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Erythrocyte omega-3 fatty acids are inversely associated with incident dementia: Secondary analyses of longitudinal data from the Women's Health Initiative Memory Study (WHIMS)

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Abstract

OBJECTIVE—To assess whether red blood cell (RBC) docosahexaenoic acid and eicosapentaenoic acid (DHA+EPA) levels have a protective association with the risk of dementia in older women.

METHODS—RBC DHA+EPA levels were assessed at baseline, and cognitive status was evaluated annually in a cohort of 6706 women aged ≥ 65 years who participated in the Women's Health Initiative Memory Study (WHIMS). Cox regression was used to quantify the association

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E.M.A. contributed to the statistical analysis and interpretation of the data, and drafting and revising the manuscript.

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W.S.H. contributed to the design of the study, the acquisition of the data, and drafting and revising the manuscript.

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Supplemental data: WHIMS omega3 FAs and dementia appendix.docx

between RBC DHA+EPA and the risk of probable dementia, independent of major dementia risk factors.

RESULTS—During a median follow-up period of 9.8 years, 587 incident cases of probable dementia were identified. After adjusting for demographic, clinical, and behavioral risk factors, a one standard deviation increase in DHA+EPA levels was associated with a significantly lower risk of dementia (HR = 0.92, 95% CI: 0.84, 1.00; $p < 0.05$). This effect estimate did not meaningfully change after further adjustment for baseline cognitive function and *APOE* genotype. For women with high DHA+EPA exposure (1 SD above mean) compared to low exposure (1 SD below mean), the adjusted 15-year absolute risk difference for dementia was 2.1% (95% CI: 0.2%, 4.0%). In secondary analyses, we also observed a protective association with longitudinal change in Modified Mini-Mental State (3MS) Exam scores, but no significant association with incident MCI, PD/MCI, or baseline 3MS scores.

DISCUSSION—Higher levels of DHA+EPA may help protect against the development of dementia. Results from prospective randomized controlled trials of DHA+EPA supplementation are needed to help clarify whether this association is causal.

Keywords

All cognitive disorders/dementia; Alzheimer's Disease; Cohort studies; Omega-3 fatty acids; Women; biomarkers

INTRODUCTION

With the aging of the U.S. population, developing interventions to prevent and treat AD/D has become an increasingly important public health priority. Due to their longer life expectancy, the disease burden of AD/D is especially high for women. For a woman aged 65 years, the subsequent lifetime risk of dementia is 20%. [1]

No truly effective pharmacological or non-pharmacological strategy exists for the prevention and/or treatment of Alzheimer's disease and related dementias. [2, 3] Interest in a potential role of long-chain, marine omega-3 fatty acids (FA) including eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) has grown based on a variety of observations. These include the structural presence of DHA in neural tissues, [4] the anti-inflammatory properties of these FAs, [5] and the ability of the DHA metabolite resolvin-D1 to increase phagocytosis of amyloid- β by monocytes. [6] In addition, epidemiologic studies have shown that diets richer in fish are associated with reduced risk for dementia [7, 8] and less neuropathology. [9]

In the present study, we evaluated the association between DHA+EPA exposure and incident dementia in the Women's Health Initiative (WHI) Memory Study (WHIMS), a large and well-characterized cohort of older U.S. women. Secondary analyses explored relationships with other cognitive outcomes, including incident mild cognitive impairment (MCI) and longitudinal changes in Modified Mini-Mental State (3MS) scores. We hypothesized that higher levels of RBC DHA+EPA would have protective associations with these outcomes.

METHODS

Study Population

This study was a secondary analysis of longitudinal data collected for the WHIMS study cohort. WHIMS was an ancillary study of cognitive outcomes in 7479 older women who participated in the WHI randomized trials of hormone therapy (HT). In the trials, women with a prior hysterectomy were randomized to receive 0.625 mg conjugated equine estrogens (CEE) or placebo daily, and women with an intact uterus received a combination of 0.625 mg CEE and 2.5 mg progestin (CEE+P) or placebo daily.[10, 11] All participants gave informed consent, and institutional review boards approved the study protocols. At the time of enrollment in WHIMS, participants were 65–80 years of age and free of dementia. The CEE+P and CEE-only trials were terminated in 2002 and 2004, respectively, when it was determined that the risk-benefit ratio was unfavorable for participants assigned to active treatment.[12, 13] Observational follow-up continued after the end of the trial interventions.

