT, Word Fluency Test.
[2] Covariate measured at visit 1.
[3] $\bar{x}$ ± SD (all such values).
[4] $P < 0.05$ for null hypothesis that means (Student's t test) or percentages (chi-square test) are equal between the 2 groups.
[5] Covariate measured at visits 1 through 4.
[6] Covariate with other time frame.

fatty acids in that particular fraction). In contrast to what was noted in Table 2, Table 3 shows that the greater DHA+EPA was associated with less decline in verbal fluency (WFT) for both cholesteryl ester and phospholipid fractions (Odds ratios were: 0.74 (0.57, 0.97) and 0.73 (0.58, 0.93), respectively).

Subgroup analysis by selected cardiovascular, genetic, and other health conditions associated with greater oxidative stress indicated that some of these factors may modify the effect of DHA+EPA on the main outcomes of interest. In particular, when the outcome was decline in verbal fluency (WFT), DHA+EPA was mostly protective among hypertensive subjects for both the cholesteryl ester and phospholipids fraction and among dyslipidemic subjects for the phospholipids fraction only. In addition, a higher plasma phospholipids concentration of n−3 HUFAs was protective against decline in verbal fluency only among subjects with a lower score on depressive symptoms. These significant findings are based on likelihood ratio test at type I error of 0.10. For all other domains of cognition (DWRT and DSST/

TABLE 2
Plasma concentrations of fatty acid groups by cognitive decline status and adjusted odds ratios (ORs) for decline in global cognitive functioning by 1-SD changes in fatty acid concentration: Atherosclerosis Risk in Communities Study, 1987–1998[1]

	Global cognitive decline (Reliable Change Index < −1.645)		
Fatty acid	No decline (n = 2111)	Decline (n = 140)	OR (95% CI)
Plasma cholesteryl esters			
Total SFAs	17.93 ± 1.98[2]	18.16 ± 1.93	1.11 (0.95, 1.31)
Stearic acid (18:0)	0.89 ± 0.19	0.86 ± 0.15	0.85 (0.70, 1.03)
Palmitic acid (16:0)	10.02 ± 0.79[3]	10.22 ± 0.83	1.28 (1.07, 1.54)[3]
Total MUFAs	15.91 ± 1.96	16.04 ± 2.04	0.69 (0.46, 1.02)
Oleic acid (18:1n−9)	15.83 ± 1.95	15.96 ± 2.03	0.70 (0.47, 1.04)
Total PUFAs	65.86 ± 3.73	65.26 ± 3.86	0.55 (0.37, 0.81)[3]
Total n−6 PUFAs	64.39 ± 3.82	63.76 ± 3.92	0.54 (0.36, 0.82)[3]
AA (20:4n−6)	8.27 ± 1.67[3]	8.72 ± 1.77	1.21 (1.00, 1.47)[3]
Linoleic acid (18:2n−6)	54.26 ± 4.64[3]	53.13 ± 4.91	0.64 (0.49, 0.83)[3]
Total n−3 PUFAs	1.42 ± 0.43	1.46 ± 0.39	1.08 (0.92, 1.26)
EPA (20:5n−3)	0.55 ± 0.29	0.57 ± 0.25	0.84 (0.66, 1.05)
DHA (22:6n−3)	0.45 ± 0.16	0.47 ± 0.17	1.18 (0.97, 1.44)
Linolenic acid (18:3n−3)	0.41 ± 0.10	0.41 ± 0.09	0.97 (0.82, 1.16)
DHA+EPA	1.01 ± 0.40	1.04 ± 0.36	1.09 (0.94, 1.27)
(DHA+EPA)/AA	0.12 ± 0.05	0.12 ± 0.04	0.96 (0.80, 1.15)
Plasma phospholipids			
Total SFAs	49.36 ± 2.95	49.31 ± 2.75	0.98 (0.82, 1.16)
Stearic acid (18:0)	13.31 ± 1.18	13.15 ± 1.31	1.04 (0.84, 1.29)
Palmitic acid (16:0)	25.36 ± 1.61[3]	25.72 ± 1.88	1.24 (1.05, 1.47)[3]
Total MUFAs	9.23 ± 1.09	9.22 ± 1.13	0.97 (0.82, 1.16)
Oleic acid (18:1n−9)	8.50 ± 1.09	8.50 ± 1.15	1.00 (0.84, 1.18)
Total PUFAs	41.91 ± 1.67	41.92 ± 1.65	0.99 (0.84, 1.17)
Total n−6 PUFAs	38.20 ± 1.78	38.08 ± 1.74	0.93 (0.79, 1.10)
AA (20:4n−6)	11.47 ± 1.93[3]	11.92 ± 1.98	1.16 (0.93, 1.43)
Linoleic acid (18:2n−6)	21.96 ± 2.60[3]	21.37 ± 2.68	0.87 (0.70, 1.09)
Total n−3 PUFAs	3.59 ± 1.05	3.71 ± 1.07	1.11 (0.95, 1.30)
EPA (20:5n−3)	0.57 ± 0.31	0.57 ± 0.25	0.96 (0.80, 1.16)
DHA (22:6n−3)	2.87 ± 0.88	2.98 ± 0.94	1.13 (0.96, 1.32)
Linolenic acid (18:3n−3)	0.14 ± 0.05	0.14 ± 0.04	1.03 (0.87, 1.22)
DHA+EPA	3.44 ± 1.05	3.56 ± 1.07	1.11 (0.95, 1.29)
(DHA+EPA)/AA	0.31 ± 0.11	0.31 ± 0.11	0.99 (0.83, 1.18)

