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n-3 fatty acids, hypertension and risk of cognitive decline among older adults in the Atherosclerosis Risk in Communities (ARIC) study

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Abstract

Objective—Recent research indicates that n-3 fatty acids can inhibit cognitive decline, perhaps differentially by hypertensive status.

Design—We tested these hypotheses in a prospective cohort study (ARIC). Dietary assessment using an FFQ and plasma exposure by gas chromatography were completed in 1987-89 (visit 1), while cognitive assessment with three screening tools DWRT, DSST/WAIS-R and WFT, was completed in 1990-92 (visit 2) and 1996-98 (visit 4). Regression calibration and simulation extrapolation (SIMEX) were used to control for measurement error in dietary exposures.

Setting—Four US communities: Forsyth County (NC), Jackson (MS), suburbs of Minneapolis (MN), and Washington County (MD).

Subjects—Men and women aged 50-65 years at visit 1 with complete dietary data (n=7,814); White men and women in same age group in the MN field center with complete plasma fatty acid data (n=2,251).

Results—Findings indicated that increase by 1 SD of dietary long chain n-3 fatty acids (% of energy intake) and balancing long-chain n-3/n-6 decreased the risk of six-year cognitive decline in verbal fluency subjects with odds ratios of 0.79 (95%CI: 0.66-0.95) and 0.81 (95% CI: 0.68-0.96),

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respectively among hypertensives. An interaction with hypertensive status was found for dietary long-chain n-3 fatty acids (in grams/day) and WFT decline (LR test p=0.06). This exposure in plasma cholesteryl esters was also protective against WFT decline, particularly among hypertensives (OR: 0.51, p<0.05).

Conclusion—One implication from our study is that diets rich in fatty acids of marine origin should be considered for middle aged hypertensive subjects. To this end, randomized clinical trials are needed.

Keywords

Fatty acids; cognitive decline; hypertension; regression calibration; simulation extrapolation; dietary assessment

The proportion of the US population ages 65 and over was 12.3 percent in 2000 based on recent United Nations estimates and is projected to increase rapidly in the coming decades to reach 20 percent by 2050 [1]. As populations age, all cognitive disorders, including dementia, become more common. Recent research indicates that *n*-3 fatty acids may be important in preventing cognitive decline. So far, epidemiological evidence, although inconclusive, suggests a protective effect of *n*-3 fatty acid intake in the diet [2-4]. Essential fatty acids are linked to several biochemical and biophysical functions, including structural integrity and fluidity of membranes, enzyme activities, lipid-protein interactions and serving as precursors for eicosanoids such as prostaglandins, leukotrienes and thromboxanes [5]. The fatty acid composition of neuronal cell membrane phospholipids reflects their intake in the diet [6] and fish oils, which contain high levels of C_{20} and C_{22} polyunsaturated fatty acids (PUFAs), exert the most profound influence on brain PUFA concentration. According to experimental animal studies, there is a plausible pathway by which hypertension and low dietary *n*-3 fatty acid intake may interact in increasing the risk of cognitive decline. In fact, hypertensive rats tended to have lower brain MUFAs and PUFAs than normotensive rats [7], possibly due to pressure-induced endothelial dysfunction at the blood-brain barrier or exhausted astrocytic metabolism. Oxidative stress which accompanies high blood pressure leads to increased peroxidation of unsaturated fatty acids and a reduction in their concentration in the brain represents an alternative explanation.

Despite animal experimental evidence for a possible biological interaction between dietary intake of *n*-3 fatty acids and hypertensive status [7-10] in affecting cognitive decline, no epidemiological study to date has attempted to test this hypothesis. The present observational prospective study assessed the effect of low *n*-3 fatty acid status on six-year cognitive decline in men and women aged 50 years and older. A secondary objective was to explore whether hypertensive subjects would benefit to a larger extent than normotensive subjects from this increased intake.

Data and Methods

Study Sample

Atherosclerosis Risk in Communities (ARIC) is an ongoing prospective cohort study aimed at investigating the etiology of atherosclerosis and its clinical sequelae and the longitudinal

impact of variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time. In each of four US communities--Forsyth County (NC), Jackson (MS), suburbs of Minneapolis (MN), and Washington County (MD)-- 4,000 adults aged 45-64 years were examined four times, three years apart (visits 1 through 4). Three out of the four cohorts represented the ethnic mix of their communities, while at Jackson, MS, only African American residents were recruited [11]. Out of the total sample examined at baseline (N=15,792) we restricted these analyses to 11,557 individuals aged 50 years or older at baseline. Out of the total sample examined at baseline (N=15,792), we restricted these analyses to 11,557 individuals aged 50 years or older at baseline since research clearly shows that risk of cognitive decline in general, and of dementia in particular, is negligible prior to the age of 60 years (which is the age at which the youngest individuals in this cohort were re-examined in visit 4) [12]. Eligibility for these analyses further required survival through visit 4 (n=8,346), complete data on cognitive functioning at visits 2 (1990-92) and 4 (1996-98) yielding a sample size of 8,012 and also complete dietary intake at visit 1 (1987-89), which yielded n=7,814 men and women. Of these, plasma fatty acid data at visit 1 was available on a sub-set of the Minneapolis cohort, MN (n=2,251).

