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Original Contribution

Associations of the Ratios of *n*-3 to *n*-6 Dietary Fatty Acids With Longitudinal Changes in Depressive Symptoms Among US Women

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In the present study, we examined longitudinal changes in self-reported depressive symptoms (and related domains) in relation to baseline intakes of *n*-3 fatty acids (absolute and relative to *n*-6 fatty acids). Sex-specific associations were evaluated in a prospective cohort of adults ($n = 2,053$) from Baltimore, Maryland, who were 30–64 years of age at baseline and were followed for a mean of 4.65 (standard deviation, 0.93) years (2004–2013). Using mean intakes of *n*-3 and *n*-6 fatty acids reported on two 24-hour dietary recalls, we estimated the ratios of *n*-3 to *n*-6 fatty acids for both highly unsaturated fatty acids (≥20 carbon atoms) (HUFAs) and polyunsaturated fatty acids (≥18 carbon atoms) (PUFAs). Outcomes included total and domain-specific scores on the 20-item Center for Epidemiologic Studies-Depression scale. Based on mixed-effects regression models, among women, both higher *n*-3 HUFA:*n*-6 PUFA and *n*-3 PUFA:*n*-6 PUFA ratios were associated with a slower rate of increase in total Center for Epidemiologic Studies-Depression scores over time. Higher *n*-3 HUFA:*n*-6 HUFA ratios were associated with slower increases in somatic complaints in men, whereas among women, higher *n*-3 HUFA:*n*-6 PUFA and *n*-3 PUFA:*n*-6 PUFA ratios were both linked to putative longitudinal improvement in positive affect over time. Among US adults, *n*-3:*n*-6 dietary fatty acid ratio was associated with longitudinal changes in depressive symptoms, with a higher ratio linked to a slower increase in depressive symptoms over time, particularly among women.

adults; depressive symptoms; diet; longitudinal studies; *n*-3 fatty acids

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; CES-D, Center for Epidemiologic Studies-Depression; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HUFA, *n*-3 highly unsaturated fatty acids; LA, linoleic acid; PUFA, *n*-3 polyunsaturated fatty acids.

Unipolar depression, a potentially life-long illness (1–3), currently stands among the most prevalent diseases in the health care spectrum (4). In the United States, its lifetime prevalence is 12% among men and 21% among women (4). The *n*-6:*n*-3 fatty acid ratio is a putative risk factor for the development of elevated depressive symptoms, other mental disorders, and cardiovascular disease (5, 6). It is believed that humans evolved from having a diet with an initial *n*-6:*n*-3 fatty acid ratio equal to 1 (5). However, Western diets are characterized by a high *n*-6:*n*-3 ratio, ranging from 10:1 to 20:1 (5, 6).

Linoleic acid (LA; 18:2*n*-6) and α-linolenic acid (ALA; 18:3*n*-3) are *n*-6 and *n*-3 fatty acids, respectively, that are essential for humans. They are precursors to arachidonic acid (AA; 20:4*n*-6), eicosapentaenoic acid (EPA; 20:5*n*-3), and docosahexaenoic acid (DHA; 22:6*n*-3). AA and EPA compete to synthesize eicosanoids, which regulate blood pressure, platelet aggregation, vasoconstriction, and chemotaxis. The chemical structure is formatted as X:Y*n*-Z, where X is the total number of carbon atoms, Y is the number of double bonds, and Z is the carbon number with first double bond starting from the methyl end. Eicosanoids from AA are particularly proinflammatory, whereas EPA and DHA affect normal functional development of the brain, membrane receptor function, neurotransmitter metabolism, and neuroprotection (7).