[1] n = 2251. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Each fatty acid exposure was included in a separate multivariate logistic model. Both covariates considered and the control for confounding are described in Subjects and Methods.

[2] $\bar{x}$ ± SD (all such values).

[3] $P < 0.05$ for null hypothesis that means are equal between the 2 groups (Wald test).

WAIS-R), there were weak to null effects of DHA+EPA on cognitive decline in both fraction (cholesteryl esters and phospholipids), overall and across subgroups.

DISCUSSION

This is one of the few published reports on the association of plasma fatty acids with cognitive decline among middle-aged and older adults using a prospective cohort design. Our findings indicated global cognitive decline was affected by a greater relative amount of palmitate (an SFA) in both fractions. In cholesteryl esters, risk of global cognitive decline was increased by a greater arachidonic acid concentration (an n−6 HUFA) and a lower amount of linoleic acid (an n−6 medium-chain PUFA), even after control for several demographic, behavioral, and nutritional factors. These factors were shown previously to influence plasma fatty acids (48) as well as cognitive decline (49). It is worth noting that most of our models did not retain >2 potential confounders, which most often were other fatty acid groups in the plasma fraction. More important, lower concentrations of our main exposure of interest—n−3 HUFAs (mainly DHA and EPA)—was associated with a higher risk of decline in verbal fluency, particularly among hypertensive and dyslipidemic subjects and in those who were less depressed at baseline. These interactions, however, were only consistent between cholesteryl ester and phospholipid fractions of plasma for hypertensive status, which may suggest a specific mechanism involving high blood pressure. No effect was observed on delayed word recall

TABLE 3
Multivariate logistic regression of plasma n−3 highly unsaturated fatty acids (HUFAs) on cognitive decline: a subgroup analysis from the Atherosclerosis Risk in Communities Study, 1987–1998[1]