Outcome assessment

Three measures of cognitive functioning were made only for visits 2 and 4 among the total ARIC cohort, and these measures relied on the following instruments: Delayed Word Recall Test (DWRT) Delayed Word Recall Test (DWRT) [14]; the Digit Symbol Substitution portion of the Revised Weschler Adult Intelligence Scale (DSST/WAIS-R) [15], and Word Fluency Test (WFT) of the Multilingual Aphasia Examination, also known as the controlled oral word association [16].

The *Delayed Word Recall Test (DWRT)*: This screening tool assesses verbal learning and recent memory. It requires the respondent to recall 10 common words after a 5-minute 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 seconds. The 6-months test-retest reliability of DWRT was previously shown to be high among 26 normal elderly individuals (Pearson correlation coefficient, r=0.75) [14].

The *Digit Symbol Substitution (DSST/WAIS-R):* This test is a paper-and-pencil test requiring timed translation of numbers 1 through 9 to symbols using a key. The test measures psychomotor performance and is relatively unaffected by intellectual ability, memory, or learning for most adults[16]. It appears to be a sensitive and reliable marker of brain damage[17]. The test score can range between 0 and 93 and it reflects the correctly translated number of digit-symbol pairs within a time limit of 90 seconds. Short-term test-retest reliability over 2-5 weeks has been found to be high in individuals aged 45-54 years (r=0.82) [15].

The *Word Fluency Test (WFT):* This test requires subjects to record as many words as possible using the initial letters F, A and S and to list these words, the subject is given only 60 seconds per letter. The total score corresponds to the total number of words generated during these three trials. The test is particularly sensitive to linguistic impairment [1618] and early mental decline in older persons [19]. It is also a sensitive marker of damage in the left

lateral frontal lobe [1618]. The immediate test-retest correlation coefficient based on an alternate test form has been found to be high (r=0.82) [20].

Preliminary analysis suggested that while visits 2 and 4 scores of DSST and WFT had a correlation coefficient close to 0.5, correlations between DSST and DWRT were 0.4 and between DWRT and WFT around 0.4. However, cognitive changes (V4-V2) in each of these scales had much weaker correlations with each others ranging between 0.06 and 0.09.

Cutoff points were determined for decline in each of three cognitive status tests using the Reliable Change Index (RCI) method in order to correct for measurement error and practice effects [21]. RCI is defined as $((X_2-X_1)-(M_2-M_1))/S.E.D.$, where X_1 is the individual's score at baseline, X_2 the individual's score at follow-up, M_1 and M_2 are the group mean pretest and follow-up scores respectively, and S.E.D. the observed standard error of the difference scores. Scoring below an RCI of -1.645 was regarded as a "statistically reliable" deterioration in the test scores.

A composite measure of the three RCIs to assess global cognitive decline (GCD) was created using principal components analysis (PCA), a data reduction technique [22]. Similarly, the cutoff point of the composite score for statistically reliable global cognitive decline was chosen to be -1.645.

Exposure Assessment

Usual dietary intake was estimated from an interviewer-administered 61-item semi-quantitative food frequency questionnaire (FFQ) previously developed and validated by W. Willet and colleagues against multiple food records among a sub-sample of the Nurse's Health Study cohort [23]. Dietary intake of essential fatty acids and their elongated and desaturated products were expressed as percent of total energy intake and grouped under four main categories, as suggested by Lands and colleagues [2425]: **(3P)** n-3 C_{18} polyunsaturated fatty acids: 18:3+18:4n-3 **(6P)** n-6 C_{18} polyunsaturated fatty acids: 18:2+18:3n-6 **(3H)** n-3 C_{20} and C_{22} highly unsaturated fatty acids (HUFAs): 20:5+22:5+22:6n-3 and **(6H)** n-6 HUFAs: 20:3+20:4+22:4+22:5n-6. Sums of fatty acid intake as percent of energy included (3)= (3P)+(3H) and (6)=(6P)+(6H). Ratios of interest included (3P)/(6P), (3H)/(6H) and (3P+3H)/(6P+6H) also denoted as 3/6. In multivariate models, all these variables were standardized by subtracting each observation from the variable mean and dividing the difference by the standard deviation. Hence, the main exposures of interest were 3P, 3H, 3 (as percent of energy intake), 3P/6P, 3H/6H, 3/6, and total 3H (in grams per day). Adjustment was made for the other fatty acid variables, and total energy intake was considered as a potential confounder to emulate a multivariate nutrient density model[26].

