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Relation of serum BDNF to major depression and exploration of mechanistic roles of serum BDNF in a study of vitamin D3 and omega-3 supplements for late-life depression prevention

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AUTHOR CONTRIBUTIONS

Drs. Vyas and Okereke had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Okereke, Reynolds, Mischoulon, Chang, Cook, Manson

Acquisition, analysis, or interpretation of data: All authors

Initial drafting of the manuscript: Vyas, Okereke

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CONFLICT OF INTEREST

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Dr. Chang receives royalties from Up-to-Date.

Dr. Buring's spouse was on the Scientific Advisory Board of Pharmavite LLC during the trial.

Dr. Mora has served as consultant to Pfizer and Quest Diagnostics.

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Dr. Reynolds receives payment from the American Association of Geriatric Psychiatry as Editor-in-Chief of the American Journal of Geriatric Psychiatry, royalty income for intellectual property as co-inventor of the Pittsburgh Sleep Quality Index and, in the past, a one-time honorarium from Merck for consultation on care pathways for insomnia. Dr. Reynolds also receives royalty income from Oxford University Press and from Up-to-Date.

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Abstract

This study: 1) examined cross-sectional and longitudinal relations of serum brain-derived neurotrophic factor (BDNF) to late-life depression (LLD); 2) tested effects of vitamin D3 and omega-3s on change in BDNF; 3) explored modifying or mediating roles of BDNF on effects of vitamin D3 and omega-3s for LLD. We selected 400 adults from a completed trial of vitamin D3 and omega-3 supplements for LLD prevention. BDNF was measured using an enzyme-linked immunosorbent assay. We administered semi-structured diagnostic interviews and Patient Health Questionnaire [PHQ]-9 to ascertain outcomes at baseline (depression caseness vs. non-caseness; PHQ-9) and at 2-year follow-up among baseline non-depressed individuals (incident vs. no incident MDD; change in PHQ-9). At baseline, while there were no significant differences in mean serum BDNF comparing depression cases and non-cases, being in the lowest vs. highest serum BDNF quartile was significantly associated with worse depressive symptoms. There were no significant longitudinal associations between serum BDNF and LLD. Neither supplement significantly affected change in BDNF; serum BDNF did not appear to modify or mediate treatment effects on LLD. In conclusion, we observed significant cross-sectional but not longitudinal associations between serum BDNF levels and LLD. Vitamin D3 or omega-3s did not alter serum BDNF over 2 years.

Keywords

BDNF; depression; vitamin D3; omega-3s; geriatric

1. INTRODUCTION

Major depressive disorder (MDD) affects millions worldwide and is a major cause of later-life disability. Clinical heterogeneity and biological complexity of MDD pose significant challenges in discovering reliable clinical biomarkers (Strawbridge et al., 2017). Furthermore, the identification of minimally-invasive, cost-effective, and easy-to-obtain biomarkers can serve as potential targets for detection, treatment, and prevention of late-life depression (LLD). Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, is synthesized in the brain, can cross the blood-brain barrier, and is abundantly distributed throughout the peripheral tissues. BDNF plays a pivotal role in neuronal differentiation, maintenance, and survival, as well as in the synaptic plasticity of neurons – all biological processes implicated in the development of MDD (Bathina and Das,

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2015; Duman et al., 2000; Duman and Monteggia, 2006). Thus, low serum levels of BDNF have promise as a biomarker in MDD. In meta-analyses of observational studies, depressed patients had lower serum BDNF compared to non-depressed individuals; however, clinical heterogeneity of participants and study designs limit the ability to draw uniform conclusions across these studies (Bocchio-Chiavetto et al., 2010; Molendijk et al., 2014; Shi et al., 2020). Furthermore, little is known about whether serum BDNF can predict new onset of MDD and change in mood in older adults.

The role of nutraceuticals, such as vitamin D3 and marine omega-3 fatty acids (omega-3s) supplements, have been previously investigated for prevention of LLD in randomized clinical trials (RCTs); the majority of RCTs found no benefits of either supplement on LLD prevention (Liao et al., 2019; Vellekkatt and Menon, 2019). Experimental rat model studies found that supplementation with vitamin D3 may increase serum BDNF levels by activating BDNF gene expression pathways (Khairy and Attia, 2021). Additionally, prior animal studies observed that vitamin D3 may indirectly modulate apoptosis and age-related pro-inflammatory states via stimulation of BDNF production (Briones and Darwish, 2012; Nagatsu and Sawada, 2005). Similarly, evidence suggests that dietary omega-3s enhance BDNF synthesis by activating BDNF transcription through PI3K/Akt signaling pathway and thereby increasing BDNF levels (Knöchel et al., 2015; Wu et al., 2008). However, limited trial evidence exists on whether vitamin D3 and omega-3s can alter serum BDNF in a well-characterized sample of older adults (Gravesteijn et al., 2022). Also, knowledge gaps remain regarding serum BDNF as a biologic modifier or mediator, which could inform how vitamin D3 and omega-3s influence LLD risk and for whom (Papakostas and Fava, 2008).

