See corresponding editorial on page 7.

Brain atrophy in cognitively impaired elderly: the importance of long-chain ω-3 fatty acids and B vitamin status in a randomized controlled trial[1,2]

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ABSTRACT

Background: Increased brain atrophy rates are common in older people with cognitive impairment, particularly in those who eventually convert to Alzheimer disease. Plasma concentrations of omega-3 (ω-3) fatty acids and homocysteine are associated with the development of brain atrophy and dementia.

Objective: We investigated whether plasma ω-3 fatty acid concentrations (eicosapentaenoic acid and docosahexaenoic acid) modify the treatment effect of homocysteine-lowering B vitamins on brain atrophy rates in a placebo-controlled trial (VITACOG).

Design: This retrospective analysis included 168 elderly people (≥70 y) with mild cognitive impairment, randomly assigned either to placebo (n = 83) or to daily high-dose B vitamin supplementation (folic acid, 0.8 mg; vitamin B-6, 20 mg; vitamin B-12, 0.5 mg) (n = 85). The subjects underwent cranial magnetic resonance imaging scans at baseline and 2 y later. The effect of the intervention was analyzed according to tertiles of baseline ω-3 fatty acid concentrations.

Results: There was a significant interaction (P = 0.024) between B vitamin treatment and plasma combined ω-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) on brain atrophy rates. In subjects with high baseline ω-3 fatty acids (>590 μmol/L), B vitamin treatment slowed the mean atrophy rate by 40.0% compared with placebo (P = 0.023). B vitamin treatment had no significant effect on the rate of atrophy among subjects with low baseline ω-3 fatty acids (<390 μmol/L). High baseline ω-3 fatty acids were associated with a slower rate of brain atrophy in the B vitamin group but not in the placebo group.

Conclusions: The beneficial effect of B vitamin treatment on brain atrophy was observed only in subjects with high plasma ω-3 fatty acids. It is also suggested that the beneficial effect of ω-3 fatty acids on brain atrophy may be confined to subjects with good B vitamin status. The results highlight the importance of identifying subgroups likely to benefit in clinical trials. This trial was registered at www.controlled-trials.com as ISRCTN94410159. *Am J Clin Nutr* 2015;102:215–21.

Keywords: B vitamin, brain atrophy, homocysteine, mild cognitive impairment, ω-3

INTRODUCTION

Mild cognitive impairment (MCI)[8] is a syndrome characterized by a subtle decline in cognitive function and is considered a transitory state between normal aging and clinical dementia and Alzheimer disease (AD) (1, 2). A modest rate of brain atrophy is observed in normal aging. However, in subjects with MCI, dementia, or AD, the brain atrophy rates are markedly faster (3–5). Furthermore, in MCI, the rate of atrophy is usually higher in the subgroup that eventually converts to AD (6). There are no available cures for AD, but an alternative approach is strategies to delay disease progression at an early stage. Cranial MRI is established as a method to monitor disease progression (3, 4, 7, 8). Effective interventions may be detected by a slowing of brain atrophy rate.

The role of ω-3 fatty acids in cognitive decline and dementia is debated. Epidemiologic evidence is consistent with a protective role of dietary intake of fish oils rich in ω-3 fatty acids such as EPA and DHA (9, 10). Case-control studies have revealed associations between DHA or EPA and brain volume and lower degrees of white matter hyperintensities (11, 12). In prospective studies, red blood cell DHA and EPA concentrations

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[2] Supplemental Tables 1–3 and Supplemental Figure 1 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[8] Abbreviations used: AD, Alzheimer disease; MCI, mild cognitive impairment; PEMT, phosphatidylethanolamine *N*-methyltransferase; SAH, *S*-adenosylhomocysteine; tHcy, total homocysteine; VITACOG, Homocysteine and B Vitamins in Cognitive Impairment.

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were positively correlated with higher total brain and hippocampal volumes 8 y later (13), and higher relative concentrations of plasma EPA were associated with a reduced brain atrophy rate in the medial temporal lobe (14). However, results from randomized clinical trials including ω-3 supplementation are not equally convincing (9, 15). One reason for this discrepancy may be a failure to identify the relevant subgroups that are likely to benefit from supplementation (16).