	Cognitive decline (Reliable Change Index<−1.645)			
	DWRT OR (95% CI)	DSST/WAIS-R OR (95% CI)	WFT OR (95% CI)	Global cognitive decline OR (95% CI)
Plasma cholesteryl esters				
All subjects	1.02 (0.85, 1.21)	1.00 (0.83, 1.20)	0.74 (0.57, 0.97)[2]	1.09 (0.94, 1.27)
Normotensive	1.07 (0.85, 1.36)	1.07 (0.84, 1.36)	0.99 (0.71, 1.38)	1.16 (0.95, 1.41)
Hypertensive	0.94 (0.72, 1.23)	0.90 (0.67, 1.21)	0.55 (0.36, 0.84)[3]	1.05 (0.81, 1.36)
ApoEε4 (no allele)	1.05 (0.84, 1.31)	0.97 (0.76, 1.25)	0.61 (0.43, 0.87)	1.08 (0.90, 1.30)
ApoEε4 (1 or 2 alleles)	0.99 (0.72, 1.35)	1.10 (0.82, 1.46)	0.80 (0.45, 1.36)	1.01 (0.90, 1.30)
Normal lipid profile	0.94 (0.73, 1.20)	1.04 (0.83, 1.29)	0.87 (0.66, 1.15)	1.10 (0.93, 1.30)
Dyslipidemia	1.16 (0.87, 1.53)	0.92 (0.66, 1.28)	0.49 (0.28, 0.87)	1.05 (0.76, 1.44)
No stroke or TIA	1.02 (0.85, 1.23)	1.02 (0.84, 1.23)	0.74 (0.55, 1.00)	1.10 (0.94, 1.29)
Stroke or TIA	0.96 (0.46, 1.99)	0.86 (0.44, 1.66)	0.72 (0.38, 1.38)	0.95 (0.58, 1.55)
Nondiabetic	1.00 (0.81, 1.22)	0.99 (0.80, 1.24)	0.79 (0.59, 1.05)	1.13 (0.96, 1.34)
Type 2 diabetes mellitus	1.09 (0.79, 1.49)	0.96 (0.67, 1.38)	0.65 (0.32, 1.33)	0.93 (0.61, 1.42)
Hypocoagulable profile	1.02 (0.85, 1.22)	1.00 (0.82, 1.22)	0.79 (0.59, 1.05)	1.13 (0.97, 1.32)
Hypercoagulable profile	0.98 (0.53, 1.82)	1.08 (0.65, 1.80)	0.55 (0.27, 1.11)	0.83 (0.48, 1.43)
Depressive symptoms (≤10)	1.03 (0.85, 1.27)	1.03 (0.83, 1.27)	0.62 (0.43, 0.88)	1.13 (0.95, 1.35)
Depressive symptoms (>10)	0.99 (0.71, 1.39)	0.95 (0.65, 1.37)	0.97 (0.73, 1.31)	0.99 (0.74, 1.34)
Good pulmonary function	1.07 (0.88, 1.29)	0.96 (0.77, 1.20)	0.77 (0.66, 1.04)	1.07 (0.89, 1.28)
Poor pulmonary function	0.86 (0.56, 1.31)	1.13 (0.82, 1.54)	0.61 (0.31, 1.20)	1.16 (0.88, 1.53)
Plasma phospholipids				
All subjects	0.87 (0.71, 1.06)	1.02 (0.84, 1.24)	0.73 (0.58, 0.93)[2]	1.11 (0.95, 1.29)
Normotensive	0.98 (0.73, 1.32)	1.04 (0.78, 1.38)	0.89 (0.65, 1.23)	1.18 (0.95, 1.48)
Hypertensive	0.78 (0.59, 1.03)	0.97 (0.74, 1.28)	0.64 (0.44, 0.91)[3]	1.06 (0.84, 1.33)
ApoEε4 (no allele)	0.95 (0.73, 1.23)	0.97 (0.75, 1.25)	0.68 (0.51, 0.91)	1.14 (0.95, 1.38)
ApoEε4 (1 or 2 alleles)	0.77 (0.55, 1.08)	1.18 (0.85, 1.64)	0.61 (0.35, 1.08)	0.86 (0.61, 1.22)
Normal lipid profile	0.77 (0.59, 1.01)	0.99 (0.78, 1.26)	0.85 (0.66, 1.10)	1.13 (0.94, 1.35)
Dyslipidemia	1.06 (0.77, 1.46)	1.04 (0.74, 1.44)	0.49 (0.30, 0.84)[3]	1.09 (0.80, 1.49)
No stroke or TIA	0.87 (0.71, 1.07)	1.02 (0.83, 1.25)	0.74 (0.57, 0.96)	1.10 (0.93, 1.30)
Stroke or TIA	0.91 (0.44, 1.84)	1.00 (0.57, 1.76)	0.61 (0.34, 1.11)	1.10 (0.72, 1.68)
Nondiabetic	0.85 (0.69, 1.06)	1.01 (0.81, 1.27)	0.75 (0.58, 0.97)	1.17 (0.99, 1.38)
Type 2 diabetes mellitus	0.92 (0.60, 1.40)	0.99 (0.66, 1.46)	0.73 (0.37, 1.43)	0.90 (0.57, 1.42)
Hypocoagulable profile	0.87 (0.71, 1.07)	0.97 (0.79, 1.19)	0.74 (0.58, 0.96)	1.10 (0.95, 1.31)
Hypercoagulable profile	0.83 (0.44, 1.56)	1.58 (0.89, 2.80)	0.69 (0.30, 1.23)	1.19 (0.77, 1.86)
Depressive symptoms (≤10)	0.80 (0.63, 1.02)	1.01 (0.81, 1.27)	0.62 (0.46, 0.84)	1.08 (0.89, 1.32)
Depressive symptoms (>10)	1.04 (0.74, 1.45)	1.01 (0.69, 1.48)	1.00 (0.71, 1.39)[3]	1.18 (0.91, 1.52)
Good pulmonary function	0.89 (0.72, 1.12)	0.96 (0.77, 1.20)	0.79 (0.61, 1.02)	1.09 (0.91, 1.31)
Poor pulmonary function	0.78 (0.50, 1.20)	1.21 (0.83, 1.78)	0.52 (0.28, 0.96)	1.17 (0.86, 1.59)