Thus, we addressed these knowledge gaps among an in-clinic sample of 400 participants in VITAL-DEP (VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention) study (Okereke et al., 2018). Our study objectives were to: 1) examine cross-sectional and prospective relationships between serum BDNF and LLD; 2) test effects of vitamin D3 and/or omega-3s on change in serum BDNF over 2 years; 3) explore potential moderating or mediating roles of BDNF on the effects of vitamin D3 or omega-3s for LLD prevention.

2. METHODS

2.1. Source of participants

Participants were members of the VITAL-DEP (NCT01696435), depression prevention ancillary study to VITAL (NCT01169259). VITAL tested vitamin D3 (2000 IU/day) and/or omega-3s (1 g/day) in a 2 x 2 factorial design for primary prevention of cardiovascular disease and cancer in 25,871 US adults (men aged ≥50 and women aged ≥55 years); dosing and constituents of the study agents in VITAL-DEP were constrained to being the same as in the parent trial. The detailed protocols of VITAL and VITAL-DEP trials are described elsewhere (Manson et al., 2012; Okereke et al., 2018). A sub-cohort of 1054 VITAL participants from the New England region were enrolled for detailed, in-person phenotyping at baseline (period: January 2012 and March 2014) and 2 years later (period: January 2014 – April 2016) at the Clinical and Translational Science Center (CTSC) in Boston, MA, USA. VITAL participants also provided fasting blood samples during CTSC

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visits. All participants provided written informed consent, and approvals were obtained from the institutional review board of Mass General Brigham.

All 1,054 VITAL-CTSC participants were invited to take part in the 45-minute VITAL-DEP assessment, which featured a semi-structured psychiatric diagnostic interview, neuropsychological testing, and self-report questionnaires on mood and other health factors. The purpose of this assessment was to identify individuals at risk for incident MDD at a 2-year follow-up (Okereke et al., 2018). Of 1,046 participants who completed the baseline assessment, 326 were ineligible for a 2-year follow-up due to a baseline history of MDD and/or other major psychiatric exclusions (Figure S1). Of the remaining 720 participants, 662 (91.9%) completed follow-up MDD assessments.

2.2. Sample selection

Among 326 participants with a history of depressive and/or other major psychiatric disorders and with a CTSC blood sample at baseline, we randomly selected 100 participants with a history of depression for baseline serum BDNF assay; the sample was balanced by sex and randomized treatment agents. The depression cases included current, past, or recurrent MDD, current or past major depressive episode, or dysthymia.

Among participants who were free of any major psychiatric disorders at baseline, who completed 2-year follow-up assessments, and who provided blood samples at both baseline and 2-year follow-up visits, we selected 300 participants for baseline and year-2 serum BDNF assays. For this selection, we included all 34 incident MDD cases (identified during the 2-year VITAL-DEP follow-up assessments) to increase the power for longitudinal analysis; the other 266 participants did not have incident MDD over 2 years and were balanced by sex and treatment agents (See Figure S1).

2.3. Assessment of depression outcomes

The Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV was used to ascertain MDD and psychiatric diagnosis status at baseline and 2-year follow-up. Participants also completed self-report measures (including Patient Health Questionnaire [PHQ]-9; *range 0-27 points*) (Kroenke et al., 2001) at baseline and 2-year CTSC visits. In the main VITAL trial, participants also completed the PHQ-8 (*range 0-24 points*) (Kroenke et al., 2009) at baseline and annually thereafter over a median 5 years of follow-up.

2.4. Serum BDNF assays

Samples were thawed, aliquoted, and sent to the Rifai laboratory at Boston Children's Hospital for serum BDNF assay measured by a commercially available enzyme-linked immunosorbent assay kit (Quantikine™) (Crombie et al., 2021); assays were performed according to the manufacturer's instructions. To minimize batch effects, all baseline and year 2 sample pairs were shipped and measured at the same time in a manner blinded to the analyzing lab. Among 36 blinded QC replicates randomly distributed across the plates, the average coefficient of variation (CV) was 5.8%; within-pair CV was 3.1%.

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2.5. Statistical analyses

2.5.1. Samples for cross-sectional and longitudinal analyses—To mitigate risk of selection bias, we selected at random a sub-set of 266 participants who had no major psychiatric disorders at baseline and did not develop MDD over 2 years, along with a random selection of 14 of the 34 participants who had no history of major psychiatric disorders at baseline but who developed incident MDD over 2 years. The random selection of 14 of the 34 participants was performed to match the MDD incidence rate observed in the overall VITALDEP CTSC sub-cohort (i.e., 34/720*300) and to preserve the caseness in the baseline sample. Thus, we performed the baseline *cross-sectional analyses* in a randomly selected sample of 100 participants with a history of depression and 280 (266 + 14) participants without depression or other major psychiatric history at baseline.