Homocysteine is a nonessential, sulfur-containing amino acid synthesized endogenously from methionine. Raised plasma total homocysteine (tHcy) is a recognized modifiable risk factor for cognitive impairment, dementia, and AD (10, 17, 18). The atrophy rate of the brain is faster at low plasma vitamin B-12 concentrations (19) and at high plasma tHcy concentrations (20, 21). Results from Homocysteine and B Vitamins in Cognitive Impairment (VITACOG), a randomized clinical trial with homocysteine-lowering B vitamins in older people with MCI, showed that treatment with high doses of B vitamins markedly reduced the global brain atrophy rate, as well as atrophy rates in those gray matter regions most commonly associated with AD (20, 21).

Multiple links between ω-3 fatty acids and homocysteine have been suggested. There is an inverse correlation between tHcy and plasma concentrations of ω-3 fatty acids (22, 23), and B vitamins are important for the methylation and assembly of phospholipids (24, 25). The purpose of this study was to determine whether the plasma long-chain ω-3 fatty acid status modifies the effect of high-dose B vitamin supplementation on brain atrophy rates in elderly subjects with MCI.

METHODS

Participants

This retrospective study was conducted as a part of the VITACOG trial (registered at www.controlled-trials.com as ISRCTN94410159). The study received approval of the Oxfordshire National Health Service research ethics committee A (COREC 04/Q1604/100) and was carried out according to the principles of the Declaration of Helsinki. Participants were recruited in the Oxford area between April 2004 and November 2006, and all participants gave their written informed consent. The study protocol and participants, along with inclusion and exclusion criteria, and details on randomization have been described in detail previously (20). In short, 646 volunteers aged >70 y were assessed for eligibility and a diagnosis of MCI. After random assignment of eligible MCI subjects and some withdrawals, 133 subjects received 0.8 mg folic acid, 0.5 mg vitamin B-12, and 20 mg vitamin B-6 (TrioBe Plus; Meda AB/Recip AB) once daily for 24 mo, and 133 received placebo, using a parallel design. Adherence was assessed by counting tablets returned and by measurement of blood vitamin concentrations after the completion of the study (20). Of the 223 subjects completing the trial (110 in the B vitamin group and 113 in the placebo group), 187 had given their written consent to undergo MRI scans at baseline and follow-up. Of these, 180 completed scans at both time points. In the present study, we only included participants who completed the trial with technically suitable MRI scans at both occasions (n = 168). Of these, 85 were in the B vitamin group, and 83 were in the placebo group.

MRI scans

The MRI protocol used in the VITACOG study has been described elsewhere (20). Briefly, baseline and follow-up volumetric cranial MRI scans were carried out at the Oxford Centre for Clinical Magnetic Resonance Research by using a 1.5T MRI system (Sonata; Siemens Medical Solutions). Whole-brain atrophy per year was estimated from magnetic resonance images taken at baseline and follow-up by using the fully automated SIENA protocol (26). Normalized brain volume at baseline was estimated from a single image by using a cross-sectional method (SIENAX).

Biochemical assays

Plasma was prepared from nonfasting blood samples collected at baseline and after 2 y of intervention. Total fatty acid concentrations were analyzed by gas chromatography coupled to mass spectrometry by using a modified in situ transesterification protocol for fatty acid methyl ester preparation (see supplemental material). Plasma samples were stored between 4 and 6 y at −80°C before analysis. Under these conditions, fatty acid compositions in serum have been shown to be stable for up to 10 y (27). Fatty acid concentrations were expressed in absolute concentrations (μmol/L) unless otherwise stated. The between-day CVs for DHA and EPA were 4.7% and 3.9%, respectively, and <10% for the remaining fatty acids (**Supplemental Table 1**). *ApoE* genotype, plasma tHcy, folate, and vitamin B-12 were analyzed as previously described (19).