For the present study, we excluded women who were missing biomarker data on omega-3 FA exposure, had no longitudinal data available on cognitive status following enrollment, or who had missing covariate values (Figure 1). Median length of follow-up was approximately 10 years (maximum =20.8) in WHIMS and its subsequent extension study (WHIMS-ECHO).

Outcomes

WHIMS participants' cognitive status was evaluated at annual follow-up assessments. Participants were classified as having probable dementia (PD), mild cognitive impairment (MCI), or no cognitive impairment based on criteria from the *DSM-IV*[14] During the WHIMS study period (1995–2007), participants were screened in person for dementia with the 100-point Modified Mini-Mental State (3MS) exam on an annual basis. Those who screened positive underwent additional cognitive testing and clinical evaluation.[15] All potential cases were centrally adjudicated based on neuropsychiatric test results, questionnaire data, and relevant contextual information on recent health events (e.g., stroke). Participants were classified as having probable dementia (PD), mild cognitive impairment (MCI), or no cognitive impairment based on criteria from the *DSM-IV*[14] Details on outcome ascertainment and case adjudication have been described previously.[10, 11, 16] After the original in-person follow-up phase of WHIMS finished, extended follow-up (2008–2016) was conducted under the WHIMS-ECHO (Epidemiology of Cognitive Health Outcomes) protocol where cognitive evaluation was conducted by telephone interview. Participants were screened for dementia with the Modified Telephone Interview for Cognitive Status (TICS-M).[17] The Dementia Questionnaire,[18] a validated instrument that assesses cognitive, behavioral, and functional status, was administered to participants who screened positive. In addition, Dementia Questionnaire proxy interviews were used if a woman's cognitive status could not be assessed directly. As occurred during WHIMS, potential cases were centrally adjudicated based on *DSM-IV* criteria using on all available information, which included scores from the TICS-M and Dementia Questionnaire, and information on recent health events.

In the present study, the primary endpoint was incident PD ascertained over the WHIMS and WHIMS-ECHO study periods. Secondary endpoints included a composite endpoint of PD or MCI, MCI alone, longitudinal changes in 3MS scores, and 3MS scores at baseline. Participants were censored at their last cognitive assessment.

Exposure Assessment

Participants' baseline omega-3 FA status was assessed using a validated RBC biomarker.[19, 20] RBC FA composition was analyzed using gas chromatography with flame ionization detection. The individual RBC FAs were quantified and expressed as a percent of total identified FAs. The primary exposure of interest was the combination of RBC DHA and EPA content. RBC DHA+EPA, referred to as "the omega-3 index" in prior literature, has been shown to be associated with reduced risks of dementia and cardiovascular disease, and with larger cerebral and hippocampal tissue volumes in prior research.[19, 21–23] The intra-assay coefficient of variation for RBC DHA+EPA was <5.0%. There were 7299 participants with analyzed RBC samples. During aliquoting, the RBC samples were stored improperly at –20 C for a period of approximately two weeks, causing oxidative degradation of the polyunsaturated FAs before measurement. The original FA levels were estimated with multiple (i.e., 10) imputations using independent data on FA degradation rates and the length of time the samples were exposed to –20 C, as described previously by Pottala et al.[24] After correction and the exclusion of samples not meeting quality-control criteria described by Pottala et al., 7082 participants were eligible for analysis. Among these women, correlations between the corrected RBC biomarkers and participants' intakes of DHA and EPA, as estimated from food frequency questionnaires, were similar to those reported in a sample of participants in the Nurses' Health Study.[25, 26] Descriptive statistics on the RBC FA profiles for the study cohort are provided in Appendix Table A1. Parameter estimates were then pooled using Rubin's technique to account for the uncertainty in the corrected DHA+EPA measurements.[27]

Covariates

Covariates reflecting participant HT trial assignment, demographics, health conditions, and health behaviors were selected *a priori* based on their importance as predictors of cognitive and cardiovascular health. (See Tables 1 and 2.) As with DHA+EPA status, all covariates were assessed at study entry.

