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Effect of Omega-3 Fatty Acids, Lutein/Zeaxanthin, or other Nutrient Supplementation on Cognitive Function: The AREDS2 Randomized Clinical Trial

the Age-Related Eye Disease Study 2 (AREDS2) Research Group, **Emily Y. Chew, MD**[1], **Traci E. Clemons, PhD**[2], **Elvira Agrón, MA**[1], **Lenore J. Launer, PhD**[3], **Francine Grodstein, ScD**[4], and **Paul S. Bernstein, MD, PhD**[5]

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Abstract

Importance—Observational data have shown high dietary intake of saturated fat and low intake of vegetable were associated with increased risk of Alzheimer disease.

Objective—To test the effects of oral supplementation with nutrients on cognitive function.

Design, Setting and Participants—In the double-masked (blinded) randomized controlled clinical trial, the Age-Related Eye Disease Study 2, retinal specialists in 82 academic and community medical centers in the United States enrolled and followed participants who were at risk for developing late age-related macular degeneration (AMD) from October 2006 to December 2012. In addition to annual eye examinations, several validated cognitive function tests were

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Conflict of Interest

Emily Y. Chew, Elvira Agrón, and Lenore Launer, are employees of the National Institutes of Health that sponsored the study. Emily Y. Chew, Elvira Agrón, Traci E. Clemons, Lenore Launer, and Fran Grodstein have no financial or other conflicts of interest. Paul Bernstein: reported serving as a consultant for Kemin Health, Kalsec, DSM, and Science Based Health.

1. Emily Y. Chew: literature search, study design, data collection, analyses, interpretation, writing manuscript. Had full access to the study data.
2. Traci E. Clemons: study design, data collection, analyses, interpretation, writing manuscript and critical review of manuscript. Had full access to the study data.
3. Elvira Agron: data analyses, interpretation, figures, and critical review of the manuscript
4. Lenore J. Launer: data interpretation, writing and critical review of the manuscript
5. Francine Grodstein: study design, data interpretation, writing and critical review of the manuscript
6. Paul Bernstein: data collection and interpretation, critical review of the manuscript

administered via telephone by trained personnel at baseline and every 2 years during the 5 year study.

Interventions—Long-chain polyunsaturated fatty acids (LCPUFAs) (1 g) and/or lutein (10 mg)/zeaxanthin (2 mg) versus placebo were tested. All participants were also given varying combination of vitamins C, E, beta-carotene, and zinc, known as the Age-Related Eye Disease Study supplement.

Main Outcomes and Measures—The main outcome was the yearly change in composite scores determined from a battery of cognitive function tests from baseline. The analyses, which were adjusted for baseline age, sex, race, history of hypertension, education, cognitive score and depression score, evaluated the differences in the composite score between the treated vs. untreated groups. The composite score provided an overall score for the battery, ranging from −22 to 17, with higher scores representing better function.

Results—While 89% (3741/4203) AREDS2 participants consented to the ancillary cognitive function study, 93.6% (3501/3741) underwent cognitive function testing. The mean (SD) age of the participants was 72.7 (7.7) years and 57.5% were female. There were no statistically significant differences in change in scores for participants randomized to the supplements vs. those that were not. The yearly change in the composite cognitive function score was −0.19 (99% Confidence Interval [CI]: −0.25 to −0.13) for participants randomized to LCPUFAs and was −0.18 (99% CI: −0.24 to −0.12) for those randomized to no LCPUFAs (difference in yearly change: −0.03, 99% CI: −0.20 to 0.13, p=0.63). Similarly, the yearly change in the composite cognitive function score was −0.18 (99%CI: −0.24 to 0.11) among those randomized to receive lutein/zeaxanthin vs −0.19 (99%CI: −0.25 to −0.13) among those randomized to not receive lutein/zeaxanthin (difference in yearly change: 0.03, 99% CI: −0.14 to 0.19, p=0.66).

Conclusions and Relevance—Among older persons with AMD, oral supplementation with LCPUFAs and lutein/zeaxanthin had no statistically significant effect on cognitive function.

Introduction

The prevalence of Alzheimer disease, estimated to have affected 5.2 million people in the United States in 2013, may triple in the next four decades.[1] Epidemiologic studies have suggested that diets high in omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) have a protective role in maintaining cognitive function.[2] Docosahexaenoic acid (DHA), a component of omega-3 LCPUFA, is an essential structural component of the brain cells, and low levels of DHA have also been found in persons with Alzheimer disease.[3] For these reasons, omega-3 LCPUFAs were tested for the treatment of dementia. However, numerous randomized controlled clinical trials failed to show omega-3 LCPUFAs to be effective for treating dementia.[4,5]

Similarly, observational data suggested that high dietary intake or high plasma levels of antioxidants may also be associated with better cognitive performance,[6,7,8] while randomized clinical trials did not support this hypothesis.[9,10] Results of a randomized trial of beta-carotene suggested that this carotenoid might be important in the treatment of dementia, depending on the duration of supplementation.[11] A possible role for lutein and zeaxanthin in the treatment of cognitive impairment in the older adults has also been raised,

in one small randomized clinical trial of lutein combined with omega-3 LCPUFAs in 49 women with limited follow-up.[12]

The Age-Related Eye Disease Study 2 (AREDS2),[13] a controlled randomized clinical trial (registered at clinicaltrials.gov: NCT00345176) of omega-3 fatty acids and/or lutein/zeaxanthin supplements for the treatment of age-related macular degeneration (AMD) and cataract, provided an opportunity to evaluate the role of these oral supplements in preventing cognitive decline. AREDS2 enrolled one of the largest numbers of study participants for cognitive function testing at baseline and every 2 years in one of the longest duration of study follow-up (median of 5 years), providing a more definitive result on the effects of oral nutritional supplementation on cognition.

Method

The study design has been published1024 (protocol available in supplementary materials).[13] AREDS2 limited enrollment to people at high risk of progressing to late AMD, those with either bilateral large drusen or large drusen in one eye and late AMD in the fellow eye. The clinical research was conducted according to the Declaration of Helsinki and all Institutional Review Boards approved the AREDS2 research protocol. All participants provided written informed consent for AREDS2. At the time of randomization, study participants were asked whether they agree to be contacted for the ancillary study. Within 3 months following randomization, verbal informed consent was obtained and written materials regarding the AREDS2 Cognitive Function Study were provided to the study participants. This study, supported by National Institutes of Health (NIH), was required to gather information on race and ethnicity. Using guidelines from the *NIH Health Policy on Reporting Race and Ethnicity Data*: *Subjects in Clinical Research*, self-reported race and ethnicity of the AREDS2 participants were collected with two ethnic categories (Hispanic or Latino and Not Hispanic or Latino) and five racial categories (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, and White). Participants were able to select more than 1 racial category.