As described in sample selection, we selected 300 participants for baseline and year-2 serum BDNF assays. Of 300 participants, we included n=288 for *longitudinal analyses*. We excluded prevalent selective serotonin reuptake inhibitor (SSRI) users (n=10) or those with missing self-reported information on SSRI use (n=2) at baseline, as SSRIs are frequently prescribed for depression and other psychiatric disorders (Edinoff et al., 2021), and evidence suggests that SSRIs increase BDNF levels by enhancing BDNF gene expression (Björkholm and Monteggia, 2016; Zhou et al., 2017). Thus, we excluded participants who were prevalent SSRI users or those with missing information on SSRI use to mitigate potential bias in the estimates for longitudinal analyses.

2.5.2. Descriptive analyses—We examined the distribution of baseline serum BDNF using a box plot and histogram and found this variable to be normally distributed.
In the sample eligible for cross-sectional analyses (n=380), we compared participants' characteristics according to depression status using two-sample t-tests for normally distributed continuous variables, Wilcoxon's rank-sum tests for non-normally distributed continuous variables, and chi-square tests for difference in proportions.

We also compared baseline characteristics according to quartiles of baseline serum BDNF in the sample eligible for longitudinal analyses (n=288); p-values for trend were calculated using: Jonckheere-Terpstra test for non-parametric continuous variables; Cochran-Armitage trend test for binary categorical variable; Cochran Mantel-Haenszel statistics for a categorical variable with more than 2 levels.

2.5.3. Cross-sectional analyses—We constructed multivariable logistic regression models to examine the cross-sectional associations between serum BDNF level and depression status (i.e., participants with a history of depression vs. those without any history of major psychiatric disorders). Furthermore, we used multivariable negative binomial models for examining the cross-sectional associations between quartiles of serum BDNF and PHQ-9 scores; percent differences and 95% confidence intervals (CIs) were reported. The choice of covariates for inclusion in multivariable models was based on prior evidence (such as age, SSRI use) and on evidence of $p<0.15$ on stepwise covariate selection (such as sex, Black vs. non-Black, and history of diabetes) (Bursae et al., 2008); see supplement for more details on stepwise approach.

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2.5.4. Longitudinal analyses—We used multivariable logistic regression models to determine odds ratio (OR) and 95% CI of incident MDD over 2 years by a median of baseline serum BDNF. After using measures for goodness of fit and Voung test (Akaike, 1973; Voung, 1989), we determined that the negative binomial model, compared to the zero-inflated negative binomial model, was most appropriate to the PHQ-9 distribution; thus, we used repeated measures negative binomial regression to estimate the association between baseline serum BDNF level and percent differences in change in PHQ-9 over 2 years; time was modeled as an indicator variable, and the p-interaction was reported using time-x-quartiles of serum BDNF. All longitudinal models were adjusted for age, sex, and treatment agents.

2.5.5. Testing treatment agents' effects on serum BDNF—We examined effects of vitamin D3 or omega-3s, vs. placebos, on 2-year change in serum BDNF using repeated measures response profile models; the mean difference in change in serum BDNF between treatment vs. placebo groups was assessed using a time-x-treatment interaction.

2.5.6. Exploratory analyses of modification and mediation—We probed effect modification by baseline serum BDNF (i.e., ≥ or <median level) of the effects of vitamin D3 or omega-3s, vs. placebos, on longitudinal depression outcomes by using multiplicative interaction terms (median BDNF category-x-treatment); stratified results were presented. Analyses of mediation of treatment effects on change in PHQ-9 by a 2-year change in serum BDNF were also pre-specified using %Mediate macro (Lin et al., 1997).

2.5.7. Post-hoc analyses—1) To mitigate potential bias due to the MDD case selection, we reran the longitudinal analyses addressing the relation of baseline serum BDNF and change in PHQ-9 over 2 years and of treatment effects on change in serum BDNF over 2 years in a random stratified sample; 2) The relation of baseline serum BDNF and change in PHQ-8 over 5 years (long-term change) was examined using repeated measures negative binomial regression; 3) We probed modification by baseline serum BDNF for treatment effects on long-term change in PHQ-8 by using multiplicative interaction terms (BDNF quartile category x treatment); stratified results were presented. We conducted mediation analyses by a 2-year change in serum BDNF for treatment effects on long-term change in PHQ-8 (Lin et al., 1997); 4) We addressed whether the treatment effects on 2-year change in serum BDNF differed across age groups (<65 vs. 65+ years), sex (female vs. male), or race/ethnicity (Black vs. non-Black adults); p-interaction was reported from the test of the subgroup-x-treatment-x-follow-up time interaction term in the model; 5) There is a lack of an evidence-based optimal cut-point of serum BDNF for LLD prediction. Thus, we employed a robust bootstrap technique for receiver operating characteristic (ROC) curve analysis to evaluate the performance of serum BDNF for discriminating incident vs. no incident MDD (Thiele and Hirschfeld, 2021); the longitudinal analysis addressing the relation of serum BDNF to incident MDD was rerun using an optimal cut-point of baseline serum BDNF.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA), and R. Tests were two-sided; $p<0.05$ was used for statistical significance. There was no control for multiple hypothesis testing, and no formal adjustment was made to the p-values. Thus,

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exploratory analyses of modification and moderation effects of serum BDNF, and post-hoc analyses, including those regarding subgroups, should be interpreted with caution.