Twelve-hour fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted fatty acid analysis of plasma phospholipid and cholesteryl ester fractions on visit 1 blood specimens. The procedure is described in detail elsewhere [27]. The identity of 28 fatty acid peaks were revealed 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 percent of all

fatty acids) was calculated by integrating the area under the peak, dividing the result by the total area for all fatty acids, and multiplying by 100. Data from the chromatogram were transferred electronically to a computer for analysis. Plasma exposures are expressed as % of total fatty acids in each fraction and were grouped similarly to dietary exposure. Test-retest reliability coefficients (individuals sampled 3 times, 2 weeks apart) for various plasma fatty acids ranged from 0.50 to 0.93 for cholesteryl esters to 0.89 for phospholipids[28]. However, only 3H and the ratio of 3H/6H were considered in these analyses.

Replicate dietary measures

Dietary intake was assessed among the surviving ARIC sample at visit 3 (1992-94), using the same semi-quantitative food frequency questionnaire that was administered at baseline. At visit 2, a sub-sample of ARIC (around 10% of the original sample) was asked to repeat the FFQ. As stated earlier, of our eligible subset with baseline data on exposure and complete outcome data (n=7,814), 657 had data on visit 2 exposure, 7,482 had complete data at visit 3, while 634 had both.

Covariates

Most covariates considered as potential confounders were measured at visit 1, although some were defined according to criteria that spanned all four visits. Covariates can be subdivided into socio-demographic, genetic, health behaviors and nutritional. Age, gender, ethnicity and education were all reported by the respondent. Apo E genotype was categorized as 0 to indicate the absence of an ε4 allele vs.1 to denote carrier status for at least one ε4 allele. Among the behavioral factors (all measured at visit 1), smoking was represented on a three-level categorical scale, namely: never smoked, smoked previously and current smoker. Food frequency questionnaire derived values of alcohol (grams/day) and caffeine (mg/day) were considered as well. Physical activity was assessed using a questionnaire developed by Baecke and colleagues, including 16 items about usual exertion [29]. A validated index of physical activity was derived at visit 1, summing sports, work and leisure indices which ranged from a score of 1 (low) to 5 (high) [30]. Body mass index at visit 1 was computed by dividing weight in kilograms by the height-squared (in square meters). Baseline dietary intake of antioxidants and other micronutrients (mainly Vitamins B_6, B_{12} and folate) was considered as well [23]. The association of these covariates with our outcome has been previously documented by similar cohort studies based on ARIC data [31-34].

Our main effect modifier, hypertension, was operationalized using measured systolic and diastolic blood pressure at each visit as well as use of anti-hypertensive medication over the past two weeks. Seated blood pressure levels were calculated as the average of the second and third of three consecutive measurements with a random-zero sphygmomanometer and hypertension was defined as ≥ 140 mm Hg. for SBP and ≥ 90 mm Hg. for DBP or the use of anti-hypertensive medication during the past two weeks prior to examination on any of visits 1 through 4.

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= decline) and assessed statistical significance of differences using independent samples *t*-test at an alpha level of 0.05. We computed odds ratios of decline with increase in each exposure by 1 SD through a multivariate logistic regression analysis. Control for confounding was accomplished using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimated effect of the exposure by more than 5% were retained in the final model [35].

Hypertension was considered as a potential effect modifier. Likelihood ratio tests were used to assess statistical significance of interaction between exposure and hypertensive status at a type I error level of 0.20, after obtaining the final parsimonious model [3637]. The multivariate models can be summarized by equations (1) and (2):

$$\text{Logit}[\Pr(Y=1|Q,Z)]=\beta_0+\beta_1 Q_1+\sum \beta_{2i}Q_{2i}+\sum \beta_j Z_j \quad (1)$$

$$\text{Logit}[\Pr(Y=1|Q,Z,H)]=\beta_0+\beta_1 Q_1+\sum \beta_{2i}Q_{2i}+\beta_3 H+\sum \beta_j Z_j+\gamma Q_1\times H \quad (2)$$

In the above equations, Q_1 is the main exposure of interest as derived from the food frequency questionnaire, Q_{2i} are the other fatty acids that might act as confounders, Z_j is a vector of potential confounders that are assumed to be perfectly measured (i.e. no error variance associated with them), and H is the potential effect modifier "hypertensive status" also assumed to be perfectly measured. The same process was used with the plasma exposures in cholesteryl esters and phospholipids. To correct for measurement error in dietary exposure, a sensitivity analysis was conducted for models **(1)** and **(2)** whereby regression calibration and simulation extrapolation were applied to the final parsimonious models for each outcome/exposure pair [3839]. In a multivariate setting, both RCAL and SIMEX rely on the method of moments and attempt to estimate the error variance in the error-prone exposure and adjust the exposure-outcome effect using different procedures. In both cases, replicate measures of FFQ measurements at visits 2 and 3 were used for the correction. The two methods are described in more detail in the online Appendix. Statistical analyses were conducted using STATA ver. 9.0 [40].