[1] OR, odds ratio; DWRT, Delayed Word Recall Test; DSST/WAIS-R, Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale–Revised; ApoE, apolipoprotein E; TIAs, transient ischemic attacks. WFT, Word Fluency Test. Cognitive decline was measured per 1-SD increase in n−3 HUFA exposure. Both covariates considered and the control for confounding are described in Subjects and Methods.

[2] $P < 0.05$ for testing the null hypothesis that regression coefficient $\beta_1 = 0$ (Wald test).

[3] $P < 0.10$ for testing the null hypotheses that interaction term (n−3 HUFA × binary subgroup variable) coefficient γ is equal to 0 when the likelihood ratio test is used.

(DWRT) or psychomotor speed (through DSST/WAIS-R test) among any of the subgroups considered. Additional linear regression analyses of the association between n−3 fatty acids and baseline cognitive scores in the total sample shows a positive and significant relation in the case of verbal fluency and global cognitive functioning for both cholesteryl esters and phospholipids. In our logistic regression models of cognitive decline and n−3 fatty acids (Tables 2 and 3), those specific outcomes retained the baseline score on selection of potential confounders.

Our study included a group of white, middle-aged men and women residing in the suburbs of Minneapolis. They were in general highly educated; very few had less than a high school education. Because education tends to affect most neuropsychological tests, having a relatively homogenous group remained an advantage, even as we were able to control for educational level in the analysis. Average changes in cognitive scores over a period of 6 y were relatively small when looking at the range of values that each test can take. Another strength was the use of the RCIs with a cutoff of −1.645, which was validated in previous neuropsychological research as a method to reflect statistically reliable deterioration in the test scores, given that RCIs capture participants with changes in cognitive function tests that probably lie outside the range of random error. RCIs were also shown to adjust for measurement error and practice effects (32).

The study had a few limitations. First, it made use of tissue composition of fatty acids, which does not necessarily reflect the proportion of fatty acids in the diet. Nevertheless, the ARIC Study had also collected dietary intake data by using a semiquantitative FFQ at the same baseline visit (ie, visit 1). A study by Ma et al (35) showed that intake of DHA and EPA in the ARIC Study was highly correlated with their concentrations in both cholesteryl esters and phospholipids. Previous studies also indicated that plasma concentrations of HUFAs may also be a good reflection of long-term dietary intake (48, 50–53). In addition, repeated measures of a subsample of the plasma specimens collected by the ARIC Study Minnesota center indicated that fatty acid concentration in both fractions is reliable over the short and long term (reliability coefficient for most fatty acids: ≥ 0.65), which indicates little intraindividual variability (36). It was shown that low reliability may lead to attenuation of causal effects and this fact should also be taken into consideration (54). However, the main advantage of using a biomarker rather than a self-report of intake is that we are certain that errors in the exposure are independent of errors in our outcome measure. This independence of exposure errors from outcome status would in most cases lead to an attenuation of effect in the presence of measurement error and hence lead to an odds ratio that is biased toward the null value of 1. Another limitation is that the sum of fatty acids in each fraction is 100. Therefore, a higher percentage of a specific group (eg, SFAs) will automatically reflect a lower percentage of another. Hence, there is a problem of interdependence which makes it difficult to interpret the effect of a single constituent or group of constituents. We are also unable to translate these findings quantitatively into dietary recommendations (eg, number of servings of fish per week). In terms of timing of the exposure, one limitation is that it does not reflect the period in which cognitive decline is taking place but rather predates it by 3 y. A final limitation of the current study is the relatively narrow scope of the cognitive assessment. A more detailed assessment might have revealed associations undetected by the current measures.