3. RESULTS

3.1. Baseline characteristics

In the total sample (n=400), baseline serum BDNF was normally distributed [mean (standard deviation): 36.4 (7.6) ng/mL]; the median age (interquartile range) was 65.1 (60.4-69.2) years; 50.5% were females; and 20.8% were racial/ethnic minorities. Table 1 represents descriptive characteristics by baseline depression status in the sample eligible for cross-sectional analyses (n=380). Compared to those without any history of major psychiatric disorders, participants with a history of depression were slightly younger, had higher body mass index; reported lower leisure-time physical activity, had higher percentages of past/current cigarette smoking, ~2-fold higher prevalence of diabetes, and higher median PHQ-9 score. Notably, there was no difference in baseline serum BDNF by depression status.

Table S1 presents descriptive characteristics by quartiles of serum BDNF in the sample eligible for longitudinal analyses (n=288). Females had significantly higher serum BDNF than males (p-trend <0.001). There were no noticeable differences in serum BDNF by other demographic, lifestyle/behavioral, health-related, or depression variables.

3.2. Cross-sectional relations of serum BDNF to depression outcomes

Table 2A shows the mean difference in serum BDNF between participants with a history of depression vs. those without a history of major psychiatric disorders at baseline; there was no significant difference between the groups [adjusted regression coefficient (β) (95% CI): 1.11 (−0.59 to 2.80); p=0.20]. Table 2B shows results for differences in total depressive symptom levels, as indicated by the PHQ-9 score, by quartiles of serum BDNF; participants in the lowest vs. highest quartile of serum BDNF (14.9 – 31.5 ng/mL vs. 40.9 – 69.2 ng/mL) had an 80% higher severity of depressive symptoms after adjusting for age, sex, Black vs. non-Black, history of diabetes and SSRI use (percent differences=80%; 95% CI (12% to 188%); p-value=0.01); overall trend across serum BDNF quartiles was non-monotonic and appeared meaningful (p-trend=0.06).

3.3. Longitudinal relations of baseline serum BDNF levels to depression outcomes over 2 years

In the sample eligible for longitudinal analysis (n=288), over 2 years, 28 participants developed incident MDD, and 260 participants remained depression-free. The median serum BDNF at baseline was 35.5 ng/mL. Comparing participants with above vs. below the median of baseline serum BDNF levels, there was no statistically significant difference in the likelihood of incident MDD (Table 3A). Furthermore, being in the lowest vs. highest quartiles of baseline serum BDNF was not associated with a mean difference in change in PHQ-9 over 2 years (p-trend=0.82) (Table 3B).

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3.4. Effects of treatment agents on change in serum BDNF levels over 2 years

We did not observe a statistically significant mean difference in 2-year change in serum BDNF levels among those randomized to treatment agents vs. placebos [adjusted mean difference (95% CI)=0.51 (−0.59 to 1.60) for vitamin D3 vs. placebo; −0.62 (−1.71 to 0.48) for omega-3s vs. placebo] (Table 4).

3.5. Exploratory analyses of modification and mediation effects of serum BDNF

Modification analyses revealed that baseline serum BDNF (i.e., above vs. below median) did not significantly modify the estimates for the effects of treatment agents vs. placebos on risk of MDD or on mean differences in change in PHQ-9 over 2 years (all p-interactions>0.05) (Table 5A and 5B). Because we did not observe any evidence in this sample of effects of vitamin D3 or omega-3s on 2-year change in PHQ-9 or of an association between 2-year change in serum BDNF and 2-year change in PHQ-9 (results are not shown here), we concluded that 2-year change in serum BDNF did not mediate the treatment effects for 2-year change in mood.

3.6. Post-hoc analyses

These results showed: 1) Results of baseline serum BDNF and 2-year change in PHQ-9 and of treatment effects on 2-year change in BDNF were non-significant in a random stratified sample (results are not shown); the estimates were comparable to our primary analyses and may mitigate the possibility of bias due to the MDD case selection; 2) Being in the lowest vs. highest quartiles of baseline serum BDNF was not associated with long-term (5+ years) change in PHQ-8 (Table S2); 3) baseline serum BDNF did not significantly modify the effects of vitamin D3 or omega-3s on average mean difference in change in PHQ-8 score over 5 years of follow-up (Table S3). Further, because we did not observe any evidence of treatment effects on long-term change in mood, or of an association between a 2-year change in serum BDNF and a 5-year change in PHQ-8 (results are not shown), we concluded that a 2-year change in BDNF did not mediate the treatment effects for long-term change in mood; 4) No significant variations by age, sex, and race were found in the treatment effects on change in serum BDNF over 2 years (all p-interactions>0.05; stratified results are not shown here); 6) The optimal cut-off of serum BDNF to discriminate MDD cases from those who remained depression-free over 2 years, as determined by ROC analysis (Thiele and Hirschfeld, 2021), was 36.1 ng/mL (area under curve=0.54; sensitivity=60.7%; specificity=55.0%) (Figure S2); the result of optimal serum BDNF category and incident MDD was similarly null to those results of a median category of serum BDNF and incident MDD.