In sensitivity analyses, baseline 3MS score and *APOE* status were added as potential covariates. Because 3MS score at baseline may reflect the accrued effects of higher DHA +EPA levels over one's lifetime, we did not adjust for 3MS in our main analyses. *APOE* genotype could be imputed only for white participants based on genome-wide association study (GWAS) results. *APOE* status was not included as a covariate in our main analyses because it was available only for a subset (80%) of otherwise eligible participants.

Several tests for effect modification were performed in pre-specified secondary analyses. In these analyses, we evaluated whether the relationship between DHA+EPA and cognitive status was modified by *APOE* genotype, baseline cognitive function (as measured by the 3MS), HT trial assignment, and time since trial enrollment.

Statistical Methods

Kaplan-Meier survival analysis and Cox proportional hazards regression were used to evaluate the relationship between RBC DHA+EPA levels at baseline and time-to-incident-PD. DHA+EPA was analyzed as a continuous variable in our regression models. Three Cox regression models with increasing degrees of covariate adjustment were used to estimate the effect of DHA+EPA on the relative hazard of PD. In model 1, we adjusted for trial assignment and patient demographics; in model 2 we additionally adjusted for cardiometabolic comorbidities; in model 3 we additionally adjusted for health behaviors (Tables 1–2). This approach was repeated for the survival analyses of the MCI and composite PD/MCI endpoints. Univariate survival plots were inspected to check for meaningful violations of the proportional hazards assumption. Changes in risk estimates and in cohort composition over the course of the study period were evaluated to assess the possible impact of loss to follow-up on our results.

To describe differences in absolute risk of PD across levels of RBC DHA+EPA, we used model 3 to estimate the predicted probability of PD for all cohort members at year 15 of follow-up under two DHA+EPA exposure scenarios: (a) one standard deviation (SD) above the mean (6.79% RBC content) and (b) one SD below the mean (3.75% RBC content). This contrast was intended to represent, at the population level, the estimated impact of DHA +EPA biostatus on the cumulative incidence of PD after adjustment for baseline covariates. Bootstrapping with 1000 replicate samples was used to estimate 95% confidence intervals for the risk difference.

Linear mixed models were used to estimate the relationships between DHA+EPA and baseline 3MS scores and the change in 3MS scores over the follow-up period. All covariates in the Cox survival analyses were included as fixed effects and as interactions with time in the 3MS models; in addition, random intercept and time effects were included to account for within-person correlations in test scores.

A two-tailed p value <0.05 was considered statistically significant. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

After restricting to WHIMS participants with information on baseline RBC DHA+EPA levels and longitudinal data on cognitive status, 6706 women were eligible for analysis (Figure 1). At enrollment, the median participant age was 70 years (range: 63, 81) and median 3MS score was 96 (range: 66, 100). Mean RBC DHA+EPA content was 5.27% (SD = 1.52%). Compared to women in the lowest DHA+EPA tertile, women in the highest tertile were slightly older, twice as likely to have graduated from college (40% vs. 22%), and more likely to be non-white (19% vs. 8%). They were also slightly healthier in terms of diabetes prevalence, average body weight, and current smoking status. There was no significant univariate association between DHA+EPA tertile and global cognitive function, as measured by the 3MS, at baseline (Table 1).

Primary Endpoint: Incident Probable Dementia

Over a median of 9.8 years of follow-up (maximum = 20.8), 587 incident PD cases were identified. The Kaplan-Meier cumulative incidence of PD in the WHIMS cohort was 1.5% at year 5, 4.0% at year 10, and 11.7% at year 15. In unadjusted analyses, higher DHA+EPA levels were associated with a lower risk of incident PD, but this association was not significant (log-rank test p value: 0.15; see Figure 2). In Cox proportional hazard models, after adjustment for trial assignment and demographic, health status, and health behavior variables (model 3), a 1-SD increase in DHA+EPA was associated with a reduced risk of incident dementia (HR = 0.92, 95% CI: 0.84, 1.00; $p < 0.05$). This effect estimate did not change meaningfully after further adjustment for baseline 3MS score or *APOE* genotype. Full results are provided in Table 2.