Results from numerous studies, including cross-sectional studies (8–16), prospective cohort studies (17–22), and clinical

trials (23–28) have suggested an inverse relationship between *n*-3 fatty acid intake and elevated depressive symptoms or major depressive disorder in apparently healthy individuals outside the context of postpartum depression. Unfortunately, the ratio between *n*-3 and *n*-6 fatty acids was not examined in several studies (8, 9, 11–14, 18, 20–22, 29–37). No association was found in other studies (e.g., 29, 33–36, 38–42), whereas conclusions from recent meta-analyses provided conflicting evidence (43–45). Importantly, few studies thus far had a prospective cohort or nested case-control design with sample sizes greater than 1,000 (18, 20–22, 37, 46, 47). A few researchers have evaluated sex-specific influences of *n*-3 fatty acid intakes on depressive symptoms (13, 15, 21, 22, 35, 46). In a recent cross-sectional analysis, Beydoun et al. (15) showed a direct association of intake of *n*-3 fatty acids with positive affect and an inverse association with depressive symptoms overall, specifically among women. To our knowledge, no other researchers have examined whether domains of depressive symptoms were related to *n*-3:*n*-6 fatty acid ratios (15).

In the present study, we examined longitudinal changes in self-reported depressive symptoms measured using the Center for Epidemiologic Studies-Depression (CES-D) scale in a sample of 2,053 middle-aged adults residing in Baltimore, Maryland, and the associations of those changes with absolute *n*-3 fatty acid intake and *n*-3:*n*-6 fatty acid ratios. Importantly, the analysis was designed to assess sex differentials in the associations between both total and subdomain scores for depressive symptoms and fatty acids.

METHODS

Database and participants

Initiated in 2004, the Healthy Aging in Neighborhoods of Diversity Across the Life Span (HANDLS) Study recruited a fixed cohort of socioeconomically diverse participants. Participants represent African-American and white subjects who were 30–64 years old at baseline and living in Baltimore, Maryland, based on an area probability sample of 13 neighborhoods (groups of contiguous census tracts). Data for the initial wave (visit 1) were collected in 2 phases. Phase 1 consisted of screening, recruitment, the first 24-hour dietary recall interview, and a household interview; phase 2 consisted of examinations in mobile medical research vehicles, including a second 24-hour dietary recall interview obtained on the medical research vehicles. Written informed consent was obtained from all participants after they were given access to a protocol booklet written in layman's terms and a video that described all procedures and future re-contacts. The follow-up examinations (visit 2) were performed on the medical research vehicles and included many of the instruments used and obtained at visit 1. Approval for all waves of data collection was obtained from the MedStar Health Institutional Review Board. All participants provided written informed consent at each examination.

Longitudinal data from the baseline (visit 1, 2004–2009) and follow-up (visit 2, 2009–2013) waves were used. The time between waves ranged from less than 1 year to approximately 8 years, with a mean of 4.65 (standard deviation, 0.93) years. Initially, 3,720 participants were included (sample 1). In the present study, we restricted data to participants for whom we had 2 days of dietary recall data and CES-D data from visit 1, visit 2, or both. Of the total of 3,720 HANDLS Study participants, 1,543 had missing dietary data (either 1 recall or both) at visit 1, and thus 2,177 (58.5%) had complete baseline phase 2 examinations and nonmissing dietary data with two 24-hour recalls (sample 2). Among sample 2 participants, CES-D scores from visits 1 and 2 were complete for 1,284 individuals, whereas 461 had only CES-D data from visit 1and 308 had only CES-D data from visit 2. Finally, 124 participants had missing data on CES-D scores for both visits but had complete dietary data. Thus, the final sample (sample 3) consisted of 2,053 individuals with complete CES-D data from at least 1 of 2 visits. Compared with the remaining participants in sample 1, there were fewer participants in sample 3 who lived above the poverty line (56% vs. 62%) and more women (57% vs. 52%); there were no significant differences by age or race/ethnicity.

Assessment of depressive symptoms

Depressive symptoms were measured using the CES-D scale at both baseline and the follow-up waves. The CES-D is a 20-item self-reported symptom-rating scale used to assess affective and depressed mood (48). A score of 16 or higher on the CES-D is a commonly used indicator for elevated depressive symptoms (49), which are highly predictive of clinical depression based on the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (50). Four CES-D domains were derived and shown to have an invariant factor structure across the National Health and Nutrition Examination Survey I and pilot HANDLS Study data (51). Total and domain-specific CES-D scores were examined for somatic complaints, depressive affect, positive affect, and interpersonal problems (51).