4. DISCUSSION

This study provides new results regarding the longitudinal associations between serum BDNF and LLD, the effects of two nutrient supplements (vitamin D3 and omega-3s) on serum BDNF, and potential modification and mediation by serum BDNF for effects of these supplements on LLD. There were no significant cross-sectional differences in mean serum BDNF comparing participants with a history of depression and those without any history of psychiatric disorders; however, being in the lowest vs. highest quartile of baseline

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serum BDNF (14.9 – 31.5 ng/mL vs. 40.9 – 69.2 ng/mL) was significantly associated with worse depressive symptoms. In longitudinal analyses, there were no significant associations between serum BDNF and incident MDD or change in PHQ-9 over 2 years. Compared to their placebos, vitamin D3 or omega-3s had no significant effects on change in serum BDNF, and serum BDNF did not appear to modify or mediate effects of either nutraceutical on LLD in exploratory analyses.

Converging lines of evidence suggest that BDNF plays a pivotal role in the pathophysiology of LLD (Duman et al., 2000; Duman and Monteggia, 2006; Karege et al., 2002). Prior findings regarding lower serum BDNF in depressed compared to non-depressed individuals were compelling; yet, other studies have reported null associations (Ohio et al., 2021; Ziegenhorn et al., 2007), and we did not observe differences in mean serum BDNF comparing participants with depression history vs. those without a history of major psychiatric disorders in our sample. Differences in clinical characteristics and study methods may account for heterogeneity in reported findings in the literature (e.g., age and sex distribution of sample; study setting [community-based vs. tertiary-care clinic]; depression outcome used; matching criteria used for sample selection; psychiatric comorbidity in the sample; concomitant use of antidepressants or other psychotropic drugs) (Bocchio-Chiavetto et al., 2010; Bus et al., 2011; Molendijk et al., 2014; Shi et al., 2020; Trajkovska et al., 2007). Separately, our finding suggesting an inverse cross-sectional association between serum BDNF level and depressive symptom severity level is consistent with some prior studies but not all (Caldieraro et al., 2017; Jevtovic et al., 2011; Molendijk et al., 2011). Explanations for such discrepancies may include differences in the mood scales used, sampling of participants at various stages of disease progression, and influences of potential measured or unmeasured confounders (e.g., seasonality, physical activity).

In this study, we also probed the clinical utility of serum BDNF as a potential predictive biomarker for longitudinal depression outcome, but we did not observe any evidence that baseline serum BDNF predicted MDD risk or change in mood scores in this sample. Although the multivariable odds ratio of incident MDD, compared to those who remained depression-free, was not statistically significant, it was nonetheless almost two-fold higher, raising the possibility of a true relationship. Genetic inheritance and epigenetic mechanisms (e.g., DNA methylation and histone modification) that regulate BDNF expression have been implicated in the development of MDD (Hing et al., 2018). Furthermore, emerging literature suggests that variability in BDNF could be associated with aging-related processing and signaling pathways and may promote changes in cellular and molecular aging makers, and thus, could affect the MDD risk (Miranda et al., 2019; Molinari et al., 2020). Although DNA genotyping and cellular and molecular aging markers were not profiled in our sample, the possibility of increased MDD risk through BDNF polymorphisms or aging-related pathways cannot be excluded, and future studies may address these mechanisms.

Our results regarding the effects of vitamin D3 or omega-3s supplementation on 2-year change in serum BDNF represent a novel contribution to the literature and we also newly probed the question of variation by age, sex, and race/ethnicity in this prospective association; however, all results were null. These null findings in a 2-year RCT of adults contrast with experimental rat models, which suggest that vitamin D3 and omega-3s have

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neuroprotective effects by activating the BDNF transcription pathways (Gravesteijn et al., 2022; Kiraly et al., 2006; Wu et al., 2008). In the literature, there were 3 prior small-scale and short-term intervention studies in generally healthy older adults (*n<100; intervention period:≤6 months*) testing the effects of vitamin D3 or omega-3s supplements on alteration in serum BDNF concentrations, and none found significant intervention effects on change in BDNF (Gravesteijn et al., 2022). Separately, our findings also suggest that serum BDNF did not appear to modify or mediate the longitudinal relations of treatment agents with LLD.