AREDS2 was a randomized, double-masked, placebo-controlled, 2 × 2 factorial trial evaluating the risks and benefits of adding omega-3 long-chain polyunsaturated fatty acids (LCPUFAs), specifically docosahexaenoic acid (DHA) (350 mg) and eicosapentaenoic acid (EPA) (650 mg) and/or lutein/zeaxanthin (10 mg/2mg), to the original AREDS formulation, or one of the variations of the AREDS formulation, for the treatment of AMD. Study participants were randomly assigned in a 1:1:1:1 allocation to take one of the following study supplements daily: 1) placebo; 2) DHA/EPA; 2) lutein/zeaxanthin; or 4) DHA/EPA and lutein/zeaxanthin. The investigational products matched the placebos in size, shape and taste. The original randomization scheme is displayed in the consort graph of our previous publications of the primary results and in the supplementary materials (e-Figure 1).[14]

Because they are known to be at high risk for developing late AMD, all AREDS2 participants were also offered the original or a modified version of the AREDS formulation. A second randomization was conducted to evaluate the effect of eliminating beta-carotene from the AREDS supplements (beta-carotene vs. no beta-carotene) and the effect of

comparing 80 mg of zinc vs. 25 mg of zinc. Those who consented to the optional secondary randomization were randomly assigned to: 1) AREDS formulation (vitamins C 500 mg, E 400 IU, beta-carotene 15 mg, zinc oxide 80 mg, and cupric oxide 2 mg), 2) AREDS formulation minus beta-carotene, 3) AREDS formulation with low zinc (25 mg zinc), or 4) AREDS formulation minus beta-carotene and low zinc. Current smokers and former smokers who had quit within 1 year before randomization and who agreed to this secondary randomization were randomized to one of the two groups without beta-carotene because beta-carotene supplementation has been shown to increase the risk of lung cancers in cigarette smokers.[15,16] Participants, who did not consent to this secondary randomization, were provided with the original AREDS supplements, if they were not current smokers or had not smoked within the past year. The consort figure for the secondary randomization is available in a previously published report.[14]

The primary and secondary randomizations were stratified by clinical center and AMD category (bilateral large drusen or large drusen one eye and advanced AMD in fellow eye) using randomly permuted blocks of varying sizes. Each treatment was assigned 5 bottle numbers. Bottle numbers were issued via an electronic randomization system for each participant once study eligibility was verified. The assigned bottle number was used to distribute the study treatment(s). Coordinating center personnel involved in creating the randomization system had access to the bottle number/treatment assignments. Participants and study personnel were masked (blinded) to treatment assignment. Participants received their study supplements or matching placebos at each annual visit. Compliance with the study supplements was measured with annual pill count. In a subset of participants, the serum levels were also evaluated for compliance at baseline and years 1, 3 and 5. Follow-up study visits were scheduled annually with telephone contacts by study coordinators at 6 months between study visits and at 3 months post randomization to collect information on AMD treatment and adverse events. Investigators, also masked to all medical data and treatment assignments conducted the analyses.

Cognitive function tests

AREDS2 utilized a cognitive battery similar to the one used in Age-Related Eye Disease Study (AREDS) administered by certified personnel on the telephone over a period of 30 minutes (e-methods, supplementary materials).[9] The cognitive function test administrators, who were masked to the participant's study supplement assignment and medical history, received extensive initial training as well as certification on interview techniques and scoring of responses. Subsequently, quarterly reviews of 3 randomly selected interviews on audiotape from each interviewer were centrally conducted to provide feedback regarding the interview and scoring techniques for quality assurance. The first administration of the AREDS2 telephone battery instruments was within 3 months after randomization to the primary AREDS2 protocol and approximately every 2 years thereafter.

The AREDS Telephone Battery was originally found to be an appropriate substitute for participants who were unable to complete an in-clinic assessment of cognitive function.[17] All tests have been validated and used in previous studies with cognitive function testing. An abbreviated version of the cognitive battery consisting of the Hearing Handicap Inventory,

CES-D, and TICS (approximately 10 to 15 minutes to administer) was administered to participants who had time constraints or other concerns about the full-length battery. The AREDS2 cognitive battery consisted of 8 tests of cognitive function which were administered after each participant was tested for hearing and depression at each telephone call for cognitive function testing. The order of testing is listed in the text box. The ranges of the values for these individual tests are described in the supplementary materials.

Outcome Measurements

The primary outcome in the planned ancillary study of cognition evaluates the yearly change in the composite scores of the cognitive function tests (numbered 3 to 10 above). The higher the score the better the cognition thus negative values represent worsening of the cognitive function testing. We assessed the difference in the yearly change of the scores by treatment main effects, focused mainly on omega-3 LCPUFAs vs. no omega-3 LCPUFAs, as stated a priori. Secondary analyses included lutein/zeaxanthin vs. no lutein/zeaxanthin, high zinc vs. low zinc, and beta-carotene vs. no beta-carotene as these nutrients were previously explored in other studies. However, this ancillary study was not sufficiently powered to evaluate these additional nutrients.

The change in the TICS total score, a comprehensive testing of all the domains over the course of the study from baseline was assessed.[24] A composite score was constructed by converting the test results of the eight cognitive tests listed above into z-scores and then adding the z-scores. The composite score provided an overall score for the battery, ranging from −22 to 17, with higher scores representing better function.[17] This methodology has been used in published studies of cognitive function testing.[25,26] We also individually evaluated the yearly change in the cognitive function scores for the eight cognitive function tests from baseline to 2 and 4 years. Composite scores were analyzed only in those participants who completed the entire battery of cognitive function tests while those who had TICS only were analyzed for all participants who had TICS scores available in the entire cohort.

Statistical Analyses

The primary hypothesis stated at the beginning of AREDS2 ancillary cognitive function study was to test whether omega-3 LCPUFAs (the main effects of those who were randomized to omega-3 LCPUFAs vs. those not randomized to omega-3 LCPUFAs) would have any effect on cognitive function. It was estimated that 15 to 35% of the AREDS2 participants would have score above the normal range for the TICS. We assumed that at least 3,000 AREDS2 participants (1500 assigned to omega-3 LCPUFAs and 1500 assigned to no omega-3 LCPUFAs). The study would have at least 85% power to detect an odds ratio of 0.75 as measured by the TICS assuming a two-sided $\alpha = 0.05$ and a control group (no omega-3 fatty acids) rate of participants with a score above the normal range. The study is powered to evaluate only the main effects of the nutrients but since this is a factorial design, studies of interaction were also conducted to assess for potential interactions.