Results

Characteristics of study subjects

Table 1 shows the characteristics of study subjects according to availability of dietary and plasma fatty acid data as well as cognitive assessment data at both points in time. Subjects in the plasma fatty acid group consisted of whites residing in the suburbs of Minneapolis. They were in general more educated than the dietary group, which was a mix from all ARIC centers. They had a lower proportion of women (50.7% vs. 54.63%), a lower prevalence of the ApoEε4 allele (28.8% vs. 30.0%), a higher proportion "ever smoked" status (59.5% vs. 55.4%), and greater consumption of alcohol and caffeine. Some differences were noted for

other behavioral and nutritional factors as well. Hypertensive status was particularly high in the dietary group (56%) compared to 49% among the plasma group. Raw mean scores of baseline cognitive function were greater among those in the plasma compared to the dietary group and average declines between visits 2 and 4 were found to be steeper in the former. Table 2 summarizes the distribution of dietary and plasma fatty acid exposures considered. Standard deviations (SD) for Q_1, M and N are of particular importance in interpreting multivariate logistic regression analysis. Q_2 and Q_3 represent replicate measurements on Q_1 at visits 2 and 3 which were used to correct for measurement error in the exposure.

Multivariate analysis findings: dietary exposures

Multivariate logistic regression of the relationship between dietary exposure and cognitive decline is presented in Table 3. Results indicate that risk of clinically significant decline in DWRT over the period of 6 years was reduced modestly with every standard deviation increase in long-chain n-3 fatty acid intake (*3H*) as % of total energy intake. This was observed in the total population and among the hypertensive subgroup. For DSST/WAIS-R, although the ratio *3H/6H* was protective against decline, this effect did not reach statistical significance. However, the likelihood ratio test indicated a significant level of interaction between this exposure and hypertensive status, shown by the variation in effect across strata of the effect modifier (1.09 among normotensives *vs.* 0.88 among hypertensives). Risk of decline in WFT was reduced by long chain and all types of n-3 fatty acid intake (*3H* and *3*) as % of total energy intake, and by the ratio of 3H/6H and 3H in grams/day. This relationship was stronger among hypertensive subjects and a significant interaction was noted for *3H* in grams/day (LR test p=0.06). No statistically significant results were observed for global cognitive decline or other dietary exposures. After adjusting for measurement error in the dietary covariate using regression calibration, loss of precision in measures of effect was observed. In most cases, bias seemed to be towards the null when comparing naïve and calibrated estimates. In few instances, significance of odds ratios was preserved, such as in the case of *3H, 3H(grams/day), 3* and *3H/6H* with WFT decline as the outcome among the hypertensive stratum (data not shown). In these four exposures respectively, the calibrated odds ratios for WFT decline in the hypertensive stratum with their 95% CI were 0.68 (0.49, 0.95), 0.69 (0.47,1.00), 0.65 (0.43, 1.00) and 0.70 (0.52, 0.95). Using SIMEX for selected associations, similar results were obtained. Figure 1 shows two examples of stratified models 3.2b and 3.2g of Table 3 (3H and ratio of 3H/6H's effect on decline in WFT). The figures show the extent to which naïve estimates are biased towards the null when compared to the corrected regression coefficients using the SIMEX method. This method is described in detail in the online appendix.

Multivariate analysis findings: plasma exposures

Multivariate logistic analyses of the plasma fatty acid data (Table 4) indicated generally lower odds of cognitive decline among subjects with a higher concentration of long chain n-3 fatty acid in their plasma cholesteryl esters and phospholipids, and an elevated ratio of long chain *n*-3/*n*-6 fatty acids. An interaction was noted between WFT for absolute 3H in cholesteryl esters (OR: 0.74 among normotensives *vs.* 0.51 among hypertensives) without reaching statistical significance at the level of 0.10 (p=0.25). The same pattern was observed for the ratio of 3H/6H in cholesteryl esters and both exposures in the phospholipid fraction.