Our study had notable strengths. The cohort is well-characterized, features diverse racial and ethnic minority representation, and has well-validated and rigorously adjudicated depression measures at baseline and follow-up. Another advantage was that we performed BDNF assays in serum rather than plasma samples, since serum BDNF may represent a long-term measure of brain BDNF levels (Fujimura et al., 2002). Also, the study contributes new information to the literature by addressing: the association between baseline serum BDNF and shorter-term (2 years) and longer-term (5+ years) changes in mood; the effects of vitamin D3 or omega-3s supplementations on change in serum BDNF over 2 years; exploration of modification and mediation effects of serum BDNF on the treatment effects on short-term and long-term change in mood.

This study also has limitations. First, the incidence rate of MDD over two years was low (~5%); however, this is consistent with the low incidence of MDD in samples of generally healthy and high-functioning community-recruited (i.e., not a hospital or clinic-based) participants (Bot et al., 2019; de Koning et al., 2019). Second, interpretation of our findings is constrained to the use of serum/blood-based BDNF markers and may not generalize to results that may be observed when using BDNF in cerebrospinal fluid (CSF); it is still unclear whether peripheral BDNF concentrations reflect its levels in the central nervous system. Nonetheless, BDNF has been reported to be able to pass through the blood-brain barrier, and modest, positive correlations have been shown between serum and CSF BDNF levels (Klein et al., 2011). Third, study participants were members of a long-term randomized trial cohort and may therefore be healthier than older adults in the general community; while this may affect generalizability, it does not detract from the internal validity of the findings. Fourth, evidence suggests that doses of ≥1.5 g/day omega-3s may be necessary for LLD prevention (Bai et al., 2018); however, due to the fixed-dose design in VITAL, we were unable to test effects of high-dose omega-3s or of different balances of EPA vs. DHA on change in serum BDNF levels.

In conclusion, we observed a significant cross-sectional association between serum BDNF with severity of depressive symptoms, but not with depression case status. In longitudinal analyses, there were no significant associations between serum BDNF with incident MDD or change in mood over 2 years. Furthermore, daily supplementation of vitamin D3 and/or omega-3s did not alter serum BDNF over 2 years, and serum BDNF did not appear to modify or mediate longitudinal associations between vitamin D3 or omega-3 supplements and LLD.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- There is an urgent need to identify biomarkers for major depressive disorder.
- Low serum BDNF has promise as a biomarker for major depressive disorder.
- Low serum BDNF was associated with worse depressive symptoms at baseline.
- Serum BDNF did not predict the new onset of major depressive disorder.
- Vitamin D and omega-3s did not alter BDNF levels over 2 years.

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Table 1.

Participants' characteristics in a random sample at baseline, stratified by depression case status.

Baseline characteristics	Sample (n=380)	Depression cases[a] (n=100)	Non-depressed individuals (n=280)	P-value
Age at CTSC, median (IQR)	64.9 (60.3 - 69.2)	63.6 (58.8 - 66.9)	65.3 (60.7 - 69.5)	0.01[b]
Sex, n (%)				0.73
Male	188 (49.5)	48 (48.0)	140 (50.0)	
Female	192 (50.5)	52 (52.0)	140 (50.0)	
Race/ethnicity, n (%)				0.98
Non-Hispanic white	297 (78.2)	78 (78.0)	219 (78.2)	
Black	40 (10.5)	11 (11.0)	29 (10.4)	
Others[d]	43 (11.3)	11 (11.0)	32 (11.4)	
Education, n (%)				0.49
≤college degree	182 (48.0)	51 (51.0)	131 (47.0)	
Post-college education	197 (52.0)	49 (49.0)	148 (53.1)	
Income, n (%)				0.18
<$50,000	77 (23.3)	25 (28.4)	52 (21.4)	
$50,000+	254 (76.7)	63 (71.6)	191 (78.6)	
BMI, kg/m^2, median (IQR)	27.5 (24.4 – 30.6)	28.2 (25.1 - 31.6)	27.3 (24.4 – 29.9)	0.05[b]
PA,[e] MET-hours/week, median (IQR)	21.5 (8.0 - 38.0)	18.3 (7.2 - 30.6)	21.7 (8.2 - 40.6)	0.07[b]
Alcohol use, n (%)				0.13
Rare/never	69 (19.3)	25 (26.3)	44 (16.7)	
Monthly	32 (8.9)	7 (7.4)	25 (9.5)	
Weekly	154 (43.0)	42 (44.2)	112 (42.6)	
Daily	103 (28.8)	21 (22.1)	82 (31.2)	
Smoking use, n (%)				0.03
Never	197 (52.1)	42 (42.0)	155 (55.8)	
Past	163 (43.1)	50 (50.0)	113 (40.7)	
Current	18 (4.8)	8 (8.0)	10 (3.6)	