To quantify the absolute risk reduction associated with higher levels of DHA+EPA, we contrasted the projected cumulative incidence of PD if all participants had a DHA+EPA level 1-SD above the sample mean ("high exposure"), or 1-SD below the sample mean ("low exposure"). The HR for this 2-SD contrast, based on regression model 3, was 0.84 (95% CI: 0.71, 1.00; $p < 0.05$). The 15-year cumulative incidence of PD was estimated to be 12.1% with high DHA+EPA exposure compared to 14.2% with low DHA+EPA exposure (absolute risk difference = 2.05%, 95% CI: 0.24%, 3.97%).

Several possible sources of effect modification of the relationship between RBC DHA+EPA and the relative hazard of PD were explored. We found no evidence for effect modification (on the log HR scale) by HT trial assignment (CEE-only trial: $p = 0.98$; CEE+P trial: $p = 0.57$, baseline 3MS score ($p = 0.40$), or *APOE* genotype ($p = 0.32$). A marginally significant interaction between DHA+EPA and years since trial enrollment was observed ($p = 0.11$): the inverse association between a 1-SD increase in DHA+EPA and PD risk was stronger during the initial WHIMS follow-up period (HR = 0.85, 95% CI: 0.73, 1.00) than during the post-trial WHIMS-ECHO period (HR = 0.95, 95% CI: 0.86, 1.05; see Table 2).

Secondary Analyses: Probable Dementia and Mild Cognitive Impairment

In additional secondary analyses, we evaluated the relationship between baseline RBC DHA +EPA levels and incident MCI and a composite PD/MCI endpoint. There was no significant relationship between higher DHA+EPA levels and the risk of MCI or PD/MCI (Table 2). When follow-up was restricted to the initial WHIMS period, marginally significant protective associations were observed for MCI (HR = 0.90, 95% CI: 0.81, 1.01) and PD/MCI (HR = 0.91, 95% CI: 0.83, 1.01).

Secondary Analyses: RBC DHA and EPA Analyzed Separately

We also evaluated RBC DHA and EPA, considered separately, as predictors of incident PD, MCI and PD/MCI. Relationships between DHA and EPA and the study endpoints were similar to the findings for RBC DHA+EPA, although the standard errors for the HRs were larger when DHA and EPA were analyzed separately (Table 2).

Secondary Analyses: 3MS Scores

The 100-point Modified Mini-Mental State (3MS) exam was administered annually to WHIMS participants. Scores through the 11th year of follow-up were included in the present study. Results from our mixed effects linear regression models indicated that higher levels of RBC DHA+EPA were associated with lower 3MS scores at baseline (−0.109, 95% CI: −0.187, −0.032, per 1-SD increase), and with a slower rate of decline in 3MS scores over the follow-up period (0.045, 95% CI: 0.022, 0.067, 3MS points per year, per 1-SD increase). Full results for the 3MS outcomes are provided in Table 3.

DISCUSSION

In this cohort of 6706 older and relatively healthy women who participated in WHIMS, we found that higher baseline levels of RBC DHA+EPA were associated with a lower risk of incident PD and a slower rate of cognitive decline over a median follow-up period of 10 years. This relationship was independent of a variety of known risk factors and predictors for dementia. Our findings add to a growing literature supporting the hypothesis that long-chain omega-3 FAs may be protective against cognitive deterioration.

Strengths of our study include the fact that it is the largest cohort to date in which the association between AD/D and biomarkers of omega-3 FA status has been assessed (as opposed to estimated fish or omega-3 FA intake based on self-reported food intake data). With a maximum length of follow-up of 15 years, our study is also one of the longest cognitive outcome studies to date that has assessed biomarkers of omega-3 FA status. In addition, the well-characterized WHIMS cohort includes data on many important demographic and clinical characteristics, allowing us to adjust for key AD/D risk factors.

The protective association between RBC DHA+EPA and the HR for PD was more pronounced earlier in the follow-up period (WHIMS HR: 0.85) compared to later (WHIMS-ECHO HR: 0.95). Beyond chance, there are several possible explanations for this. It could be that higher DHA+EPA levels delayed dementia onset, but eventually, predisposed individuals did go on to develop the disease. In a time-to-event analysis of a given risk factor, HRs for later periods can become attenuated as the individuals most susceptible to the outcome event are “nudged” into having an earlier event by the risk factor, while susceptible individuals lacking that risk factor remain in the cohort for continued follow-up.[28] Secondly, because RBC omega-3 FAs were assessed only at baseline, it is possible that the association with cognitive outcomes was attenuated over time due to exposure misclassification, as participants’ dietary practices drifted from baseline.