Statistical analysis

The main objective of this retrospective study was to investigate the effect of B vitamin treatment on brain atrophy rates as a function of baseline ω-3 fatty acid status. Combined ω-3, here defined as the sum of DHA and EPA, was used along with DHA and EPA separately. Because fatty acid concentrations demonstrated a skewed distribution, all fatty acid concentrations were transformed by using the natural log before analyses. Paired t tests were used to investigate whether ω-3 fatty acids changed between baseline and follow-up in subjects treated with B vitamins and placebo. Independent-samples t tests were used for comparisons between groups and partial correlations for analysis of the relation between baseline fatty acid concentrations and atrophy rates. Comparisons of correlations were performed by using the Fisher r-to-z transformation. For the main objective of the study, the linear regression model was used as the main statistical model with brain atrophy as the dependent variable and B vitamin treatment and ω-3 fatty acid status, as expressed in tertiles, as the main predictors or independent variables. The model also adjusted for age, sex, initial brain volume, *ApoE* status, education level, and baseline levels of diastolic blood pressure, triglyceride concentration (log), creatinine, and tHcy (log) [see Smith et al. (20) for why these variables were chosen]. Diagnostic checks to assess assumptions of the linear model were carried out along with outliers and leverage analysis. To test that the effect of treatment on brain atrophy is dependent on the concentration of ω-3 fatty acids within the linear regression model, we used a global Fisher test (F test) to test the null hypothesis that all interaction terms are equal to 0. Two interaction terms were included in the linear regression model: the

TABLE 1
Characteristics of participants in the VITACOG study[1]

	All (n = 168)	Placebo (n = 83)	B Vitamins (n = 85)
Age,[2] y	76.6 (75.9, 77.3)	76.2 (75.3, 77.2)	77.0 (75.8, 78.1)
Women, n (%)	102 (60.7)	52 (62.7)	50 (58.8)
Brain volume,[2] mL	1381 (1369, 1393)	1376 (1360, 1391)	1387 (1368, 1405)
BMI,[2] kg/m^2	26.0 (25.4, 26.5)	26.6 (25.7, 27.6)	25.3 (24.6, 26.0)[3]
Systolic BP,[2] mm Hg	148 (144, 151)	147 (143, 152)	148 (143, 153)
Diastolic BP,[2] mm Hg	80 (78, 82)	80 (78, 83)	80 (77, 82)
Depression score (GDS)[2]	6.5 (5.8, 7.2)	7.5 (6.4, 8.9)	5.6 (4.5, 6.4)[3]
Alcohol consumption,[2] U/wk	8.1 (6.6, 9.6)[4]	7.2 (5.1, 9.2)	9.0 (6.8, 11.2)
Antidiabetic drugs, n (%)	14 (8.3)	10 (12)	4 (4.7)
Smoker, anytime, n (%)	81 (48.2)	43 (51.8)	38 (44.7)
Use of B vitamins, n (%)	31 (18.5)	17 (20.5)	14 (16.5)
Use of fish oils, ω-3, n (%)	67 (39.9)	31 (37.3)	36 (42.4)
ApoE4 carriers, n (%)	51 (30.4)	29 (35)	22 (25.9)
tHcy, baseline,[5] μmol/L	11.3 (10.8, 11.8)	11.3 (10.6, 12.0)	11.3 (10.6, 12.0)
tHcy, follow-up,[5] μmol/L	10.3 (9.8, 10.8)	12.1 (11.4, 12.9)[6]	8.7 (8.3, 9.2)[3,6]
Vitamin B-12, baseline,[5] pmol/L	331 (313, 350)	333 (310, 357)	330 (303, 360)
Vitamin B-12, follow-up,[5] pmol/L	497 (462, 535)	366 (335, 400)[6]	672 (626, 722)[3,6]
Folate, baseline,[5] nmol/L	23.3 (21.2, 25.7)	24.2 (21.4, 27.5)	22.4 (19.4, 25.9)
Folate, follow-up,[5] nmol/L	45.4 (39.9, 51.6)	24.9 (21.4, 29.1)	82.1 (74.6, 90.4)[3,6]
ω-3, baseline,[5] μmol/L	472 (439, 508)	488 (442, 539)	457 (411, 508)
ω-3, follow-up,[5] μmol/L	465 (434, 499)	479 (433, 530)	452 (410, 499)
EPA, baseline,[5] μmol/L	177 (161, 195)	181 (158, 208)	173 (151, 197)
EPA, follow-up,[5] μmol/L	177 (162, 195)	181 (158, 207)	174 (153, 198)
DHA, baseline,[5] μmol/L	288 (270, 307)	299 (276, 325)	277 (252, 306)
DHA, follow-up,[5] μmol/L	280 (264, 298)	290 (266, 316)	271 (248, 296)