For each test of cognitive function, changes in score from baseline at the study visits 2 and 4 years were evaluated using a mixed model regression. The models take into account the

repeated measures of the participants and the unequal time spacing and number of cognitive tests for each participant by using a spatial power covariance structure. The models provide estimates of yearly change in score that can be associated with the AREDS2 study supplement assignment and each covariate. The models were adjusted for baseline age, sex, race, history of hypertension, education, baseline cognitive score and baseline depression score. Age, baseline score and baseline depression score were treated as continuous variables, and the others as categorical variables with race dichotomized to white vs. non-white. A repeated measures logistic regression was used to analyze the outcome of TICS<30. To account for the correlation of multiple visits per participant an autoregressive correlation structure was used. To account for multiplicity of analyses, we used 99% confidence intervals (CI) rather than the conventional 95% CI. Because 36 models were conducted, to be statistically significant, the p-value would need to be <0.001.

Participants were excluded from the analyses if they are missing baseline cognitive function tests, any follow-up test, incomplete test, or missing baseline demographics data. All analyses were conducted under the principle of intention to treat and no data imputation was applied. All analyses were conducted using SAS version 9.3 (SAS Institute Inc.).

Results

A total of 4203 participants were enrolled between October 2006 and September 2008 at 82 clinical sites across the United States and followed until December 2012 in AREDS2. Enrollees had to satisfy the specified inclusion and exclusion criteria.[13] Of the 4203 AREDS2 participants enrolled, 3741 (89%) consented to the full Cognitive Function Study while 462 declined and 3501/3741 (93.6%) underwent cognitive function testing (Figure 1, CONSORT figure). Of the 3501 who consented, 2991 (85%) participated in this study by completing at least 1 full interview and 510 (15%) had the shortened battery (e-Table 1, online). Among those who consented for the present study, we excluded those with no baseline test, no follow-up test, or missing demographic data or incomplete tests, leaving us with 3073 participants for analyses. Of the three possible interviews for cognitive function during the course of the study (baseline, years 2 and 4), 2831 of 3501 (81%) study participants contributed to all 3 interviews while 410 (12%) had 2 interviews and 260 (7%) had one interview only (e-Table 1 online). The mean (SD) age of the participants in the Cognitive Function Study was 72.7 (7.7) years and 57.5% were female.

AREDS2 participants included in the analyses of the Cognitive Function Study were younger, more likely to be white, and more likely to have a higher level of education (e-Table 2, online). Among those who participated, there was a fairly comparable distribution of the treatment assignments. However, within those who did not participate, there was a relatively greater proportion assigned to lutein/zeaxanthin (e-Table 2, online). Baseline serum levels of the nutrients evaluated were comparable across treatment groups. Table 1 describes the comparable baseline characteristics of the participants who were randomly assigned to omega-3 LCPUFAs (DHA/EPA) or to no omega-3 LCPUFAs (DHA/EPA).

Adherence to Study Medication and Loss to Follow-up Rate

Evaluation of compliance with the primary study supplements showed that 1439/1736 (82.9%) assigned to omega-3 LCPUFAs, and 1419/1688 (84.1%) assigned to NO omega-3 LCPUFAs were adherent to their study drugs at least 75% of the time and taking 75% or more of their study drugs. Similarly, 1432/1690 (84.7%) of those assigned to lutein/zeaxanthin and 1494/1724 (86.7%) of those NOT assigned to lutein/zeaxanthin took 75% or more of the study medications at least 75% of the time. In addition, serum levels of 545 AREDS2 participants who were randomized to the treatments showed a 2-fold or greater increase in the serum levels of omega-3 LCPUFAs and/or lutein/zeaxanthin compared to those participants who were not assigned to the respective nutrients.

Cognitive Function Testing Scores

The baseline cognitive function scores for all the participants for the various analyses are displayed in e-Table 3, online. The baseline scores from the cognitive function tests were comparable across the randomized groups of the main effects of omega-3 LCPUFAs vs. no omega-3 LCPUFAs (Table 2). Similar balanced distribution was seen across all the randomized groups of all the other nutrients evaluated (data in supplementary materials, e-Table 4, online).

Overall Effects of the Nutrient Supplementation on Cognitive Function

Table 3 demonstrates the demographics and risk factors at baseline for the participants included in the TICS scores analyses and/or the composite scores analyses. The higher the cognitive function score, the better the cognition. For the outcome of the change at years 2 and 4 from baseline, calculated as yearly change, negative values signal a decrease in cognitive function when compared with baseline (Table 4). In general, the cognitive function testing scores decreased over time for the AREDS2 participants.

AREDS2 participants who achieved higher scores at baseline on both the TICS and the composite score were more likely to be female and to be white. Participants with higher levels of education also achieved higher scores on testing. Persons without a history of hypertension, congestive heart failure, coronary heart disease, myocardial infarction, and stroke were more likely to score higher in both TICS and composite scores.

Main Effects of Omega-3 Long-Chain Polyunsaturated Fatty Acids (LCPUFAs)

The yearly change in the composite cognitive function score was −0.19 (99% Confidence Interval [CI]: −0.25 to −0.13) for participants randomized to LCPUFAs and was −0.18 (99% CI: −0.24 to −0.12) for those randomized to no LCPUFAs (difference in yearly change of −0.03, 99% CI: −0.20 to 0.13, p=0.63) (Figures 2A). The difference in the yearly change of the TICS between the omega-3 LCPUFA treatment groups was −0.10 (99% CI: −0.24 to 0.04, p=0.07) (Figure 2A). Further evaluation of the TICS score for the odds for doing poorly was conducted using a binary outcome of TICS score <30, which is considered to be less than “normal”. Figure 3 show that the odds of having TICS score <30 was 1.12 (99% CI: 0.91 to 1.39, p=0.15). The yearly changes of the various studies that contributed to the composite scores ranged from −0.10 to 0.17; none of these changes were statistically significant (figure 2A).

Main Effects of Lutein and Zeaxanthin

The yearly change in the composite cognitive function score was −0.18 (99%CI: −0.24 to 0.11) among those randomized to receive lutein/zeaxanthin vs −0.19 (99%CI: −0.25 to −0.13) among those randomized to not receive lutein/zeaxanthin (difference in yearly change of 0.03, 99% CI, −0.14 to 0.19), p=0.66) (Figure 2B). Similarly, the difference in the yearly TICS score change between the lutein/zeaxanthin treatment groups was 0.01 (99% CI: −0.16 to 0.13, p=0.80) (Figure 2B) and the odds of having TICS score of <30 was 1.08 (99% CI: 0.87 to 1.33, p=0.35), Figure 3. The results of the tests ranged from −0.32 to 0.41 and none was statistically significant (Figure 2B).