Discussion

This population-based prospective study conducted among middle-aged men and women at baseline showed that increased dietary intake of long chain *n*-3 fatty acids and balancing long-chain *n*-3/*n*-6 decreased the risk of cognitive decline in verbal fluency, particularly among hypertensive subjects. This finding also held for the corresponding plasma analytes in the cholesteryl ester and phospholipid fractions. This finding may be due to the higher sensitivity of WFT to early mental decline (i.e. among those aged 55 years or more at baseline) when compared to DWRT and DSST/WAIS-R [19]. Limitations of the study include the lack of psychometric diagnosis for mild cognitive impairment, which might have been a more definite and clinically relevant outcome[41]. However, the neuropsychological tests used represent some of the domains reported to be most sensitive in discriminating between normal aging and mild cognitive impairment [42]. Another limitation relates to the nature of dietary exposures in general which are often prone to measurement error both in terms of validity and short-term reliability. We corrected for validity by using an alloyed gold standard and two instrumental biomarkers but failed to correct for short-term reliability of the food frequency questionnaire due to the lack of adequate short-term replicates in ARIC. Nevertheless, a previous study by Ma and colleagues [28] provides estimate for short-term reliability of each of the fatty acids that were considered. Moreover, residual confounding due to inadequate control or measurement of potential confounders cannot be totally ruled out. It is important to note also that exposure timing (one year prior to visit 1) did not coincide with the baseline measurement of outcome (visit 2) which constitutes another major limitation.

One of the main strengths of this study is its prospective design which, as stated earlier, thus far is unique in the literature testing this particular hypothesis [43]. An evidence-based report suggested a need to look for the effect of *n*-3 fatty acids on cognitive decline by cardiovascular disease status and to define exposure in terms of absolute value of medium chain and long chain fatty acids, as well as the ratio between *n*-3 and *n*-6 fatty acids in diet and plasma [44]. All these suggestions were implemented in the present study. Moreover, this is the first study to assess effect modification by hypertensive status and to test at the population level a biological interaction documented in animal experimental work. Measurement error, which almost always accompanies dietary assessment, was corrected for in this study using regression calibration for all associations and SIMEX for a selected number of these associations.

Previous epidemiological studies have shown that the fatty acids composition of plasma differs significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment [45-47]. While the majority showed a beneficial effect of plasma and erythrocyte *n*-3 fatty acids on cognition, a case-control study – the Canadian Study of Health and Ageing – reported that the mean relative plasma concentration of *n*-3 fatty acids as well as total polyunsaturated fatty acids was higher among subjects aged 65 years or more with cognitive impairment or dementia after controlling for demographic, behavioral and genetic factors [48]. Epidemiological studies based on dietary assessments of *n*-3 fatty acids also had suggestive but somewhat controversial results. While most leaned

towards a protective effect of increasing intake of these fatty acids in the diet [2349-51], others found no effect or the opposite effect on cognitive functioning and decline [452].

One possibly important implication from our study's results is that diets rich in fatty acids of marine origin should be considered for middle aged subjects. We explored whether such an association was differential according to hypertensive status. Although no statistically significant interactions were found, the results suggest that hypertensive subjects (e.g. odds ratios for 3H and WFT were <1 with $p<0.05$) may benefit from supplementation of their diets to a larger extent than the normotensive group. These results merit replication given the large public health potential that would be associated with results that unequivocally indicate inverse association between fatty acid intake and reduced cognitive decline in the general population. The literature indicates that these fatty acids were frequently found associated with reduced risk of cardiovascular disease, including stroke [53] and coronary heart disease [5455], although thus far all the evidence is of an observational nature. They have also been associated with improved insulin sensitivity[56], reduced risk of dyslipidemia [57] and a hypocoagulable profile [58] among other health benefits. Because many of these conditions are also related to cognitive impairment, future research should focus on disentangling the direct and indirect effects of fatty acids (using plasma biomarkers) on cognition and uncover the main mechanism involved in their ability to prevent clinically significant decline in aging populations. Finally, these findings suggest the utility of randomized clinical trials that would augment intake of marine fatty acids in the treatment group and give a non-enriched diet to the placebo group while allowing for stratification by baseline hypertensive status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AD	Alzheimer's Disease
ARIC	Atherosclerosis Risk in Communities
DSST/WAIS-R	Digit Symbol Substitution Test of the Wechsler Adult Intelligence Scale – Revised
DWRT	Delayed Word Recall Test
FFQ	Food Frequency Questionnaire
GBCF	Global Baseline Cognitive Functioning
GCD	Global Cognitive Decline
HUFA	Highly Unsaturated Fatty Acids

MCI	Mild Cognitive Impairment
MUFA	mono-unsaturated fatty acids
PCA	Principal components analysis
PUFA	Polyunsaturated fatty acids
RCAL	Regression Calibration
RCI	Reliable Change Index
SIMEX	Simulation extrapolation
WFT	Word Fluency Test