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Baseline characteristics	Sample (n=380)	Depression cases[a] (n=100)	Non-depressed individuals (n=280)	P-value
History of hypertension,[f] n (%)	177 (47.0)	49 (49.5)	128 (46.0)	0.55
History of diabetes,[g] n (%)	44 (11.6)	18 (18.2)	26 (9.3)	0.02
History of high cholesterol, n (%)	140 (36.8)	42 (42.0)	98 (35.0)	0.21
PHQ-9 score, median (IQR)	1.0 (0.0 - 2.0)	1.0 (0.0 - 3.0)	0.0 (0.0 - 2.0)	0.001[b]
SSRI users, n (%)	18 (4.8)	12 (12.0)	6 (2.2)	<0.001
Serum BDNF, ng/mL, mean (SD)	36.3 (7.5)	37.4 (7.2)	36.0 (7.5)	0.11[c]
Randomization to vitamin D3 group, n (%)				0.95
Active	189 (49.7)	50 (50.0)	139 (49.6)	
Placebo	191 (50.3)	50 (50.0)	141 (50.4)	
Randomization to omega-3s group, n (%)				0.95
Active	191 (50.3)	50 (50.0)	141 (50.4)	
Placebo	189 (49.7)	50 (50.0)	139 (49.6)	

Abbreviation: BDNF, brain-derived neurotrophic factor; BMI, body mass index; PA, physical activity; PHQ, patient health questionnaire; CTSC, clinical and translational science center; IQR, interquartile range.

[a]Depression cases include current, past, or recurrent major depressive disorder, current or past major depressive episodes and dysthymia.

[b]Wilcoxon-rank sum test was performed.

[c]Two-sample t-test was performed.

[d]Others race/ethnicity group included Hispanics, Asians and Other (Native Hawaiian or other Pacific Islander, multiple race, unknown race, or unknown ethnicity).

[e]Leisure-time physical activities include walking or hiking; jogging; running; bicycling; aerobic exercise/aerobic dance/exercise machines; lower intensity exercise/yoga/stretching/toning; tennis/squash/racquetball; lap swimming; weight lifting/strength training; other exercise.

[f]Ever diagnosed with high blood pressure or ever use of anti-hypertensive medication

[g]Ever diagnosed with diabetes or current use of anti-diabetic medication.

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Table 2A.

Mean difference (95% CI) in serum BDNF (in ng/mL) comparing depression cases (n=100) vs. those without any history of psychiatric disorders (n=280).

Outcome: depression cases[a] vs. those without any history of psychiatric disorders	Mean difference (95% CI)	P-value
Model 1[b]	1.40 (−0.31 to 3.11)	0.11
Model 2[c]	1.11 (−0.59 to 2.80)	0.20

Abbreviation: BDNF, brain-derived neurotrophic factor; CI, confidence interval

[a]Depression cases include current, past, or recurrent major depressive disorder, current or past major depressive episodes, and dysthymia.

[b]Univariate model.

[c]Model adjusted by age, sex, Blacks vs. non-Blacks, history of diabetes, and SSRI use.

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Table 2B.

Percent differences in total depression severity (PHQ-9 score) according to quartiles of baseline serum BDNF (n=380).*

	Quartiles of serum BDNF at baseline				
	Highest (Q4) (*n*=95) 40.9 – 69.2 ng/mL	**Third (Q3) (*n*=95) 35.6 – 40.9 ng/mL**	**Second (Q2) (*n*=95) 31.5 – 35.5 ng/mL**	**Lowest (Q1) (*n*=95) 14.9 – 31.5 ng/mL**	**P-value for trend**
	Percent differences (95% CI)				
Model 1[a]	0% (Ref)	53% (−3% to 140%)	−7% (−42% to 49%)	54% (−2% to 142%)	0.27
Model 2[b]	0% (Ref)	54% (−3% to 145%)	9% (−33% to 77%)	80% (12% to 188%)	0.06

Abbreviation: RR, rate ratio; BDNF, brain-derived neurotrophic factor; PHQ, patient health questionnaire; CI, confidence interval

*Results from the negative binomial (NB) regression model show percent differences and 95% CIs in total depression severity on the PHQ-9 by quartiles of serum BDNF at baseline.

[a]Univariate model.

[b]Model adjusted by age, sex, race/ethnicity, history of diabetes, and SSRI use.

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Table 3A.

Odds ratio and 95% CI of DSM-IV incident MDD cases over 2 years by median category of baseline serum BDNF.

Baseline serum BDNF	Cases (*n=28*)	Non cases (*n=260*)	OR (95% CI)[a]	OR (95% CI)[b]
<35.5 ng/mL (*n=144*)	11	133	1.00 (Ref)	1.00 (Ref)
≥35.5 ng/mL (*n=144*)	17	127	1.62 (0.73 to 3.59)	1.81 (0.78 to 4.22)

Abbreviation: DSM, Diagnostic and Statistical Manual of Mental Disorders; BDNF, brain-derived neurotrophic factor; MDD, major depressive disorder; CI, confidence interval.

[a] Univariate model

[b] Models were adjusted by age at CTSC visit, sex, and treatment agents.

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Table 3B.