We also evaluated the data stratified by the dietary intake of omega-3 LCPUFAs and lutein with zeaxanthin. We found no difference among participants with varying dietary intake.

Main Effects of High Zinc vs. Low Zinc

The yearly change in the composite cognitive function score was −0.20 (99%CI: −.27 to −0.13) among those randomized to receive high zinc vs −0.19 (99%CI: −0.27 to −0.12) among those randomized to receive low zinc (difference in yearly change of −0.02/year (99% CI: −0.21 to 0.17, p=0.77) (Figure 2C). Similarly, the difference in the yearly TICS score change between the high and low zinc groups was −0.01 (99% CI: −0.18 to 0.16, p=0.89) (Figure 2C) and the odds of having TICS score <30 was 1.07 (99% CI: 0.84 to 1.36, p=0.49) (Figure 3). The results of the tests ranged from −0.23 to 0.12 and none was statistically significant (Figure 2C).

Main Effects of Beta-carotene vs. No Beta-carotene

The yearly change in the composite cognitive function score was −0.24 (99%CI: −0.32 to −0.16) among those randomized to receive beta-carotene vs −0.18 (99%CI: −0.26 to −0.10) among those randomized to not receive beta-carotene (difference in yearly change of −0.16/year [99% CI: −0.36 to 0.04, p=0.04]) (Figures 2D). Similarly, the difference in the yearly TICS score change between the beta-carotene treatment groups was −0.11 (99% CI: −0.28 to 0.07, p=0.12) (Figure 2D) and the odds of having TICS score of <30 was 1.14 (99% CI: 0.88 to 1.46, p=0.19) (Figure 3). The results of the sub-studies ranged from −0.48 to −0.01 and none was statistically significant (Figure 2D).

Discussion

After 5 years of a controlled randomized clinical trial, supplementing with omega3-LCPUFAs, specifically DHA and EPA (1 gram total), and/or lutein 10 mg and zeaxanthin 2 mg did not have a statistically significant effect on cognitive impairment in this population of persons with intermediate AMD (bilateral large drusen) or late AMD in one eye. The results of the randomization to high zinc vs. low zinc or beta-carotene vs. no beta-carotene also showed no statistically significant effect. However, worse cognitive function at study entry was associated with increasing age, lower education level, and the male gender in our baseline cross-sectional data. Other medical risk factors such as hypertension and other cardiovascular disease including stroke were associated with lower cognitive function testing scores. During the course of the study, these same risk factors as well as a lower baseline

cognitive function test score were also associated with greater decline in the cognitive function test scores; both the TICS and the composite score (Table 3).

The strengths of the study include the high compliance rates of adherence the study supplements as well as the high rates of cognitive function testing in a clinical trial with one of the largest number of study participants. The interviewers underwent extensive training as well as periodic certification to ensure quality data. The battery of cognitive function tests in AREDS2 which was conducted over the telephone without visual input is particularly appropriate for a population that has AMD. This battery of testing is similar to the battery used in other large scale studies that employed a similar telephone interview, especially using the TICS test, to evaluate cognitive function. These tests have been validated to give quality assessments.[27]

The limitations of this trial include the limited generalizability of the results because the study was conducted in a select population of well-nourished and highly educated persons with at least intermediate AMD or advanced AMD in one eye. This condition is also a neurodegenerative disease with limited information on its pathogenesis. Another limitation of this study is the possibility that at non-physiologic levels, the nutrients we tested have different biologic effects, different from those attained with dietary intake. Possibly we have started these supplements too late in the aging process, since the mean age of our population at baseline was 73.1 years. It is plausible that supplementation duration of 5 years may be insufficient as suggested by the differential effects seen in the Physicians Health Study II (PHSYII) cognitive ancillary study in those participants who had 18 years of supplementation with beta-carotene vs. those with a much shorter duration of 1 year.[11] The process of cognitive decline may occur over decades thus a short-term supplementation given too late in the disease may not be effective.

We attempted to evaluate the role of antioxidant vitamins and zinc in cognitive function testing in the original AREDS Study, which was a placebo-controlled randomized trial.[9] A limitation of the original AREDS results, however, was that cognitive testing was not conducted at baseline, thus leaving researchers unable to determine whether antioxidant or copper and zinc treatment influences the rate of cognitive decline (although baseline cognition was likely identical across assigned treatment groups).

The observational data regarding dietary intake of specific nutrients such as omega-3 LCPUFAs and antioxidants suggest strong inverse associations with dementia, yet the randomized controlled clinical trials have failed to show beneficial effects. Possibly, eating foods rather than taking any specific single supplement have an effect. Similarly, strongly suggestive observational data demonstrated a protective effect of high levels of dietary omega-3 LCPUFAs for age-related macular degeneration but the overall primary effects of the AREDS2 study of AMD also demonstrated that DHA/EPA had no effect on the treatment of AMD[14] while lutein/zeaxanthin had an incremental effect.[14,28] Similarly, we found that omega-3 LCPUFAs for the treatment of cardiovascular disease in AREDS2, another ancillary study, also did not result in a beneficial effect.[29] Possibly, we do not know the factors within the food source that may be protective. Studying dietary/food intake patterns rather than specific factors isolated with food source may better reflect nutritional

benefits as we have no knowledge of the actual factor that may make an impact or of the interactions between nutrients that influence the physiologic effects of any one nutrient may have in target tissues or pathways.

Conclusions

Among older persons with AMD, oral supplementation with LCPUFAs and lutein/zeaxanthin had no statistically significant effect on cognitive function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This study was sponsored by the National Institutes of Health, USA (see below). The principal investigator, Emily Y. Chew, MD, who is an employee of the National Institutes of Health, designed and conducted the study in a multi-centered study where investigators from academic and community medical centers collected the data. Dr. Emily Chew is responsible for the management, analysis, and interpretation of the data as well as the preparation, review, an approval of the manuscript. The decision to submit the manuscript for publication was also determined by Dr. Emily Chew, a staff of the funding agency.