Previous observational studies suggested that the biochemical composition of blood components in terms of fatty acids differs significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment. A study by Conquer et al (1) found that a lower plasma concentration of n−3 fatty acids, and in particular DHA, is associated with Alzheimer disease as well as other forms of cognitive impairment. Another case-control study, conducted by Tully et al (3), found that patients with Alzheimer disease had significantly lower concentrations of serum cholesteryl ester–EPA than did control subjects. A third recent nested case-control study found that, whereas total n−6 PUFAs in erythrocyte membranes were associated with a greater risk of cognitive decline with an odds ratio of 1.59 (95% CI: 1.04, 2.44), a higher proportion of total n−3 fatty acids were associated with a lower risk of cognitive decline, with an odds ratio of 0.59 (95% CI: 0.38, 0.93) (2). Whereas most of these studies showed an inverse association of plasma and erythrocyte n−3 fatty acids with cognition among older adults, others found either a null association for specific fatty acids or an opposite relation (55).

Our hypothesized effect of this class of fatty acids on cognition has been linked with several biologically plausible mechanisms. These include preventing vascular abnormalities (56), reducing inflammation (57), or both, as well as influencing membrane fluidity and ultimately neurotransmission (58). For example, excess n−3 HUFAs (mainly DHA and EPA) can reduce the risk of thrombosis and reduce blood pressure, although both conditions are thought to alter arterial walls and impair oxygen and nutrient supplementation needed for normal cerebral functioning (59, 60). It has also been established that n−3 HUFAs can reduce the plasma concentration of triacylglycerols (8, 61) and improve glycemic control and insulin sensitivity in type 2 diabetes (7, 62). Their ability to lower LDL cholesterol has also been shown (63), although this effect was not necessarily specific to this class of fatty acid, but rather to all PUFAs, including LA.

On the basis of our findings, reason exists to believe that subjects who are under increased oxidative stress, particularly hypertensive and dyslipidemic subjects, may benefit from enriching their diet with n−3 HUFAs, which are mostly found in cold-water fish (eg, salmon, tuna, and mackerel) and other foods of marine origin. Future research should attempt to conduct subgroup analysis by using actual markers of oxidative stress (64). In addition, a randomized trial may be warranted comparing subjects with varied levels of oxidative stress in terms of their cognitive change response to increasing dietary intake of n−3 HUFAs using a more comprehensive battery of neuropsychological tests.

The authors acknowledge the contributions of Gerardo Heiss (the Principal Investigator of the ARIC Study), particularly in data acquisition and operationalization of covariates. We also thank Philip Sloane (Department of Family Medicine, University of North Carolina–Chapel Hill) for help in the construction of the cognitive decline variables in a clinically meaningful way. In addition, we thank the staff and participants of the ARIC Study for their contributions: Diane Catellier for statistical review and Tom Mosley for performing the ARIC Study's primary publication review.