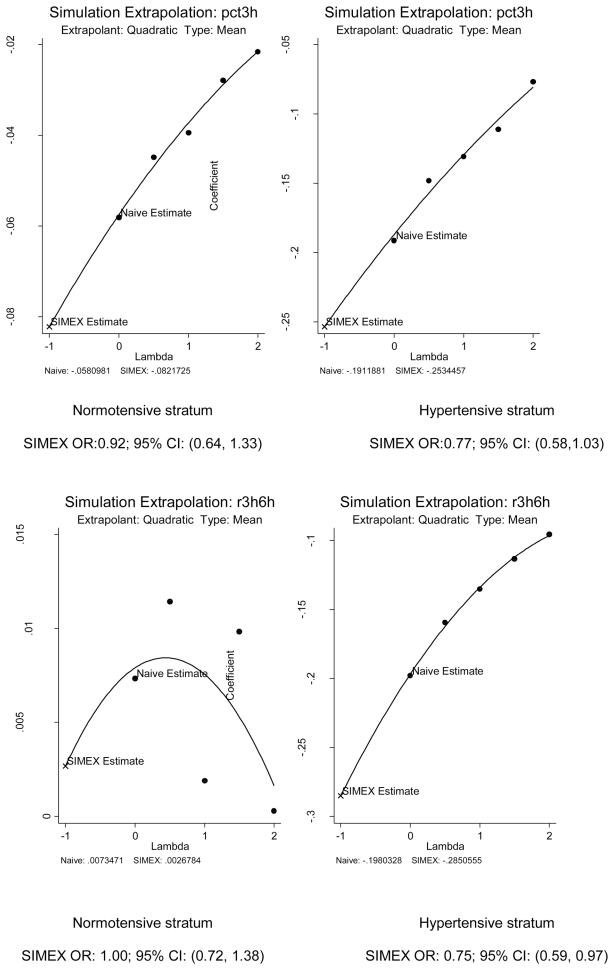

Figure 1. SIMEX plot of corrected coefficients for stratified models 3b and 3e of Table 3; ARIC (1987-98)*

*pct3h: dietary intake of long chain *n*-3 fatty acids expressed as percentage of energy intake (3H); r3hr6h: ratio of long chain *n*-3 to long chain n-6 fatty acids (3H/6H). Lambda is equivalent to $\theta = \{0.5, 1, 1.5, 2\}$ and is a scale factor used to add error to the covariate and estimate $\beta m = f(\theta, \beta m^{59})$ starting from the naïve estimate in which $\theta = 0$. Hence, the naïve estimate of the regression coefficient β is the one estimated by generalized linear models without measurement error correction. See online Appendix for more details.

Table 1

Characteristics of study subjects with complete cognitive and dietary data between visits 1 and 4 (Dietary group; N=7,814) and those with complete cognitive and plasma data (Plasma group; N=2,251); ARIC 1987-98

	Dietary group (All ARIC centers)		Plasma group (MN whites)	
Characteristics	**Mean (%)**	**(SD)**	**Mean (%)**	**(SD)**
Female	54.63		50.69 *	
Age (years) †	56.56	(4.31)	56.30 *	(4.24)
White †	81.48		100.00 *	
Education †				
Incomplete high school	20.24		6.67 *	
High School	34.06		36.18	
> High School	45.70		57.16	
Apo E ε4 allele †	30.00		28.84	
Smoking status †				
Never smoker	44.54		40.40 *	
Former smoker	35.55		41.82	
Current smoker	19.91		17.78	
Alcohol (g/day) †	5.88	(12.67)	8.08 *	(13.47)
Caffeine (mg/day) †	291.04	(290.82)	348.08 *	(325.93)
Physical activity scale †	7.06	(1.39)	7.33 *	(1.33)
Body mass index (kg/m^2) †	27.48	(4.96)	27.17 *	(4.41)
Total energy intake (Kcal/day) †	1579	(571)	1581	(559)
Vitamin A (in 1000 IUs/day) †	9.13	(6.97)	8.65 *	(6.83)
Vitamin B_6 (mg/day) †	1.75	(0.67)	1.74 *	(0.66)
Vitamin B_{12} (mcg/day) †	7.61	(4.23)	7.06 *	(3.50)
Vitamin C (mg/day) †	122.39	(80.92)	112.67 *	(69.95)
Vitamin E (mg/day) †	4.97	(3.11)	4.66 *	(3.01)
Folate (mcg/day) †	232.59	(101.18)	218.48 *	(94.97)
Hypertensive ‡	56.01		49.27 *	
Baseline cognitive scores (V2) §				
DWRT	6.61	(1.46)	6.78 *	(1.43)
DSST/WAIS-R	45.02	(13.01)	51.65 *	(10.15)
WFT	33.77	(12.06)	37.59 *	(11.52)
Cognitive change (V4-V2) §				
DWRT	-0.17	(1.56)	-0.21	(1.50)
DSST/WAIS-R	-2.80	(6.79)	-4.45 *	(6.36)

	Dietary group (All ARIC centers)		Plasma group (MN whites)	
Characteristics	**Mean (%)**	**(SD)**	**Mean (%)**	**(SD)**
WFT	-0.77	(7.86)	-1.84 *	(7.78)

* $P<0.05$ for null hypothesis that means or proportions are equal between plasma and non-plasma groups.