Drs. Emily Chew and Traci Clemons had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

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Text Box

AREDS2 cognitive battery was administered in the following order and only the tests listed from 3 to 10 were used for the scores

1. The Hearing Handicap Inventory[18] is given first as the interview is conducted by telephone.
2. The Center for Epidemiologic Studies' Depression Scale (CES-D)[19] is a test designed to assess symptoms of depression in the general population.
3. Telephone Interview Cognitive Status-Modified (TICS-M)[20] is a version of the Mini Mental State Examination. TICS-M also includes 10 words that are given early and tested for immediate and delayed recall.
4. Animal Category[21] is used together with the tests of letter fluency and alternating fluency (see below) to assess language and executive function. Participants are asked to name as many animals as possible in 1 minute.
5. Letter Fluency[21] is used as described above with animal and alternating fluency. Participants are asked to name as many words starting with the letter "F", "A", and "S" as possible in 1 minute.
6. Alternating fluency[21] is used with animal and letter fluency as described above. Participants are instructed to alternately name a word beginning with the letter "C" and a "food" category in 1 minute.
7. Wechsler Memory Scale Third Edition (WMS-III), Logical Memory Part I and Part II)[22] measures both immediate and delayed recall of two stories. The test assesses two domains of cognitive function: attention and memory.
8. Digits Backwards[23] is a task used to test the speed of processing task where the participant is asked to count as fast as they can backwards starting from 100 for 30 seconds.
9. Delayed recall of the WMS-III Recall paragraph.
10. TICS-M Recall consisted of recalling the 10 words initially read with the TICS.

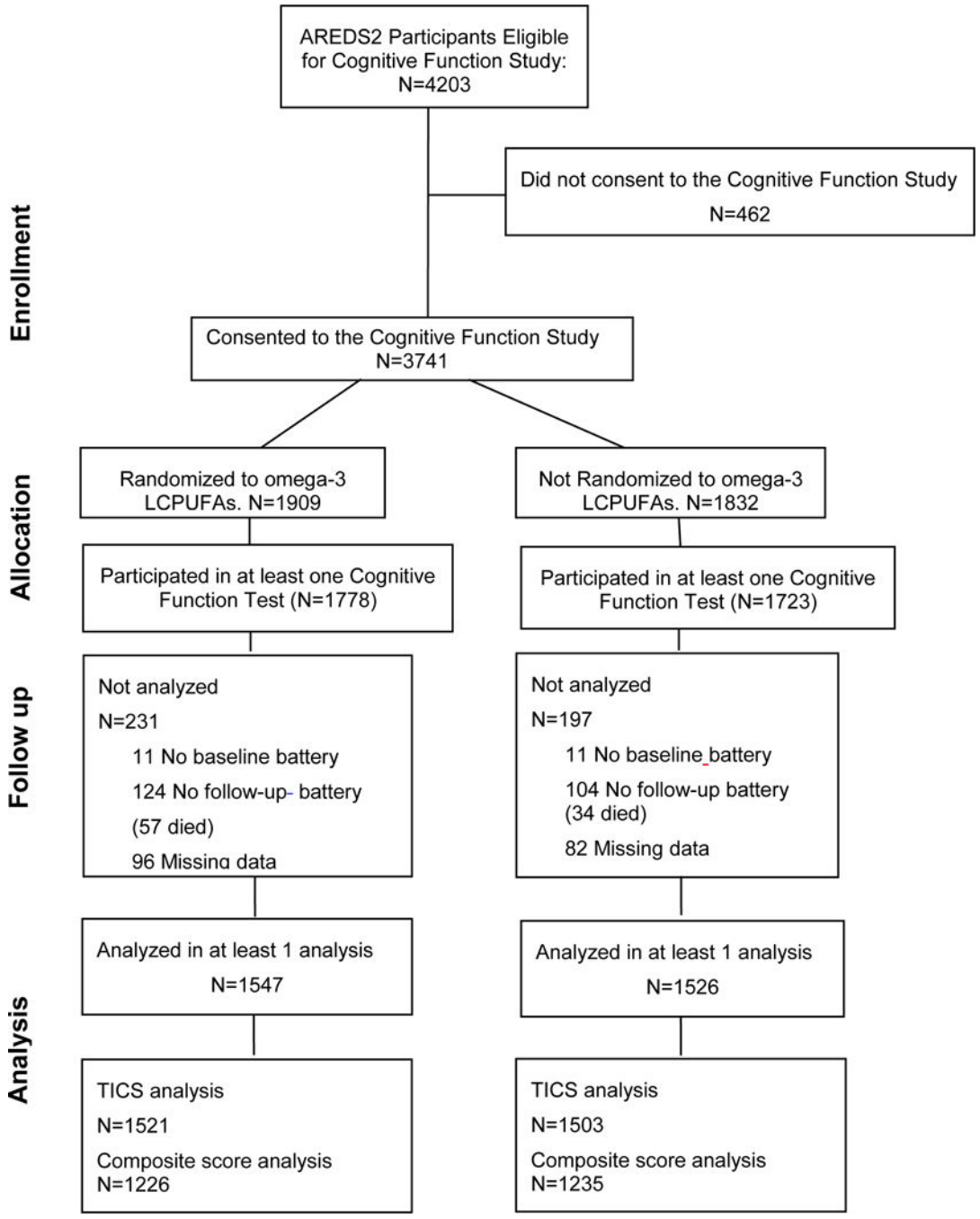
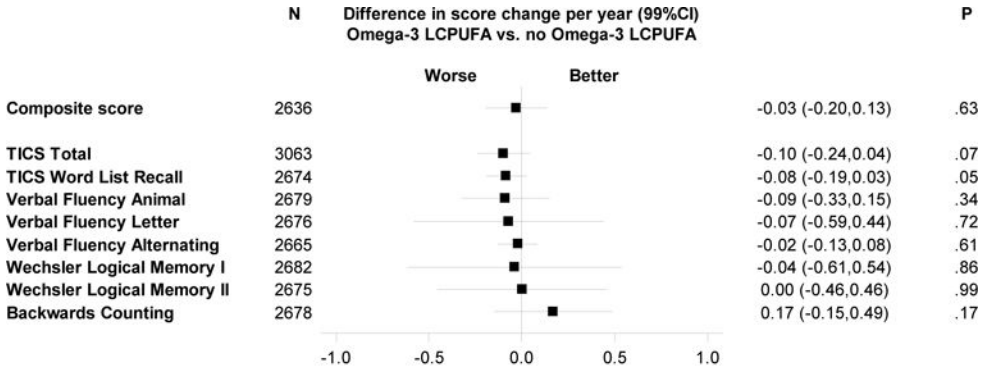