Because of the suggestion of an interaction between DHA+EPA and length of follow-up, and the likelihood that study drop-out could vary by factors related to cognitive function, we explored how the composition of the study cohort changed over time. Baseline cohort characteristics are provided in Appendix Table A2 for the entire eligible WHIMS cohort (N = 6706) and the subset of participants with continued follow-up in WHIMS-ECHO (N = 2676). Compared to the cohort as a whole, those who remained in the study for WHIMS-ECHO were more educated, healthier at baseline, and had somewhat higher RBC DHA +EPA levels. Our fully adjusted Cox regression models, which included as predictors a

larger set of demographic and health covariates related to both the risk of incident PD and the probability of censoring, are likely to be more robust against possible bias due to covariate-dependent censoring.[29]

Our results are consistent with evidence from prior epidemiologic studies supporting a protective association between omega-3 FAs and dementia. Two recent meta-analyses of observational studies found significant inverse relations between fish intake and incident dementia.[7, 8] In nine studies summarized by Lin et al.[30] and in other cross-sectional studies,[22, 31–33] DHA and EPA blood levels were inversely related to dementia, other adverse cognitive outcomes, and ischemic white matter lesions. In longitudinal cohort studies, the most (but not all) have found that biomarker measures of omega-3 FA exposure were associated with a reduced risk of incident dementia.[26, 34–39] These prior cohort studies, ranging from 246 to 2251 subjects, and 4–10 years of follow-up, were considerably smaller than the present study. Thus, our findings provide strong confirmation of these earlier reports. In prior studies, there have been findings that the relationship between omega-3 FAs and dementia pathology may vary by *APOE* genotype;[40, 41] while we did not find statistically significant evidence for interaction ($p = 0.32$), *APOE* data were only available for a subset of participants, and the possibility of clinically meaningful effect modification by *APOE* status cannot be ruled out.

Possible mechanisms to explain the apparent benefit of higher levels of the omega-3 FAs on the development of AD/D have focused on at least two pathways. First, the systemic anti-inflammatory effects of omega-3 FA[5] which are based in part on the downregulation of nuclear factor κ B[42] and in part by the production of oxygenated metabolites of EPA and DHA that actively promote the resolution of inflammation.[43] Secondly, omega-3 FAs alter membrane biophysical properties that result in alterations in ion channels, receptors, and binding between phospholipids and proteins.[44] This may have particular relevance as regards neuronal cell membranes and their interactions with A β peptides, the hallmark molecules of AD pathology. Agents or interventions that increase the binding of soluble A β oligomers (which are believed to be the cytotoxic agents rather than the fully formed fibrils[45]) to membrane phospholipids slow fibril formation and are thus considered to be a beneficial.[46] DHA has recently been shown to have this effect.[47] Hence higher membrane levels of EPA and/or DHA may favorably affect the metabolism of A β peptides and thereby reduce risk for AD/D progression.

This study had several important limitations. Unmeasured confounding cannot be ruled out as an explanation for the associations observed in our study. As shown in Table 1, RBC DHA+EPA levels were associated strongly with baseline demographic characteristics associated with cognitive outcomes: compared to women in the lowest DHA+EPA tertile, women in the highest tertile were twice as likely to have graduated from college (40% vs. 22%), and were more likely to be non-white (19% vs. 8%). We sought to adjust for these observed differences in AD/D risk factors with Cox regression, and found a significant association between RBC DHA+EPA levels and a reduced risk of dementia—independent of demographics, major risk factors for dementia and cardiovascular disease, and cognitive status at baseline (as measured by the 3MSE). However, our study was observational rather

than experimental, and our results should not be interpreted as definitive evidence of a causal relationship.

The fact that our findings were not consistent across all study endpoints is another reason to interpret our results circumspectly. Though we found a significant protective association between RBC DHA+EPA and the risk of incident AD/D and longitudinal changes in 3MSE over time, we did not find a protective association with incident MCI or 3MSE scores at baseline. A possible explanation for the lack of association with MCI is misclassification bias, as diagnostic criteria for MCI are less well defined.