[1]VITACOG subjects with available MRI data at start and finish. *ApoE4*, apolipoprotein E4; BP, blood pressure; GDS, geriatric depression scale; tHcy, total homocysteine. ω-3 denotes the sum of the amounts of EPA+DHA; VITACOG, Homocysteine and B Vitamins in Cognitive Impairment.
[2]Values are means; 95% CIs in parentheses.
[3]$P < 0.05$ (2-tailed) compared with placebo group as assessed by independent t tests.
[4]Excluding one high outlier.
[5]Values are geometric means; 95% CIs in parentheses.
[6]$P < 0.05$ (2-tailed) compared with baseline value as assessed by paired t tests.

treatment by second tertile indicator and the treatment by third tertile indicator. First tertile was then considered as reference. The F test tested the null hypothesis (H_0): treatment by second tertile interaction = treatment by third tertile interaction = 0. This has been done by using the function "linearHypothesis" in the R package "car." Because this is an interaction term, the same procedure was used to test whether the effect of baseline ω-3 fatty acids on brain atrophy is dependent on B vitamin treatment. Pairwise comparisons among the fatty acid tertiles following ANCOVA, adjusted for covariates specified above (no adjustment for multiple comparisons was made), were used to examine the differences in brain atrophy rates between the placebo group and the B vitamin–treated group.

In further analyses based on tHcy status, a threshold of 11.3 μmol/L was used to define low and high tHcy groups. This value corresponds to the median baseline tHcy concentration in the VITACOG cohort: previous studies showed that the beneficial effect of B vitamin treatment on global and regional brain atrophy (20, 21) and cognitive decline (28) was dependent on baseline tHcy. The impact of baseline tHcy status on the

TABLE 2
Plasma fatty acid concentrations (absolute) at baseline as predictors of yearly brain atrophy rate (%)[1]

	All			Placebo			B Vitamins			
	β (SE)	Partial r	P	β (SE)	Partial r	P	β (SE)	Partial r	P	P for difference
ω-3	−0.29 (0.12)	−0.21	0.009	−0.08 (0.16)	−0.06	0.618	−0.47 (0.16)	−0.36	0.002	0.047
EPA	−0.17 (0.09)	−0.17	0.037	−0.04 (0.16)	−0.04	0.738	−0.29 (0.13)	−0.27	0.019	0.129
DHA	−0.36 (0.13)	−0.22	0.005	−0.11 (0.20)	−0.06	0.592	−0.56 (0.16)	−0.39	0.001	0.026

[1]Unstandardized coefficients (β) with their SE and partial correlation coefficients with their P values. The linear regression model was adjusted for age, sex, initial brain volume, *ApoE* status, education level, diastolic blood pressure at baseline, and baseline concentrations of triglycerides (log), creatinine, and total homocysteine (log). ω-3 denotes the sum of the amounts of EPA+DHA. All fatty acid variables were entered as the natural log of the baseline concentrations in μmol/L to ensure normal distribution. P for the difference in slopes between placebo and B vitamins was calculated by using the Fisher r-to-z transformation. n = 168 (all), n = 83 (placebo), and n = 85 (B vitamins).

treatment effect according to ω-3 fatty acid tertiles was investigated by using a linear regression model with brain atrophy as the dependent variable and B vitamin treatment, ω-3 fatty acid tertiles, and tHcy status (high/low) as the main predictors or independent variables. The model adjusted for the same variables as specified above. Two 3-way interaction terms were included in the model: the treatment by tHcy status by second tertile indicator and the treatment by tHcy status by third tertile indicator. First tertile was then considered as reference. Statistical analysis was carried out by using the R statistical program version 3.03 (The R Foundation, www.R-project.org). P values (2-tailed) <0.05 ($P < 0.1$ for interaction term) were considered statistically significant.