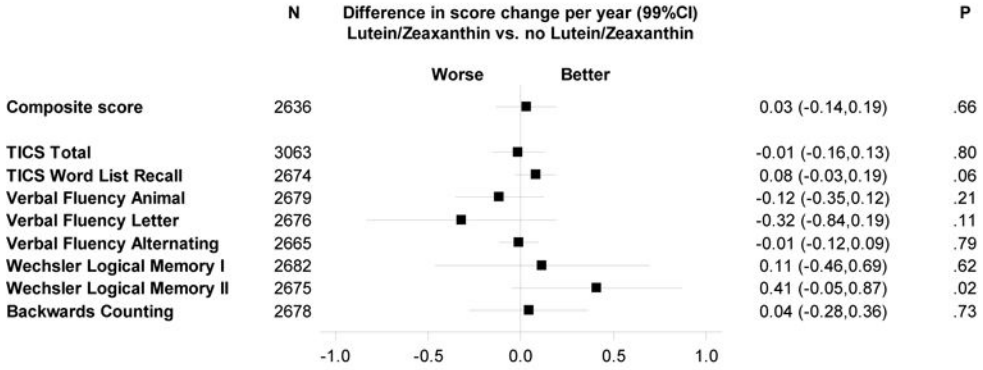
Figure 1. CONSORT Figure

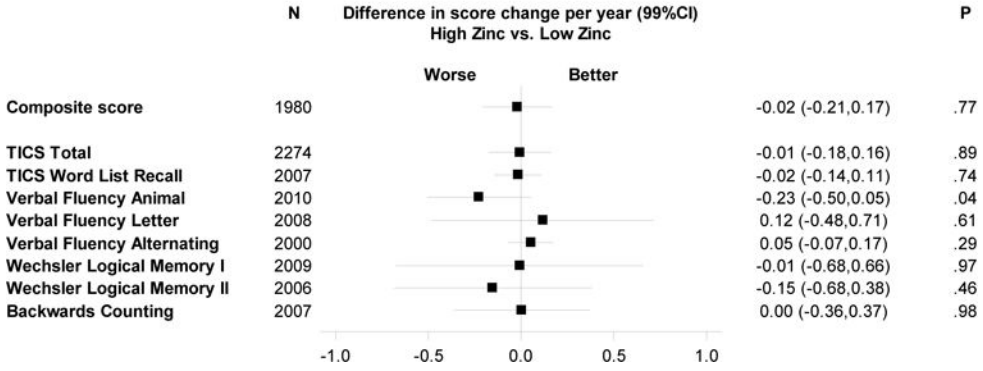
The CONSORT Figure Showing the AREDS2 participants Included in the Analyses of the the Ancillary Cognitive Function Study

LCPUFAs: Long Chain Polyunsaturated Fatty Acids

Reasons for declining consent for the Ancillary Cognitive Function Testing were not collected.







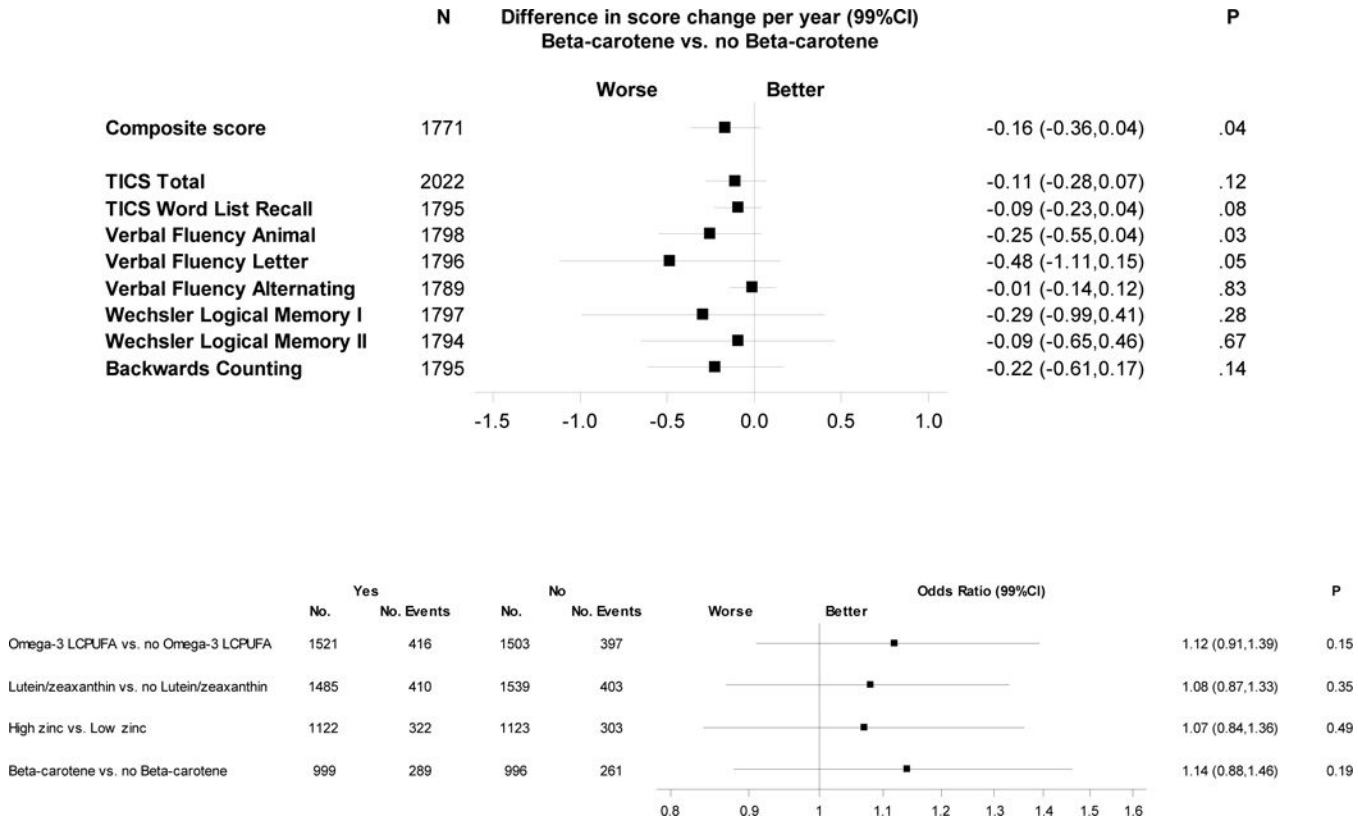


Figure 2.

Results of the mixed models regression for the change per year in cognitive function test scores from baseline for each of the nutrients tested:

A. Comparison of the main effects of omega-3 LCPUFAs vs. no omega-3 LCPUFAs.

LCPUFAs: Long Chain Polyunsaturated Fatty Acids

TICS: Telephone Interview of Cognitive Status

Composite score. A composite score was constructed by including the score of the TICS and all the cognitive tests by converting all test results into z-scores and then averaging the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

Telephone Interview Cognitive Status-Modified (TICS-M)[3] is a version of the Mini Mental State Examination. The score for TICS ranges from 0 to 39 points. See supplementary methods for explanation for the scores of the other cognitive function tests.