Difference in change in PHQ-9 score over 2-year follow-up according to quartiles of serum BDNF at baseline (n=288).

	Quartiles of serum BDNF at baseline				
	Highest (Q4) (*n*=72) 40.9 – 69.2 ng/mL	**Third (Q3) (*n*=72) 35.5 – 40.7 ng/mL**	**Second (Q3) (*n*=72) 31.1 – 35.5 ng/mL**	**Lowest (Q1) (*n*=72) 14.9 – 31.1 ng/mL**	**P-value for trend**
	RR (95% CI)[a]				
Model 1[b]	1.00 (Ref)	0.68 (0.43 to 1.08)	1.03 (0.65 to 1.65)	0.82 (0.53 to 1.27)	0.87
Model 2[c]	1.00 (Ref)	0.67 (0.42 to 1.07)	1.02 (0.63 to 1.63)	0.81 (0.52 to 1.25)	0.82

Abbreviation: RR, rate ratio; PHQ, patient health questionnaire; BDNF, brain-derived neurotrophic factor; CI, confidence interval.

[a]Analyses were from repeated measures negative binomial regression models, with follow-up time modeled as an indicator. Results show RR and 95% CI, which reflect differences in the change in severity on the PHQ-9 score over 2 years comparing quartiles of serum BDNF at baseline.

[b]Univariate model.

[c]Models were adjusted by age at CTSC visit, sex, and treatment agents.

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Table 4.

Adjusted mean difference in change in serum BDNF (in ng/mL) over 2 years comparing treatment agents to their matching placebos (n=288).

Treatment groups	Adjusted mean difference (95% CI)[a]	P-value
Vitamin D3 vs. placebo	0.51 (−0.59 to 1.60)	0.36
Omega-3s vs. placebo	−0.62 (−1.71 to 0.48)	0.27

Abbreviation: BDNF, brain-derived neurotrophic factor; CI, confidence interval.

[a]Results from the repeated measures response profile model show mean differences in the change in serum BDNF over 2 years comparing treatment agents and placebos, after controlling for age, sex, and concurrent randomization group; all participants contributed to the repeated measure analysis at one and/or both time points.

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Table 5A.

Effect of vitamin D3 or omega-3s, compared to their matching placebos, on the likelihood of DSM-IV incident MDD over 2 years by median category of serum BDNF.

1) Vitamin D3 vs. placebo

	Vitamin D3 group	Placebo group	OR (95% CI)[a]	P-interaction
	Cases/no. of participants	Cases/no. of participants		
Baseline serum BDNF				0.41
<35.5 ng/mL (*n=144*)	4/76	7/68	0.48 (0.13 to 1.76)	
≥35.5 ng/mL (*n=144*)	7/65	10/79	1.17 (0.39 to 3.53)	

2) Omega 3s vs. placebo

	Omega-3s group	Placebo group	OR (95% CI)[a]	P-interaction
	Cases/no. of participants	Cases/no. of participants		
Baseline serum BDNF				0.31
<35.5 ng/mL (*n=144*)	5/71	6/73	0.80 (0.23 to 2.80)	
≥35.5 ng/mL (*n=144*)	11/75	6/69	1.93 (0.65 to 5.77)	

Abbreviation: BDNF, brain-derived neurotrophic factor; MDD, major depressive disorder; DSM, diagnostic and statistical manual of mental disorders; OR, odds ratio; CI, confidence interval.

[a]Model was adjusted by age at CTSC visit, sex, and randomization to omega-3s group.

[a]Model was adjusted by age at CTSC visit, sex, and randomization to vitamin D3 group.

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Table 5B.

Effect of vitamin D3 or omega-3s, compared to their matching placebos, on mean difference in change in PHQ-9 score over 2 years by quartiles of serum BDNF at baseline.

	Vitamin D3 vs. placebo		Omega-3s vs. placebo	
	Mean difference (95% CI)	**P-interaction**	**Mean difference (95% CI)**	**P-interaction**
Quartiles of serum BDNF		0.89		0.42
Lowest (Q1) (*n=72*)	0.99 (0.53 to 1.85)		0.91 (0.50 to 1.65)	
Second (Q2) (*n=72*)	0.87 (0.42 to 1.79)		1.37 (0.71 to 2.66)	
Third (Q3) (*n=72*)	1.25 (0.66 to 2.36)		1.48 (0.77 to 2.83)	
Highest (Q4) (*n=72*)	0.69 (0.38 to 1.25)		1.25 (0.69 to 2.26)	

Abbreviation: BDNF, brain-derived neurotrophic factor; PHQ, patient health questionnaire; CI, confidence interval Analyses were from repeated measures negative binomial regression models, with follow-up time modeled as an indicator. Results show RR and 95% CI, which reflect differences in the change in serum BDNF over 2 years comparing treatment agents and placebos. Models were controlled for age, sex, and concurrent randomization group. P-interaction is from the test of the BDNF quartile category-x-treatment-x-time interaction term in the model.