RESULTS

Participants

Selected characteristics of the study population are summarized in **Table 1**. As previously reported (20), the baseline characteristics and losses to follow-up in the active and placebo groups were similar. Demographic comparison of those who completed the MRI scans compared with the whole cohort can be found in our 2 previous reports (20, 28). The adherence was good in both groups, as assessed by counting returned tablets and measuring plasma vitamins and related compounds (20). There were no significant safety issues and no difference in the adverse events between the intervention groups. At follow-up, the B vitamin group showed a marked improvement in plasma vitamin status, whereas the placebo group showed little or no change (20). Baseline concentrations of ω-3 fatty acids did not differ between the treatment groups and did not change significantly from baseline to follow-up in either the B vitamin or placebo group, as judged by paired t tests (Table 1).

Partial correlation analyses were performed to investigate associations between absolute ω-3 fatty acid concentrations and brain atrophy rates in the whole study group and stratified by treatment group. In the total study group, the combined ω-3, DHA, and EPA showed significant negative correlations with brain atrophy rates (**Table 2**).

In the B vitamin group, inverse correlations between the combined ω-3, DHA, and EPA and brain atrophy rates were significant (Table 2). None of these correlations were significant in the placebo group. The differences in correlation coefficients in the placebo group and the B vitamin–treated group were statistically significant for the combined ω-3 fatty acids and DHA (Table 2). Correlations of all fatty acids assayed, using both absolute and relative fatty acid concentrations, can be found in **Supplemental Tables 2** and **3**.

Effect of B vitamins on brain atrophy according to ω-3 concentration

Yearly atrophy rates in the placebo and B vitamin groups according to tertiles of baseline combined ω-3, DHA, and EPA concentrations are shown in **Figure 1**. Using linear regression, we found a significant interaction between B vitamin treatment and combined ω-3 ($P = 0.024$) and for EPA tertiles ($P = 0.085$). The interaction was not significant for DHA ($P = 0.134$). In B vitamin–treated patients, linear regression analysis revealed