† Covariate measured at visit 1.

‡ Covariate measured at visits 1 through 4: period prevalence over 9 years.

§ Covariate with other time frame.

Table 2

Distribution of fatty acid groups and ratios Q_1, M, N, Q_2 and Q_3 : Mean ±SD; ARIC (1987-1995)[1]

	Q_1		M		N		Q_2		Q_3	
	(n=7,814)		(n=2,251)				(n=634)			
Fatty acid groups and ratios *j*										
3p	0.41	±0.09	0.41	±0.10	0.14	±0.05	0.40	±0.09	0.41	±0.10
3H	0.18	±0.16	1.01	±0.39	3.44	±1.05	0.17	±0.15	0.16	±0.15
3	0.60	±0.19	1.42	±0.43	3.59	±1.05	0.57	±0.18	0.57	±0.18
3p/6p	0.10	±0.04	0.01	±0.00	0.01	±0.00	0.10	±0.02	0.11	±0.05
3H/6H	2.27	±1.87	0.11	±0.05	0.22	±0.08	2.22	±1.71	2.12	±1.69
3/6	0.15	±0.07	0.02	±0.01	0.09	±0.03	0.14	±0.07	0.15	±0.07
3H (g/day)	0.30	±0.28	—	—	—	—	0.28	±0.26	0.27	±0.29

[1] Q_1: Food frequency questionnaire measurement at visit 1 of fatty acid group intake as % of energy intake or ratio of n-3 to n-6 groups. M: biomarker of fatty acid intake in cholesteryl ester fraction of plasma; N: biomarker of fatty acid intake in phospholipid fraction of plasma; Q_2: Repeat of Q_1 measured at visit 2 among a subset of the cohort; Q_3: Repeat of Q_1 measured at visit 3 among the surviving baseline cohort.

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Table 3

Multivariate Logistic models of cognitive decline and dietary *n*-3 fatty acid exposures[†]: naïve and regression calibrated odds ratios; ARIC (1987-98)

	Statistically reliable cognitive decline (RCI<-1.64)															
	DWRT				DSST/WAIS-R				WFT				GCD			
	Naïve		RCAL[§]		Naïve		RCAL		Naïve		RCAL		Naïve		RCAL	
Dietary fatty acids (n=7,814)	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
	Models 1a-1g				Models 2a-2g				Models 3a-3g				Models 4.1a-4.1g			
Q_1																
3P	1.02	(0.93, 1.12)	1.02	(0.78, 1.34)	0.96	(0.87, 1.07)	0.89	(0.66, 1.18)	1.01	(0.92, 1.12)	1.22	(0.90, 1.64)	0.98	(0.90, 1.08)	1.00	(0.76, 1.31)
3H	0.90*	(0.81, 1.00)	0.88	(0.72, 1.05)	0.93	(0.83, 1.04)	0.96	(0.76, 1.22)	0.85*	(0.75, 0.96)	0.80	(0.62, 1.04)	0.91	(0.82, 1.02)	0.89	(0.71, 1.11)
3	0.96	(0.87, 1.06)	0.99	(0.81, 1.21)	0.90	(0.80, 1.02)	0.86	(0.62, 1.18)	0.87*	(0.77, 0.98)	0.85	(0.62, 1.17)	0.90	(0.81, 1.01)	0.85	(0.64, 1.13)
3P/6P	1.01	(0.92, 1.10)	1.00	(0.81, 1.23)	1.04	(0.94, 1.15)	1.11	(0.89, 1.39)	1.01	(0.91, 1.10)	1.21	(0.95, 1.53)	1.01	(0.92, 1.10)	1.08	(0.87, 1.35)
3H/6H	0.96	(0.87, 1.06)	0.96	(0.80, 1.16)	1.03*	(0.93, 1.13)	1.16	(0.91, 1.47)	0.86*	(0.77, 0.97)	0.84	(0.64, 1.10)	0.97	(0.88, 1.06)	0.94	(0.73, 1.21)
3/6	0.92	(0.83, 1.02)	0.89	(0.72, 1.08)	1.01	(0.92, 1.12)	1.09	(0.91, 1.30)	0.95	(0.85, 1.06)	1.04	(0.85, 1.27)	0.98	(0.89, 1.07)	1.01	(0.85, 1.21)
3H (g/day)	0.91	(0.81, 1.01)	0.89	(0.69, 1.13)	0.97	(0.87, 1.07)	1.09	(0.89, 1.36)	0.87**	(0.76, 0.99)	0.89	(0.66, 1.20)	0.96	(0.87, 1.06)	1.05	(0.85, 1.30)

* $p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (1) and (2).