In regards to randomized controlled trials (RCTs) that have tested the effects of omega-3 FA supplementation on cognitive function, a 2012 meta-analysis found no overall benefit in healthy subjects or those with frank dementia, but improvements in attention processing speed in those with CIND.[48] While most RCTs have not found a protective association, an important limitation of these studies is their short length of follow-up (2 years; typically 6 months), which may be too short to detect a measurable effect. Another hypothesis for the divergence between the RCTs and observational studies such as ours is that RBC DHA+EPA may serve as a marker not just for increased intake of DHA and EPA, but more generally for healthier dietary practices (e.g., lower intake of red meat and saturated fats, Mediterranean-style diet). To explore this hypothesis, we performed a *post hoc* sensitivity analysis in which we additionally adjusted for red meat intake based on food frequency questionnaire data[49] and RBC oleic acid content[24]—a proxy for consumption of healthy vegetable oils such as olive oil.[50] Our DHA+EPA effect estimates did not change meaningfully, suggesting that the protective association between RBC DHA+EPA and dementia risk may not simply be a substitution effect.

In conclusion, higher levels of RBC DHA+EPA were associated with a modestly lower risk of AD/D among older women. After scaling the effect estimates to reflect the contrast between having RBC DHA+EPA content 1-SD above versus 1-SD below the mean, the estimated HR and 15-year absolute risk reduction were 0.84 and 2.05%, respectively. In our view, these differences are large enough to be meaningful at the population level. Results from RCTs of omega-3 FA supplementation with extended participant follow-up are needed to help clarify whether this association is causal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

3MS	Modified Mini-Mental State
CEE	Conjugated equine estrogens
CEE+P	(CEE) + Progestin
CI	Confidence interval
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
HT	hormone therapy
HR	hazard ratio
MCI	mild cognitive impairment
PD	Probably dementia
RBC	red blood cell
SD	standard deviation
WHIMS	Women's Health Initiative Memory Study
WHIMS-ECHO	(WHIMS) Epidemiology of Cognitive Health Outcomes

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SUMMARY

This study examined the association between red blood cell (RBC) docosahexaenoic acid and eicosapentaenoic acid (DHA+EPA) levels and risk of dementia in older women. RBC DHA+EPA levels were assessed at baseline, and cognitive status was evaluated annually in a cohort of 6706 women aged 65 years who participated in the Women's Health Initiative Memory Study (WHIMS). During a median follow-up period of 9.8 years, 587 incident cases of probable dementia were identified. In the adjusted Cox regression analysis, a one standard deviation (SD) increase in DHA+EPA was associated with a significantly lower risk of dementia (HR = 0.92, 95% CI: 0.84, 1.00; $p < 0.05$). For women with high DHA+EPA exposure (1 SD above mean) compared to low exposure (1 SD below mean), the adjusted 15-year absolute risk difference for dementia was 2.1% (95% CI: 0.2%, 4.0%). Higher levels of DHA+EPA may help protect against the development of dementia.

Highlights

- We examined the association between erythrocyte EPA+DHA and risk for incident dementia in 6706 women in the USA.
- After about 10 years of follow-up and after appropriate adjustments, we found a significant, 8% decreased risk for probable dementia associated with a 1-SD increase in EPA+DHA.
- This large study confirms previous research suggesting that higher EPA+DHA levels may be protective against dementia.

Figure 1.

Flow diagram showing identification of eligible Women's Health Initiative Memory Study (WHIMS) participants.

Figure 2. Kaplan-Meier plots of dementia-free survival stratified by red blood cell docosahexaenoic acid and eicosapentaenoic acid (RBC DHA+EPA) tertile

Kaplan-Meier plots of dementia-free survival by RBC DHA+EPA levels at baseline.

Incident cases of probable dementia were counted as events. Participants were censored at loss to follow-up, the end of the study period or death. Numbers at the bottom of the graph reflect the number of participants remaining in the risk set at a given follow-up interval.

Table 1

Baseline characteristics of study participants stratified by red blood cell docosahexaenoic acid and eicosapentaenoic acid (RBC DHA+EPA) content (N = 6706).