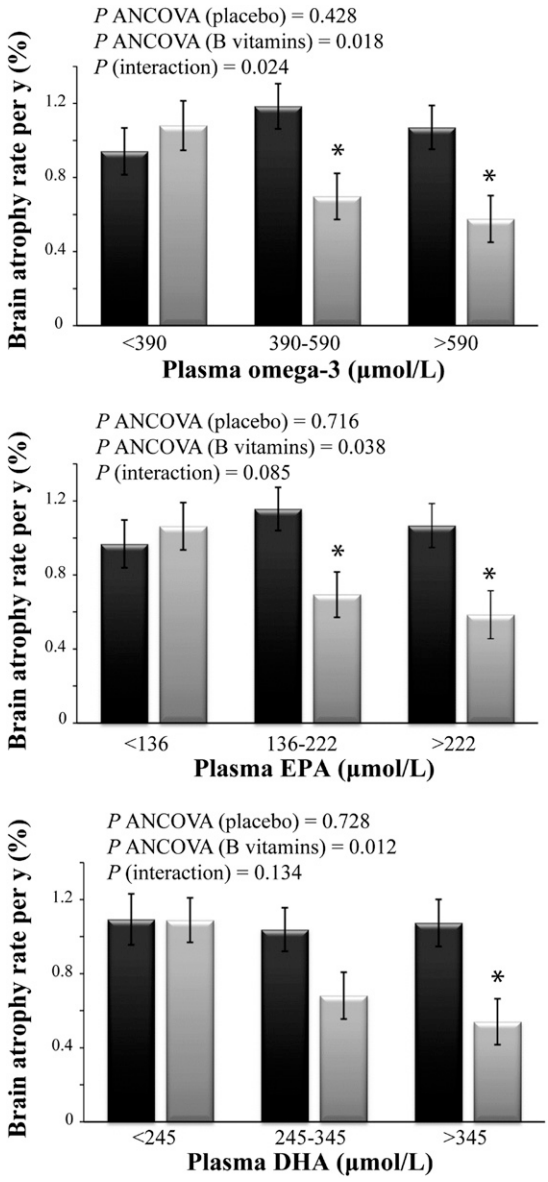

FIGURE 1. Brain atrophy rates (mean ± SEM) among subjects receiving placebo (black) and high-dose B vitamins (gray) according to tertiles of plasma baseline combined ω-3 (top), EPA (middle), and DHA (bottom), adjusted for age, sex, and initial brain volume, *ApoE* status, educational level, baseline diastolic blood pressure, and baseline plasma concentrations of triglycerides (log), creatinine, and total homocysteine (log). Interaction between treatment groups and ω-3 concentrations by tertiles were evaluated by using a linear regression model, and differences between tertiles within each treatment group were assessed by ANCOVA. *$P < 0.05$ (2-tailed) between placebo and B vitamin–treated subjects by adjusted pairwise comparisons. Group sizes in the placebo group and B vitamin group were 27–28 among the ω-3 tertiles, 26–29 among the EPA tertiles, and 24–32 among the DHA tertiles. ω-3 denotes the sum of the amounts of EPA+DHA.

significant trends for the combined ω-3 ($P = 0.018$), EPA ($P = 0.038$), and DHA ($P = 0.012$); in other words, the atrophy rate decreases with increasing ω-3 fatty acids.

Adjusted pairwise comparisons following ANCOVA showed that, in subjects with high baseline combined ω-3 (>590 μmol/L), B vitamin treatment slowed atrophy rates by 40.0% ($P = 0.023$) compared with placebo (Figure 1). A similar result was observed in those with high EPA (>222 μmol/L) (45.8% reduction, $P = 0.011$)

and for DHA (>345 μmol/L) (43.4% reduction, $P = 0.004$). B vitamin treatment had no significant effect on atrophy rates among subjects in the bottom tertiles of fatty acid concentrations (combined ω-3 <390 μmol/L; EPA <136 μmol/L; DHA <245 μmol/L).

Effect of B vitamins on brain atrophy according to baseline tHcy and ω-3 concentration

To follow up our previous results that the effect of B vitamins depends on baseline plasma tHcy (20, 28), we examined the association of baseline fatty acid with atrophy in subjects with low and high baseline tHcy values (threshold, 11.3 μmol/L). B vitamin treatment had no significant effect compared with placebo in subjects with low baseline tHcy concentrations, independent of DHA, EPA, or ω-3 concentration (**Table 3** and **Supplemental Figure 1**). In contrast, in subjects with high baseline tHcy, the effect of B vitamin treatment changed according to plasma ω-3 fatty acid concentration: the rate of atrophy was significantly slowed by treatment (by ~70%) in subjects in the upper ω-3 fatty acid tertiles (Table 3 and Supplemental Figure 1), whereas no effect was observed in the lower tertile. Group sizes in these analyses were not ideal and ranged from 4 to 23 (see Supplemental Figure 1).

DISCUSSION

In this retrospective exploratory analysis of data from a randomized, placebo-controlled trial, we observed a significant interaction effect between high-dose B vitamin treatment and ω-3 fatty acid concentrations on rate of atrophy of the whole brain. The beneficial effect of high-dose B vitamin supplementation was augmented by a high baseline status of ω-3 fatty acid. In subjects with high plasma concentrations of ω-3 fatty acids (EPA+DHA >590 μmol/L), B vitamin supplementation slowed the mean brain atrophy rate by 40% compared with subjects in the placebo group. In contrast, in subjects with low ω-3 fatty acid concentrations (<390 μmol/L), there was no beneficial effect of B vitamins on brain atrophy.

One major effect of the high-dose B vitamin treatment is to lower plasma tHcy. We found that the effect of B vitamins in the higher tertiles of ω-3 fatty acids is limited to patients with baseline tHcy concentrations above the median (≥11.3 μmol/L). In this subgroup, the brain atrophy rate among patients in the upper tertile of ω-3 fatty acid concentration (>590 μmol/L) was reduced by ~70% by B vitamin treatment compared with placebo. Although these results should be interpreted with some caution due to the small group sizes, our results indicate that the effect of B vitamins in subjects with moderate to high ω-3 fatty acid concentrations is driven mainly by beneficial effects in subjects with elevated baseline tHcy concentrations. We therefore hypothesize that low tHcy concentrations, which are the consequence of B vitamin treatment, facilitate the protective effect of ω-3 fatty acids against brain atrophy (Table 2).