Worse means a deterioration of the score for that given nutrient and better means an improved score for those randomized to omega3 fatty acids, DHA/EPA.

These analyses are adjusted for the following baseline covariates: age, gender, race, education, smoking, hypertension, congestive heart failure, and depression scale.

B: Comparison of the main effects of lutein/zeaxanthin vs. no lutein/zeaxanthin.

Lut/Zea: Lutein/Zeaxanthin

TICS: Telephone Interview of Cognitive Status

Composite score. A composite score was constructed by including the score of the TICS and all the cognitive tests by converting all test results into z-scores and then averaging the z-

scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

Telephone Interview Cognitive Status-Modified (TICS-M)[3] is a version of the Mini Mental State Examination. The score for TICS ranges from 0 to 39 points. See supplementary methods for explanation for the scores of the other cognitive function tests.

Worse means a deterioration of the score for that given nutrient and better means an improved score for those randomized to lutein/zeaxanthin.

These analyses are adjusted for the following baseline covariates: age, gender, race, education, smoking, hypertension, congestive heart failure, and depression scale.

C: Comparison of the main effects of high zinc vs. low zinc.

TICS: Telephone Interview of Cognitive Status

Composite score. A composite score was constructed by including the score of the TICS and all the cognitive tests by converting all test results into z-scores and then averaging the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

Telephone Interview Cognitive Status-Modified (TICS-M)[3] is a version of the Mini Mental State Examination. The score for TICS ranges from 0 to 39 points. See supplementary methods for explanation for the scores of the other cognitive function tests.

Worse means a deterioration of the score for that given nutrient and better means an improved score for those randomized to zinc.

These analyses are adjusted for the following baseline covariates: age, gender, race, education, smoking, hypertension, congestive heart failure, and depression scale.

D: Comparison of the main effects of beta-carotene vs. no beta-carotene.

TICS: Telephone Interview of Cognitive Status

Composite score. A composite score was constructed by including the score of the TICS and all the cognitive tests by converting all test results into z-scores and then averaging the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

Telephone Interview Cognitive Status-Modified (TICS-M)[3] is a version of the Mini Mental State Examination. The score for TICS ranges from 0 to 39 points. See supplementary methods for explanation for the scores of the other cognitive function tests.

Worse means a deterioration of the score for that given nutrient and better means an improved score for those randomized to beta-carotene.

These analyses are adjusted for the following baseline covariates: age, gender, race, education, smoking, hypertension, congestive heart failure, and depression scale.

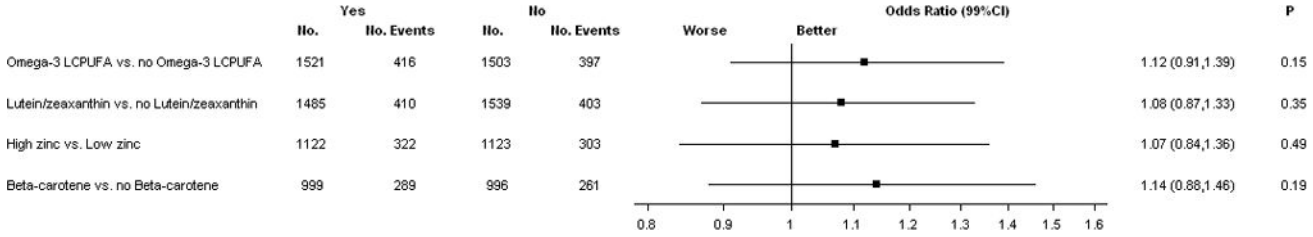


Figure 3. Results of the Odds of Having a Score of less than 30 for the Telephone Interview of Cognitive Status (TICS) for the Four Nutrients evaluated, using Repeated Measures Logistic Regression

LCPUFAs: Long Chain Polyunsaturated Fatty Acids

Lut/Zea: Lutein/Zeaxanthin

Telephone Interview Cognitive Status-Modified (TICS-M)[3] is a version of the Mini Mental State Examination. The score for TICS ranges from 0 to 39 points. A dichotomous outcome from the TICS is defined as follows: 1) TICS total < 30 points defines low cognitive function and 2) TICS total ≥30 defines normal cognitive function. “Worse” means a deterioration of the TICS score (large proportion with low cognitive function) for that given nutrient and “better” means an improved TICS (smaller proportion with low cognitive function) for that given nutrient.

Models were adjusted for: baseline age, sex, race, history of hypertension, education, baseline cognitive score and baseline depression score.

Table 1

Baseline Characteristics of AREDS2 Participants in at Least One Analysis by Assignment to Omega-3 Long-Chain Polyunsaturated Fatty Acids (LCPUFAs)

	No LCPUFAs (N=1526)	LCPUFAs (N=1547)	P-value
Baseline Characteristic	N (%)	N (%)	
Age, mean (SD), y	72.7 (7.8)	72.7 (7.7)	0.88
Female	858 (56.2)	909 (58.8)	0.16
Race			
White	1485 (97.3)	1501 (97.0)	0.85
Black	14 (0.9)	21 (1.4)	
Asian	9 (0.6)	9 (0.6)	
American Indian	2 (0.1)	3 (0.2)	
Native Hawaiian or Other Pacific Islander	2 (0.1)	1 (0.1)	
Other	14 (0.9)	12 (0.8)	
Education			
High school or less	442 (29.0)	456 (29.5)	0.08
At least some college	720 (47.2)	773 (50.0)	
Post-graduate	364 (23.9)	318 (20.6)	
Smoking status			
Never	649 (42.5)	664 (42.9)	0.53
Former	769 (50.4)	789 (51.0)	
Current	108 (7.1)	94 (6.1)	
Aspirin use	740 (48.5)	762 (49.3)	0.67
CES-D score, mean (SD)	15.7 (6.3)	16.1 (6.6)	0.08
Statin use	686 (45.0)	672 (43.4)	0.40
History of:			
Hypertension	872 (57.1)	902 (58.3)	0.51
Congestive heart failure	57 (3.7)	51 (3.3)	0.51
Coronary heart disease	154 (10.1)	130 (8.4)	0.11
Myocardial infarction	106 (6.9)	84 (5.4)	0.08
Stroke	74 (4.8)	78 (5.0)	0.80

Abbreviations: AREDS2, Age-Related Eye Disease Study 2; SD, standard deviation; CES-D, Center for Epidemiologic Studies' Depression Scale.