Dietary assessment of *n*-3 and *n*-6 fatty acids

Trained interviewers administered two 24-hour dietary recalls using the US Department of Agriculture's Automated Multiple-Pass Method. The Automated Multiple-Pass Method, a standardized 5-step process, was previously validated for intakes of protein, carbohydrates, fat and energy irrespective of a person's weight (52–54). Additional evidence supports the accuracy of the Automated Multiple-Pass Method in measuring group energy intakes in smaller samples (55, 56).

Using the US Department of Agriculture's Food and Nutrient Database for Dietary Studies, version 3 (57), we estimated nutrient intakes. The means of two 24-hour recalls were computed and used in the analysis after summing nutrient intakes for each individual per recall day.

Total *n*-3 polyunsaturated fatty acids (PUFAs) (*n*-3 fatty acids with ≥18 carbon atoms, namely ALA, DHA, EPA, and docosapentaenoic acid (DPA; 22:5*n*-3)) and *n*-3 highly unsaturated fatty acids (HUFAs) (*n*-3 fatty acids with ≥20 carbon atoms, namely DHA, EPA, and DPA) were expressed as the percentage of total energy intake and were considered as the main absolute *n*-3 fatty acid exposures of interest. We also analyzed 3 categories of relative *n*-3 fatty acid exposures.

Those were: 1) the *n*-3 HUFA:*n*-6 HUFA ratio (i.e., the ratio of DHA, EPA, and DPA to AA); 2) the *n*-3 HUFA:*n*-6 PUFA ratio (i.e., the ratio of DHA, EPA, and DPA to LA and AA); and 3) the *n*-3 PUFA:*n*-6 PUFA ratio (i.e., the ratio of ALA, DHA, EPA, and DPA to LA and AA). We computed these ratios rather than the *n*-6:*n*-3 ratio to avoid denominator values of 0 because some people in the United States (e.g., those who do not eat fish) consume negligible amounts of *n*-3 fatty acids compared with *n*-6 fatty acids and to interpret findings in the direction of an inverse association of both relative and absolute *n*-3 fatty acid intakes with a greater increase in depressive symptoms over time. It is worth noting that AA is the most common *n*-6 HUFA in the diet and that other types are found only in trace amounts (58, 59).

Covariates

Sociodemographic, lifestyle, and health-related potential confounders. The sociodemographic covariates that were considered as potential confounders were age, sex, race (white vs. African-American), marital status (married vs. unmarried), educational level (less than high school, a high school diploma, or more than high school), and poverty income ratio (<125% vs. ≥125%). Moreover, we adjusted for the following lifestyle and health-related factors: body mass index (weight (kg)/height $(m)^2$), current use of drugs (use of opiates, marijuana, or cocaine vs. none), and current smoking status (dichotomized as 0 = never or former smoker and 1 = current smoker).

Potential dietary confounders. Potentially confounding nutrients that were previously linked to depression were measured and expressed per 1,000 kilocalories of energy intake. These included vitamin B6 (mg), folate (μg), vitamin B12 (μg), total carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene) (mg), vitamin C (mg), and α-tocopherol (retinol equivalents) (35, 60–65). To emulate a multivariate nutrient density model (66), total energy intake was included as a covariate. All included covariates were measured at wave 1 and were assumed to be time-invariant.

Statistical analysis

Analyses were conducted using Stata, version 13.0 (StataCorp LP, College Station, Texas) (67). First, sociodemographic characteristics were compared by sex and CES-D score (≥16 vs. <16). Specifically, elevated depressive symptom measures were obtained from the mean of 2 CES-D scores across waves whenever available; when a score was only available from 1 wave, that value was used. Estimates of the prevalence of elevated depressive symptoms are unweighted. The mean differences between groups were tested using *t* tests and analysis of variance; relationships among categorical variables were evaluated with χ^2 tests.

Second, separate mixed-effects regression analyses were performed using CES-D scores and domains as continuous outcomes assuming missingness at random, which allowed us to use the largest possible sample size (i.e., patients with complete outcomes data from one or both waves were included). In these analyses, we examined the associations of 5 *n*-3 fatty acid dietary exposures (2 absolute and 3 relative exposures) with depressive symptoms after adjustment for potential confounders. The moderating effect of sex was tested in separate analyses by adding interaction terms to the multivariable models and stratifying by sex. Of primary interest were the main effects of each dietary exposure and their interactions with time assessed by the interval since initial measurement, as described by Blackwell et al. (68).