Long-chain ω-3 fatty acids have been associated with protective roles in dementia and AD in epidemiologic studies (see Introduction). Recently, Witte and coworkers (29) showed that daily fish-oil supplementation (880 mg DHA and 1320 mg EPA) in healthy elderly for 26 wk prevented the loss of total gray matter volume. Only 2 studies investigating ω-3 fatty acids along with B vitamins have been reported. One of these investigated a nutritional supplement that also included ω-3 fatty acids (EPA, 300 mg; DHA, 1200 mg) and B vitamins (folic acid, 0.4 mg; vitamin B-6, 1 mg; vitamin B-12, 0.003 mg) (30). The supplement produced some beneficial effects in mild AD when given for 24 wk, but this was not confirmed in a larger follow-up study (31). The second study used a 2 × 2 factorial design, with one arm including B vitamins (folate, 0.56 mg; vitamin B-6, 3 mg; vitamin B-12, 0.02 mg) and the other including ω-3 fatty acids (EPA, 400 mg; DHA, 200 mg), and found that the combination of both nutrient groups decreased the likelihood of a lower score on a temporal orientation task in a subgroup with prior stroke (32). Both studies were in populations with different characteristics and used lower doses of B vitamins compared with VITACOG, and none of these studies reported brain

TABLE 3
Brain atrophy rates per year after B vitamin treatment compared with placebo, stratified by baseline ω-3 and tHcy status[1]

	ω-3				EPA				DHA			
	Placebo	B vitamins	Difference, %	*P* value	Placebo	B vitamins	Difference, %	*P* value	Placebo	B vitamins	Difference, %	*P* value
tHcy <11.3 μmol/L												
Low tertile	1.00	1.00	0.0	0.991	0.99	0.94	5.1	0.860	1.16	1.04	10.3	0.635
Middle tertile	1.07	0.83	22.4	0.354	0.98	0.84	14.3	0.595	0.88	0.82	6.8	0.807
High tertile	0.89	0.79	11.2	0.600	0.92	0.79	14.1	0.557	0.89	0.70	21.3	0.338
tHcy ≥11.3 μmol/L												
Low tertile	1.03	1.23	−19.4	0.409	1.09	1.14	−4.6	0.821	1.07	1.10	−2.8	0.898
Middle tertile	1.39	0.54	61.2	<0.001	1.33	0.52	60.9	<0.001	1.33	0.55	58.6	<0.001
High tertile	1.48	0.47	68.2	<0.001	1.36	0.37	72.8	<0.001	1.59	0.43	73.0	0.002

[1]Brain atrophy rates per year for the placebo group and B vitamin–treated group are given in percentages. Percent difference is defined as follows: brain atrophy in placebo group minus brain atrophy in B vitamin group, divided by the atrophy rate in the placebo group, multiplied by 100. The linear regression model included treatment, age, sex, initial brain volume, *ApoE* status, education level, baseline diastolic blood pressure, and baseline plasma concentrations of triglycerides (log), creatinine, total homocysteine (median split, 11.3 μmol/L), and the tertiles of the 3 fatty acid variables. *P* values for the 3-way interactions between treatment × tHcy status (high/low) × fatty acid status (tertiles) were significant for the high tertiles: combined ω-3 ($P = 0.026$), EPA ($P = 0.055$), and DHA ($P = 0.040$). Group sizes in these analyses ranged from 4 to 23, with a median of 14 (see Supplemental Figure 1). ω-3 denotes the sum of the amounts of EPA+DHA. For ω-3, the low, middle, and high tertiles were defined as <390, 390–590, and >590 μmol/L, respectively. The corresponding values were <136, 136–222, and >222 μmol/L for EPA and <245, 245–345, and >345 μmol/L for DHA.

volume or brain atrophy data. The results of these studies are therefore difficult to compare with ours.