MAB and JSK were responsible for the conception of the study; AF was responsible for searching the literature on the validity and reliability of plasma fatty acids in the ARIC Study; MAB was responsible for data management; MAB, AF, and JSK were responsible for data analysis; JAS contributed to the description on dietary variables and to the method of assessment; AF contributed to the operationalization of the plasma fatty acids and enabled the plasma fatty acid data to be merged with other cohort data; MAB was responsible for the draft of the manuscript; JSK, JAS, and WR contributed to the writing of the manuscript; and AF reviewed the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 2000;35:1305–12.
2. Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes—the EVA Study. Am J Clin Nutr 2003;77:803–8.
3. Tully AM, Roche HM, Doyle R, et al. Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study. Br J Nutr 2003;89:483–9.
4. He K, Song Y, Daviglus ML, et al. Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke 2004;35:1538–42.
5. Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. Am J Cardiol 2004;93:1119–23.
6. He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 2004;109:2705–11.
7. Feskens EJ, Virtanen SM, Rasanen L, et al. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetes Care 1995;18:1104–12.
8. Harris WS, Windsor SL, Dujovne CA. Effects of four doses of n−3 fatty acids given to hyperlipidemic patients for six months. J Am Coll Nutr 1991;10:220–7.

9. Shahar E, Folsom AR, Wu KK, et al. Associations of fish intake and dietary n−3 polyunsaturated fatty acids with a hypocoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb 1993;13:1205–12.
10. Shahar E, Folsom AR, Melnick SL, et al. Dietary n−3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. Atherosclerosis Risk in Communities Study Investigators. N Engl J Med 1994;331:228–33.
11. Hibbeln JR, Umhau JC, George DT, Salem N Jr. Do plasma polyunsaturates predict hostility and depression? World Rev Nutr Diet 1997;82: 175–86.
12. de Champlain J, Wu R, Girouard H, et al. Oxidative stress in hypertension. Clin Exp Hypertens 2004;26:593–601.
13. Maritim AC, Sanders RA, Watkins JB III. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003;17:24–38.
14. Wada H, Hagiwara SI, Saitoh E, et al. Increased oxidative stress in patients with chronic obstructive pulmonary disease (COPD) as measured by redox status of plasma coenzyme Q(10). Pathophysiology 2006;13:29–33.
15. Shea TB, Rogers E, Ashline D, Ortiz D, Sheu MS. Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. Free Radic Biol Med 2002;33:1115–20.
16. Ruef J, Peter K, Nordt TK, Runge MS, Kubler W, Bode C. Oxidative stress and atherosclerosis: its relationship to growth factors, thrombus formation and therapeutic approaches. Thromb Haemost 1999; 82(suppl):32–7.
17. Logan AC, Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 2005;64:533–8.
18. Albers DS, Beal MF. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease. J Neural Transm Suppl 2000;59: 133–54.
19. Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. Stroke 1996;27:2230–5.
20. Blair CK, Folsom AR, Knopman DS, Bray MS, Mosley TH, Boerwinkle E. APOE genotype and cognitive decline in a middle-aged cohort. Neurology 2005;64:268–76.
21. Turner RS. Biomarkers of Alzheimer's disease and mild cognitive impairment: are we there yet? Exp Neurol 2003;183:7–10.
22. Lee Y, Aono M, Laskowitz D, Warner DS, Pearlstein RD. Apolipoprotein E protects against oxidative stress in mixed neuronal-glial cell cultures by reducing glutamate toxicity. Neurochem Int 2004;44:107–18.
23. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol 1989;129:687–702.
24. Abate C, Ferrari-Ramondo V, Di Iorio A. Risk factors for cognitive disorders in the elderly: a review. Arch Gerontol Geriatr 1998;6(suppl):7–15.
25. Knopman DS, Ryberg S. A verbal memory test with high predictive accuracy for dementia of the Alzheimer type. Arch Neurol 1989;46: 141–5.
26. Wechsler D. Manual for the Wechsler Adult Intelligence Scale–Revised. New York, NY: The Psychological Corporation, 1981.
27. Lezak MD. Neuropsychological assessment. 2nd ed. New York, NY: Oxford University Press, 1983.
28. Russell EW. WAIS factor analysis with brain-damaged subjects using criterion measures. J Consult Clin Psychol 1972;39:133–9.
29. Tranel D. Neuropsychological assessment. Psychiatr Clin North Am 1992;15:283–99.