To account for potential selection bias in mixed-effects regression models (due to the nonrandom selection of participants with complete data from the target study population), a 2-stage Heckman selection model was constructed (69) using a probit model to obtain an inverse mills ratio at the first stage (derived from the predicted probability of being selected, conditional on the covariates in the probit model, mainly baseline age, sex, race, poverty status, and educational level), as was done in a previous study (70). A type I error of 0.05 was used for all analyses, with *P* values between 0.05 and 0.10 considered as borderline significant for main effects and a *P* value <0.10 considered significant for interaction terms (71) before multiple testing correction. The latter was done using a family-wise Bonferroni procedure, with families defined by CES-D total or domain scores, assuming content-wise independence. As defined in a methodological report, a family of inferences in the context of confirmatory analysis is any group of inferences for which it is possible to combine collectively the measure of error (72). Thus, when defining a family in this case as 1 of 4 domains or the total score (i.e., total of 5 families), type I error was corrected per family by dividing it by the number of exposure variables per family (in this case 5). Therefore, for main effects, type I error was corrected to 0.05 / 5 = 0.01, whereas for interaction terms (i.e., 2-way and 3-way interactions), it was corrected to 0.10 / 5 = 0.02.

RESULTS

The unweighted prevalence of elevated depressive symptoms across waves was 29.2% among men and 37.9% among women ($P < 0.001$, χ^2 test) (Table 1). Participants with elevated depressive symptoms (CES-D score ≥16) were generally more likely to have a poverty income ratio less 125% and a lower educational level and were more likely to be unemployed compared with men and women who did not have elevated depressive symptoms (CES-D score <16). Compared with women who did not have elevated depressive symptoms, those who did were less likely to be married (21.9% vs. 31.8%; $P = 0.001$), and higher proportions were current smokers (51.5% vs. 35.7%; $P < 0.001$) and used any type of illicit drugs (42.0% vs. 31.7%; $P = 0.002$). Although the mean body mass index was significantly higher among women than among men, it was not associated with elevated depressive symptoms. Other sex differences were found for marital status (28.1% of women vs. 33.8% of men; $P = 0.011$), poverty income ratio of 125% or higher (59.8% of men vs. 53.6% of women; $P = 0.004$), employment status (53.2% of men were employed vs. 44.1% of women; $P < 0.001$), current smoking status (50.9% of men vs. 41.7% of women; $P = 0.001$), and current use of any illicit drug (57.9% of men vs. 35.6% of women; $P = 0.001$).

Of the 5 *n*-3 fatty acid exposures, there was only 1 (mean *n*-3:*n*-6 HUFA ratio) for which women had a higher score than

Table 1. Characteristics of Study Participants by Sex and Center for Epidemiologic Studies-Depression Scale Score[a], Healthy Aging in Neighborhoods of Diversity Across the Life Span Study, Baltimore, Maryland, 2004–2013