Fatty acids are delivered to various target tissues as components of phospholipids, of which phosphatidylcholine is the most abundant in plasma. Experiments in rodents have shown that phosphatidylcholine molecules enriched in DHA are distributed selectively to certain tissues, including the brain (33). It is therefore conceivable that a reduced phosphatidylcholine synthesis will affect the transport of ω-3 fatty acids to the brain, with possible implications for brain health. Indeed, low plasma concentrations of phosphatidylcholine enriched in DHA and EPA have been linked to the risk of dementia (34, 35). Phosphatidylcholine is synthesized in the liver via the cytidine 5′-diphosphate–choline dependent pathway or from phosphatidylethanolamine through 3 consecutive *S*-adenosylmethionine–dependent methylation reactions catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT). DHA content in phosphatidylcholine has been proposed as a marker of PEMT activity (36), and plasma DHA is disproportionally reduced by disruption of *PEMT* in a mouse model (37). As a consequence, PEMT activity is considered vital for the delivery and incorporation of ω-3 fatty acids into the brain (37, 38).

PEMT is inhibited by *S*-adenosylhomocysteine (SAH), the precursor of homocysteine. At high tHcy concentrations, SAH accumulates, which in turn may reduce PEMT activity. In patients with AD, there is an inverse correlation between plasma SAH and DHA concentrations in erythrocyte phosphatidylcholine, possibly because of inhibition of PEMT by SAH (39). In chick embryos, exposure to homocysteine altered brain lipid composition, with reduced concentrations of phosphatidylcholine and an increase of phosphatidylethanolamine while also reducing the proportion of DHA in brain cell membranes (40). In rats, a B vitamin–enriched diet increased plasma total DHA concentration compared with a B vitamin–deficient diet (41). These reports are consistent with the hypothesis that a good B vitamin status and low tHcy concentrations are required for an optimal utilization and distribution of ω-3 fatty acids.

Although a biochemical interaction at the level of phospholipid metabolism seems likely, there are other potential explanations for the observed interaction. For example, it is possible that both ω-3 fatty acids and B vitamins protect against hyperphosphorylation of tau, with potential consequences for tangle formation (42, 43). Also, both B vitamins and ω-3 fatty acids might attenuate inflammation associated with AD. A combination of B vitamins and ω-3 fatty acids was recently shown to reduce oxidative stress and inflammation in a rodent model of hypertension (44). Whether any of these mechanism explain the interaction reported herein is a focus for future studies.

There are some limitations of this study. In this study, we did not measure phosphatidylcholine, which is probably the best source of DHA for the brain (34, 38). In future studies, it would be valuable to also investigate the distribution of ω-3 fatty acids in the various plasma compartments and also examine the effect of B vitamins on phosphatidylcholine. Finally, our study was a randomized controlled trial with B vitamins, not ω-3 fatty acids. In future trials, it would be useful to also include treatment with ω-3 fatty acids.

In conclusion, we have shown that the effect of B vitamin supplementation on brain atrophy rates depends on pre-existing plasma ω-3 fatty acid concentrations; this finding could possibly explain why some B vitamin trials on brain function have failed. Conversely, our results suggest that tHcy status may also determine the effects of ω-3 fatty acids in cognitive decline and dementia and so could explain why some trials of ω-3 fatty acids have failed. Altogether, our results emphasize the importance of identifying subgroups in clinical trials. A randomized clinical trial of B vitamin and ω-3 fatty acid supplementation using a 2 × 2 factorial design is clearly warranted to shed light on the roles of homocysteine and ω-3 fatty acids in brain atrophy, MCI, dementia, and AD.

The authors' responsibilities were as follows—FJ, AKE, HR, and ADS: designed the research; FJ: conducted the lipid analyses and wrote the first draft of the manuscript; FJ, AO, and SMS: analyzed data; and all authors: critically reviewed the analyses and the manuscript. ADS is named as inventor on 3 patents held by the University of Oxford on the use of B vitamins to treat AD or MCI (US6008221, US6127370, and PCT/GB2010/051557); HR is named as inventor on patent PCT/GB2010/051557. Under the University of Oxford's rules, they could benefit financially if the patents are exploited. FJ, AKE, AO, and SMS reported no personal or financial conflicts of interest. None of the funders or the sponsor (University of Oxford) played any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

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