30. Benton AL, Eslinger PJ, Damasio AR. Normative observations on neuropsychological test performances in old age. J Clin Neuropsychol 1981; 3:33–42.
31. Franzen MD, ed. Multilingual aphasia examination. Kansas City, MO: Test Corporation of America, 1986.
32. Frerichs RJ, Tuokko HA. Reliable change scores and their relation to perceived change in memory: implications for the diagnosis of mild cognitive impairment. Arch Clin Neuropsychol 2006;21:109–15.
33. Stewart R, Prince M, Mann A. Age, vascular risk, and cognitive decline in an older, British, African-Caribbean population. J Am Geriatr Soc 2003;51:1547–53.
34. Kalmijn S, van Boxtel MP, Ocke M, Verschuren WM, Kromhout D, Launer LJ. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. Neurology 2004;62:275–80.
35. Ma J, Folsom AR, Shahar E, Eckfeldt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62:564–71.
36. Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62:572–8.
37. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936–42.
38. Richardson MT, Ainsworth BE, Wu HC, Jacobs DR Jr., Leon AS. Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity. Int J Epidemiol 1995; 24:685–93.
39. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
40. Consumer and Food Economics Institute. Composition of foods: raw, processed, prepared. Agriculture handbook series. Washington, DC: Agricultural Research Service, 1989.
41. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
42. Appels A, Hoppener P, Mulder P. A questionnaire to assess premonitory symptoms of myocardial infarction. Int J Cardiol 1987;17:15–24.
43. Golden SH, Williams JE, Ford DE, et al. Depressive symptoms and the risk of type 2 diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care 2004;27:429–35.
44. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993;138:923–36.
45. Selvin S. Statistical analysis of epidemiologic data. 3rd ed. Oxford, United Kingdom: Oxford University Press, 2004.
46. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol 2002;155:176–84.
47. STATA. Statistics/data analysis: release 8.2. College Station, TX: Stata Corporation, 2002.
48. Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Akabane M, Tsugane S. Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. Eur J Clin Nutr 2001;55:643–50.
49. Cerhan JR, Folsom AR, Mortimer JA, et al. Correlates of cognitive function in middle-aged adults. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Gerontology 1998;44:95–105.
50. Kuriki K, Nagaya T, Tokudome Y, et al. Plasma concentrations of (n−3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. J Nutr 2003;133:3643–50.
51. Andersen LF, Solvoll K, Drevon CA. Very-long-chain n−3 fatty acids as biomarkers for intake of fish and n−3 fatty acid concentrates. Am J Clin Nutr 1996;64:305–11.
52. Hjartaker A, Lund E, Bjerve KS. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997;51:736–42.
53. Brunner E, Stallone D, Juneja M, Bingham S, Marmot M. Dietary assessment in Whitehall II: comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. Br J Nutr 2001; 86:405–14.
54. Knuiman MW, Divitini ML, Buzas JS, Fitzgerald PE. Adjustment for regression dilution in epidemiological regression analyses. Ann Epidemiol 1998;8:56–63.
55. Laurin D, Verreault R, Lindsay J, Dewailly E, Holub BJ. Omega-3 fatty acids and risk of cognitive impairment and dementia. J Alzheimers Dis 2003;5:315–22.
56. Shi J, Perry G, Smith MA, Friedland RP. Vascular abnormalities: the insidious pathogenesis of Alzheimer's disease. Neurobiol Aging 2000; 21:357–61.
57. McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging 2001;22:799–809.
58. de Wilde MC, Hogyes E, Kiliaan AJ, Farkas T, Luiten PG, Farkas E. Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats. Brain Res 2003;988:9–19.
59. Keli SO, Feskens EJ, Kromhout D. Fish consumption and risk of stroke. The Zutphen Study. Stroke 1994;25:328–32.

60. Bonaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study. N Engl J Med 1990;322:795–801.
61. Schmidt EB, Varming K, Ernst E, Madsen P, Dyerberg J. Dose-response studies on the effect of n−3 polyunsaturated fatty acids on lipids and haemostasis. Thromb Haemost 1990;63:1–5.
62. Feskens EJ, Bowles CH, Kromhout D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. Diabetes Care 1991;14:935–41.
63. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71(suppl):228S–31S.
64. Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L. Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol Aging 2005;26:587–95. XXXX.