Characteristic	RBC DHA+EPA Tertile			P value
	Total (N=6706)	Lowest (RBC content: 2.0–4.5%; N=2235)	Middle (RBC content: 4.5–5.6%; N=2236)	
Hormone therapy (HT) trial				
Estrogen alone	2630 (39.2)	919 (41.1)	907 (40.6)	804 (36.0)
Estrogen + progestin	4076 (60.8)	1316 (58.9)	1329 (59.4)	1431 (64.0)
HT trial treatment assignment				
Active treatment	3315 (49.4)	1100 (49.2)	1140 (51.0)	1075 (48.1)
Placebo	3391 (50.6)	1135 (50.8)	1096 (49.0)	1160 (51.9)
Age in years at enrollment				
Median (min–max)	70 (63–81)	69 (63–81)	70 (64–79)	70 (63–79)
Baseline 3MS score				
Median (min–max)	96 (66–100)	96 (68–100)	96 (68–100)	96 (66–100)
APOE genotype				
No ε4 alleles	3984 (59.4)	1353 (60.5)	1384 (61.9)	1247 (55.8)
1+ ε4 alleles	1349 (20.1)	496 (22.2)	442 (19.8)	411 (18.4)
Unavailable	1373 (20.5)	386 (17.3)	410 (18.3)	577 (25.8)
Education				
College or postgraduate degree	2014 (30.0)	495 (22.1)	632 (28.3)	887 (39.7)
Some post-secondary education	2715 (40.5)	948 (42.4)	926 (41.4)	841 (37.6)
High school or less	1977 (29.5)	792 (35.4)	678 (30.3)	507 (22.7)
Race/ethnicity				
Black	456 (6.8)	61 (2.7)	145 (6.5)	250 (11.2)
White	5867 (87.5)	2058 (92.1)	1998 (89.4)	1811 (81.0)
Other	383 (5.7)	116 (5.2)	93 (4.2)	174 (7.8)
Health conditions and behaviors				
Cardiovascular disease	1344 (20.0)	443 (19.8)	452 (20.2)	449 (20.1)
Hypertension	3581 (53.4)	1208 (54.0)	1195 (53.4)	1178 (52.7)
Diabetes	744 (11.1)	255 (11.4)	267 (11.9)	222 (9.9)

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Characteristic	RBC DHA+EPA Tertile			P value
	Total (N=6706)	Lowest (RBC content: 2.0–4.5%; N=2235)	Middle (RBC content: 4.5–5.6%; N=2236)	Highest (RBC content: 5.6–15.6%; N=2235)
Current smoker	459 (6.8)	214 (9.6)	127 (5.7)	118 (5.3)
Past alcohol user	1314 (19.6)	499 (22.3)	444 (19.9)	371 (16.6)
Body-mass Index (BMI), kg/m²				
Median (min–max)	27.6 (13.8–66.7)	28.0 (13.8–66.7)	27.9 (17.0–64.9)	26.9 (15.0–66.1)
Alcohol servings per week				
Median (min–max)	0.2 (0.0–64.7)	0.0 (0.0–49.2)	0.4 (0.0–64.2)	0.4 (0.0–64.7)

Participants were ranked and divided into DHA+EPA tertiles based on the mean of the 10 imputed RBC DHA+EPA values. Summary statistics are the group median (range) for continuous variables or category count (%) for categorical variables. P values for between-group differences across tertiles are based on Kruskal-Wallis and chi-square test results for continuous and categorical variables, respectively. Participants with missing *APOE* genotype data were excluded from chi-square test for differences in *APOE* genotype across tertiles. For simplicity, the 11 educational attainment levels self-reported by WHIMS participants were categorized as follows: a doctoral degree, master's degree, some post-graduate or professional education, and college graduate as "college or postgraduate degree"; some college or associate's degree, and vocational or training school as "some post-secondary education"; and high school diploma or GED, some high school, and grade school as "high school or less."

Table 2

Associations between red blood cell docosahexaenoic acid and eicosapentaenoic acid (RBC DHA+EPA) content at baseline and the relative hazards (HRs) of incident probable dementia (PD), mild cognitive impairment (MCI), or the composite outcome (PD or MCI) during WHIMS and WHIMS-ECHO.