Characteristic	Men						Women						*P* Value[b]		
	CES-D Score <16 (*n* = 626)		CES-D Score ≥16 (*n* = 258)		All Men (*n* = 884)		CES-D Score <16 (*n* = 726)		CES-D Score ≥16 (*n* = 443)		All Women (*n* = 1,169)		Men vs. Women	Low vs. High CES-D Score Among Men	Low vs. High CES-D Score Among Women
	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%			
Overall		70.8		29.2		43		62.1		37.9		57.0			
Depressive symptoms													<0.001	<0.001	<0.001
CES-D score	8.22 (0.17)		22.8 (0.38)		12.5 (0.27)		8.00 (0.16)		24.15 (0.33)		14.12 (0.28)				
Sociodemographic and lifestyle factors															
Age, years	48.4 (0.4)		48.5 (0.5)		48.4 (0.3)		48.5 (0.4)		48.1 (0.4)		48.4 (0.3)		0.9	0.88	0.42
African-American race		58.8		61.2		59.5		59.0		55.8		57.7	0.42	0.50	0.28
Marital status													0.011	0.07	0.001
Currently married		35.8		29.1		33.8		31.8		21.9		28.1			
Missing		3.8		6.2		4.5		4.1		3.6		3.9			
Educational level													0.29	<0.001	<0.001
Less than high school		6.4		11.2		7.8		5.0		7.9		6.1			
High school graduate		55.6		67.1		58.9		53.7		65.9		58.3			
More than high school		38.0		21.7		33.3		41.3		26		35.5			
Missing										0.2		0.1			
Poverty income ratio ≥125%		63.1		51.9		59.8		59.8		43.3		53.6	0.004	0.002	<0.001
Employed													<0.001	<0.001	<0.001
Yes		59.3		38.4		53.2		51.8		31.4		44.1			
Missing		12.3		17.4		14.0		13.5		14.9		14.0			
Smoking status													<0.001	0.21	<0.001
Current smoker		49		55.4		50.9		35.7		51.5		41.7			
Missing		3.3		3.5		3.4		5.5		6.1		5.7			
Current use of illicit drugs													<0.001	0.81	0.002
Used any type		58		57.8		57.9		31.7		42.0		35.6			
Missing		6.6		7.8		6.9		8.7		7.0		8.0			
Body mass index[c]	28.1 (0.2)		27.9 (0.4)		28.0 (0.2)		31.2 (0.3)		31.1 (0.4)		31.2 (0.2)		<0.001	0.82	0.84

Table continues

Table 1. Continued

Characteristic	Men						Women						*P* Value[b]		
	CES-D Score <16 (*n* = 626)		CES-D Score ≥16 (*n* = 258)		All Men (*n* = 884)		CES-D Score <16 (*n* = 726)		CES-D Score ≥16 (*n* = 443)		All Women (*n* = 1,169)		Men vs. Women	Low vs. High CES-D Score Among Men	Low vs. High CES-D Score Among Women
	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%			
Fatty acid exposures[d]															
n-3 HUFA intake	0.09 (0.01)		0.09 (0.01)		0.09 (0.01)		0.10 (0.01)		0.10 (0.01)		0.10 (0.01)		0.38	0.81	0.87
n-3 PUFA intake	0.73 (0.02)		0.71 (0.02)		0.72 (0.01)		0.76 (0.01)		0.74 (0.02)		0.75 (0.01)		0.11	0.45	0.43
n-3 HUFA:*n*-6 HUFA ratio	2.20 (0.15)		1.97 (0.22)		2.14 (0.12)		2.66 (0.24)		2.83 (0.27)		2.72 (0.18)		0.013	0.39	0.66
n-3 HUFA:*n*-6 PUFA ratio	0.02 (0.00)		0.01 (0.00)		0.02 (0.00)		0.02 (0.00)		0.02 (0.00)		0.02 (0.00)		0.78	0.58	0.98
n-3 PUFA:*n*-6 PUFA ratio	0.11 (0.00)		0.11 (0.00)		0.11 (0.00)		0.11 (0.00)		0.11 (0.00)		0.11 (0.00)		0.66	0.6	0.98
Daily intakes of other dietary factors															
Energy, kcal	2,391 (42)		2,297 (72)		2,363 (37)		1,751 (28)		1,733 (37)		1,744 (22)		<0.001	0.25	0.69
Total carotenoids, mg/1,000 kcal	3.82 (0.19)		3.16 (0.23)		3.63 (0.15)		4.30 (0.18)		3.94 (0.23)		4.16 (0.14)		0.009	0.041	0.22
Vitamin A, retinol equivalents/ 1,000 kcal	297 (18)		317 (35)		303 (16)		345 (16)		361 (37)		351 (17)		0.05	0.57	0.66
Vitamin C, mg/ 1,000 kcal	37.6 (1.5)		33.2 (2.6)		36.3 (1.3)		42.4 (1.5)		37.4 (2.0)		40.5 (1.2)		0.018	0.13	0.04
Vitamin E, mg/ 1,000 kcal	3.1 (0.1)		2.9 (0.1)		3.1 (0.1)		3.6 (0.1)		3.3 (0.1)		3.5 (0.1)		<0.001	0.03	0.041
Vitamin B6, mg/ 1,000 kcal	0.94 (0.02)		0.88 (0.03)		0.92 (0.02)		0.95 (0.02)		0.86 (0.02)		0.92 (0.01)		0.75	0.07	0.002
Vitamin B12, μg/ 1,000 kcal	3.1 (0.2)		3.2 (0.3)		3.1 (0.2)		3.0 (0.2)		3.3 (0.4)		3.1 (0.2)		0.95	0.74	0.27
Folate, μg/1,000 kcal	180 (4)		172 (6)		178 (3)		195 (4)		179 (4)		189 (3)		0.011*	0.26	0.011

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression scale; HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acids; SE, standard error.