P-values are from χ^2 tests for categorical variables and t-test for numeric variables.

Table 2

Cognitive Function Test Scores at Baseline by Assignment to Omega-3 Long-Chain Polyunsaturated Fatty Acids (LCPUFAs)

Cognitive Function Test	No LCPUFAs[a]	LCPUFAs[a]	P-value
Composite score[b]			
N	1235	1226	
Mean (SD) Z-score	0.4 (5.4)	0.3 (5.2)	
Mean (SD) difference (99%CI)	−0.19 (−0.73, 0.36)		0.38
Telephone Interview of Cognitive Status (TICS)[c]			
N	1503	1521	
Mean (SD) points	33.0 (3.4)	33.0 (3.4)	
Mean (SD) difference (99%CI)	−0.04 (−0.35, 0.27)		0.75

[a] LCPUFAs: Long-chain polyunsaturated fatty acids.

[b] Composite score. A composite score was constructed by including the score of all 8 cognitive tests by converting all test results into z-scores and then adding the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

[c] TICS, Telephone Interview of Cognitive Status; (TICS-M)[3] is a version of the Mini Mental State Examination. The TICS score ranges from 0 to 39 points. The higher score denotes better cognitive function.

P-values are from an analysis of covariance comparing the means.

Table 3

Mean (SD) of the Telephone Interview of Cognitive Status and the Composite Score by Baseline Characteristic

	TICS[a] (N=3024)			Composite Score[b] (N=2461)		
Baseline Characteristic	**Mean**	**SD**	**P-value**	**Mean**	**SD**	**P-value**
Age tertile, y						
1: 50−70	34.0	3.1	<.001	2.3	5.0	<.001
2: 71−78	33.0	3.4		0.1	5.0	
3: 79+	31.9	3.3		−1.8	5.1	
Sex						
Female	33.4	3.3	<.001	0.9	5.1	<.001
Male	32.5	3.3		−0.4	5.4	
Race						
White	33.1	3.3	<.001	0.4	5.2	0.002
Black	31.0	3.4		−2.1	5.2	
Asian	31.8	3.9		−1.2	5.5	
American Indian	28.8	6.0		−4.5	8.2	
Native Hawaiian or Other Pacific Islander	29.3	3.1		−10.3	_[d]	
Other	30.0	3.6		−2.0	5.1	
Education						
High school or less	31.7	3.5	<.001	−2.4	5.0	<.001
At least some college	33.4	3.1		0.7	4.9	
Post-graduate	34.0	3.0		2.9	4.9	
Smoking status						
Never	33.2	3.3	0.01	0.5	5.2	0.45
Former	32.9	3.4		0.3	5.3	
Current	32.6	3.5		0.0	5.7	
Aspirin use						
No	33.1	3.4	0.20	0.5	5.4	0.21
Yes	32.9	3.3		0.2	5.1	

	TICS[a] (N=3024)			Composite Score[b] (N=2461)		
Baseline Characteristic	**Mean**	**SD**	**P-value**	**Mean**	**SD**	**P-value**
Statin use						
No	33.3	3.3	<.001	0.7	5.3	<.001
Yes	32.7	3.4		−0.1	5.2	
CES-D score≥16						
No	33.2	3.3	<.001	0.7	5.2	<.001
Yes	32.7	3.4		−0.2	5.3	
History of:						
Hypertension						
No	33.4	3.2	<.001	0.9	5.3	<.001
Yes	32.8	3.5		−0.1	5.2	
Congestive heart failure						
No	33.1	3.3	<.001	0.4	5.2	<.001
Yes	31.3	3.8		−1.6	5.2	
Coronary heart disease						
No	33.2	3.3	<.001	0.6	5.2	<.001
Yes	31.7	3.4		−2.3	4.8	
Myocardial infarction						
No	33.1	3.3	<.001	0.5	5.2	<.001
Yes	31.6	3.9		−1.9	5.6	
Stroke						
No	33.1	3.3	<.001	0.5	5.2	<.001
Yes	31.8	3.7		−1.7	5.5	

[a]TICS, Telephone Interview of Cognitive Status; (TICS-M)[3] is a version of the Mini Mental State Examination. The TICS score ranges from 0 to 39 points. The higher score denotes better cognitive function.

[b]Composite score. A composite score was constructed by including the score of all 8 cognitive tests by converting all test results into z-scores and then adding the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

[c]Parameter estimate and standard error of a linear regression.

$d_{N=1}$.

P-values are from an analysis of covariance comparing the means.

Table 4

Association of Risk Factors with the Change in the Telephone Interview of Cognitive Status (TICS) Score and Change in Composite Score. Table Shows the Estimated Score Change per Year and 99% Confidence Limits from Two Separate Mixed Models Regression.

Characteristic	TICS[a] Change per Year		Composite Score[b] Change per Year	
Age, y	−0.05 (−0.06,−0.05)	<.001	−0.05 (−0.07,−0.04)	<.001
Years from baseline	−0.11 (−0.15,−0.07)	<.001	−0.18 (−0.23,−0.14)	<.001
Score at baseline test (1 point increment)	−0.24 (−0.26,−0.22)	<.001	−0.09 (−0.11,−0.08)	<.001
CES-D[c] score at baseline (1 point increment)	−0.01 (−0.02,−0.00)	0.004	−0.01 (−0.02,0.00)	0.05
Sex				
Male	−0.30 (−0.45,−0.16)	<.001	−0.33 (−0.50,−0.16)	<.001
Female	Reference		Reference	
Race				
White	0.53 (0.10,0.97)	0.002	0.51 (−0.04,1.05)	0.02
Non-white	Reference		Reference	
Education				
At least some college	0.43 (0.25,0.60)	<.001	0.30 (0.10,0.51)	<.001
Post-graduate	0.77 (0.56,0.98)	<.001	0.50 (0.25,0.75)	<.001
High school or less	Reference		Reference	
History of hypertension				
Yes	−0.12 (−0.27,0.02)	0.03	−0.13 (−0.30,0.04)	0.05
No	Reference		Reference	

[a] TICS, Telephone Interview of Cognitive Status; (TICS-M)[3] is a version of the Mini Mental State Examination. The TICS score ranges from 0 to 39 points. The higher score denotes better cognitive function.

[b] Composite score. A composite score was constructed by including the score of all 8 cognitive tests by converting all test results into z-scores and then adding the z-scores. The composite score was computed to obtain an overall score for the battery of cognitive function tests, ranging from −22 to 17, with higher scores representing better function.

[c] CES-D, Center for Epidemiologic Studies' Depression Scale