Time period	Exposure	Model	PD* (587 cases total; 213 in WHIMS; 374 in WHIMS-ECHO)			MCI (671 cases total; 325 in WHIMS; 346 in WHIMS-ECHO)			PD or MCI (1047 cases total; 455 in WHIMS; 592 in WHIMS-ECHO)		
			HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
WHIMS (1995–2007) and WHIMS-ECHO (2008–2016)		1	0.92	0.84	1.00	0.0434	0.96	0.3751	0.95	0.89	1.02
	DHA+EPA [†] (per 1 SD)	2	0.92	0.84	1.00	0.0454	0.97	0.3880	0.95	0.90	1.02
		3	0.92	0.84	1.00	0.0498	0.97	0.4466	0.96	0.90	1.02
		S1	0.91	0.83	0.99	0.0293	0.95	0.2284	0.94	0.88	1.00
		S2	0.92	0.83	1.01	0.0929	1.00	0.9501	0.96	0.89	1.04
		3	0.92	0.85	1.01	0.0799	0.97	0.5226	0.96	0.90	1.02
WHIMS	EPA (per 1 SD)	3	0.93	0.85	1.02	0.1244	0.97	0.4376	0.97	0.91	1.03
	DHA+EPA (per 1 SD)	3	0.85	0.73	0.99	0.0429	0.95	0.4098	0.96	0.87	1.05
	DHA+EPA (per 1 SD)	3	0.95	0.86	1.05	0.3162	0.98	0.6704	0.95	0.88	1.03

Model 1 = RBC omega-3 FA content, HT trial arm (4 levels: estrogen-only trial: active treatment or placebo; estrogen and progestin trial: active treatment or placebo), age at baseline, education, race/ethnicity (black, white or other). Educational attainment was analyzed as an 11-level ordinal variable, ranging from 1 (no formal education) to 11 (doctoral degree). For simplicity, these 11 levels were collapsed to three broader categories in Table 1. Cubic spline transformations of age and education with knots at the 10th, 50th and 90th percentiles were used to allow for non-linear relationships with the study outcomes.

Model 2 = Model 1 + cardiovascular disease, hypertension, diabetes.

Model 3 = Model 2 + BMI (cubic spline transformation), current smoking status, status as prior alcohol user, current average servings of alcohol per week (cubic spline transformation).

Model S1 (sensitivity analysis 1) = Model 3 + baseline 3MS score (cubic spline transformation).

Model S2 (sensitivity analysis 2) = Model 3 + *APOE* genotype (none vs. 1+ ϵ 4 alleles). Note: the S2 sensitivity analysis included only participants for whom data on *APOE* genotype were available.

* Probable dementia was the primary endpoint.

[†] RBC DHA+EPA was the primary exposure.

Table 3

Associations between red blood cell docosahexaenoic acid and eicosapentaenoic acid (RBC DHA+EPA) content at baseline and performance on the 100-point Modified Mini Mental State Exam (3MS).

Model	Baseline cross-sectional effect estimates: mean difference in 3MS score per one standard deviation increase in RBC DHA+EPA.			Longitudinal effect estimates: mean difference in the rate of change in 3MS score per one standard deviation increase in RBC DHA+EPA.		
	Estimate	95% CI	P value	Estimate	95% CI	P value
1	-0.089	-0.165	-0.013	0.043	0.021	0.066
2	-0.094	-0.170	-0.018	0.043	0.021	0.066
3	-0.109	-0.187	-0.032	0.045	0.022	0.067

Positive mean differences an association between higher DHA+EPA levels and improved cognitive performance, negative mean differences an associated between higher DHA+EPA levels and worse cognitive performance.

Model 1 covariates: RBC DHA+EPA FA content, HT trial arm, age at baseline, education, race/ethnicity, time (linear effect and indicator variable for each annual follow-up wave). Educational attainment was analyzed as an 11-level ordinal variable, ranging from 1 (no formal education) to 11 (doctoral degree). For simplicity, these 11 levels were collapsed to three broader categories in Table 1. Cubic spline transformations of age and education with knots at the 10th, 50th and 90th percentiles were used to allow for non-linear relationships with the study outcomes.

Model 2 covariates: Model 1 covariates + cardiovascular disease, hypertension, diabetes

Model 3 covariates: Model 2 covariates + BMI (cubic spline transformation), current smoking status, status as prior alcohol user, current average servings of alcohol per week (cubic spline transformation)

Covariates were included as both main effects (cross-sectional effects) and interactions with time (effects on rate of change). To account for within-person correlations in test scores, person-level random intercept and time effects were included.