** $p<0.10$ for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test. See equation (2).

† Exposures were standardized by subtracting each observation from its mean and dividing it by its Standard Deviation. Each model (e.g. 1a) has one exposure/outcome pair.

‡ Control for confounding was done using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimate of exposure by more than 5% were retained in the final model. Covariates considered as potential confounders were: socio-demographics (age, sex, education, race); genetic factors (ApoE ε4 allele); behavioral factors (smoking, alcohol, caffeine consumption and physical activity) and nutritional factors (body mass index, caloric intake, other fatty acids, intake of antioxidants and vitamins B_6, B_{12} and folate). Hypertension was considered as a potential effect modifier separate models. Multiplicative interaction was tested using the likelihood ratio test at an alpha level of 0.10.

§ RCAL: Regression calibrated odds ratio (adjusted for measurement error in dietary fatty acids) with its 95% confidence interval, using replicate dietary fatty acid measures at visits 2 and 3 (Q_2 and Q_3).

Table 4

Multivariate Logistic models of cognitive decline and plasma *n*-3 fatty acid exposures: Interaction with hypertensive status: ARIC (1987-98)

	Statistically reliable cognitive decline (RCI<-1.64)							
	DWRT		DSST/WAIS-R		WFT		GCD	
Plasma fatty acids (n=2,251)	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Plasma cholesteryl ester[§] **(M)**								
	Models 1.1a-1.1b		**Models 2.1a-2.1b**		**Models 3.1a-3.1b**		**Models 4.1a-4.1b**	
All Subjects								
3H	1.00	(0.82, 1.21)	0.91	(0.73, 1.14)	0.63*	(0.46,0.86)	1.03	(0.87, 1.22)
3H/6H	0.92	(0.75, 1.14)	0.92	(0.74, 1.15)	0.63*	(0.45, 0.87)	0.97	(0.81, 1.16)
Normotensive stratum								
3H	1.09	(085, 1.40)	0.99	(0.74, 1.32)	0.74	(0.48, 1.12)	1.13	(0.92, 1.38)
3H/6H	1.02	(0.79, 1.32)	1.00	(0.76, 1.32)	0.73	(0.48, 1.11)	1.09	(0.88, 1.34)
Hypertensive stratum								
3H	0.89	(0.65, 1.21)	0.78	(0.55, 1.12)	0.51*	(0.32, 0.83)	0.92	(0.69, 1.22)
3H/6H	0.79	(0.56, 1.13)	0.76	(0.51, 1.14)	0.51*	(0.31, 0.84)	0.78	(0.55, 1.11)
Plasma phospholipids[§] **(N)**								
	Models 1.2a-1.2b		**Models 2.2a-2.2b**		**Models 3.2a-3.2b**		**Models 4.2a-4.2b**	
All Subjects								
3H	0.93	(0.76, 1.14)	1.04	(0.87, 1.24)	0.77*	(0.61, 0.97)	0.96	(0.80, 1.15)
3H/6H	0.89	(0.73, 1.10)	0.99	(0.82, 1.20)	0.72*	(0.55, 0.94)	0.93	(0.77, 1.13)
Normotensive stratum								
3H	1.03	(0.77, 1.36)	1.07	(0.83, 1.39)	0.83	(0.60, 1.15)	1.03	(0.77, 1.36)
3H/6H	0.99	(0.75, 1.30)	1.06	(0.83, 1.37)	0.80	(0.57, 1.13)	1.04	(0.80, 1.35)
Hypertensive stratum								
3H	0.83	(0.61, 1.11)	0.97	(0.75, 1.26)	0.74	(0.53, 1.04)	0.94	(0.73, 1.22)
3H/6H	0.77	(0.56, 1.08)	0.89	(0.66, 1.21)	0.66*	(0.43, 0.99)	0.86	(0.64, 1.15)

*$p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (1) and (2).

** p<0.10 for testing the null hypotheses that γ=0 using the likelihood ratio test. See equation (2).

† Exposures were standardized by subtracting each observation from its mean and dividing it by its Standard Deviation. Each model (e.g. 1.1a) has one exposure/outcome pair. These models are then stratified by hypertensive status for each of the two exposures.

‡ Same analytic approach was used as in Table 3.

§ Plasma cholesteryl ester levels of fatty acids (%); Plasma phospholipids levels of fatty acids. M and N in reference to standard deviations (SD) values from Table 2.