[a] CES-D scores were the mean of 2 CES-D scores across waves whenever available or the value from visit 1 or 2 when data from only 1 wave were available. Estimates of prevalence are unweighted.

[b] *P* values were based on independent sample *t* tests for continuous variables and χ^2 tests for categorical variables.

[c] Weight (kg)/height $(m)^2$.

[d] *n*-3 HUFAs included docosahexaenoic acid, eicosapentaenoic acid, and *n*-3 docosapentaenoic acid. *n*-6 HUFAs included arachidonic acid. *n*-3 PUFAs included docosahexaenoic acid, eicosapentaenoic acid, *n*-3 docosapentaenoic acid, and α-linolenic acid. *n*-6 PUFAs included arachidonic acid and linoleic acid.

Table 2. Analysis of Baseline *n*-3 Fatty Acid Exposures and Longitudinal Changes in Center for Epidemiologic Study-Depression Score Using Mixed-Effects Linear Regression Analysis, Healthy Aging in Neighborhoods of Diversity Across the Life Span Study, Baltimore, Maryland, 2004–2013

Fatty Acid Exposure	Model 1: All Participants[a] (2,052 Total Participants and 3,336 Visits)		Model 2: Men Only[a] (883 Participants and 1,409 Visits)		Model 3: Women Only[a] (1,169 Participants and 1,927 Visits)	
	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value
			% of Energy From n-*3 HUFAs*			
Fixed effect						
Intercept	16.01 (1.18)	<0.001	15.28 (1.68)	<0.001	15.90 (1.63)	<0.001
Time	1.12 (0.34)	0.001	1.27 (0.48)	0.008	0.87 (0.47)	0.06
Baseline age	−0.06 (0.02)	0.003	−0.06 (0.03)	0.033	−0.06 (0.03)	0.035
Baseline age × time	0.01 (0.01)	0.11	0.01 (0.01)	0.16	0.01 (0.01)	0.21
Sex (men vs. women)	−0.76 (0.41)	0.06				
Sex × time	−0.12 (0.12)	0.31				
n-3 HUFA	0.24 (0.81)	0.77	−0.11 (0.93)	0.91	0.45 (1.38)	0.74
n-3 HUFA × time	−0.14 (0.21)	0.51	0.06 (0.23)	0.78	−0.55 (0.41)	0.17
Random effects						
Level 1 residuals	4.03 (0.24)	<0.001	2.36 (0.52)	<0.001	4.74 (0.31)	<0.001
Level 2 residuals						
Intercept	6.66 (0.17)	<0.001	6.49 (0.24)	<0.001	6.83 (0.25)	<0.001
Linear slope	1.50 (0.06)	<0.001	1.49 (0.08)	<0.001	1.50 (0.09)	<0.001
			% Energy From n-*3 PUFAs*			
Fixed effect						
Intercept	16.43 (1.23)	<0.001	15.6 (1.7)	<0.001	16.39 (1.68)	<0.001
Time	1.15 (0.35)	0.001	1.28 (0.50)	0.011	0.89 (0.48)	0.06
Baseline age	−0.06 (0.02)	0.004	−0.06 (0.03)	0.037	−0.06 (0.03)	0.040
Baseline age × time	0.01 (0.01)	0.11	0.01 (0.01)	0.16	0.01 (0.01)	0.23
Sex (men vs. women)	−0.76 (0.41)	0.06				
Sex × time	−0.12 (0.12)	0.31				
n-3 PUFA	−0.57 (0.45)	0.20	−0.37 (0.62)	0.55	−0.72 (0.63)	0.25
n-3 PUFA × time	0.04 (0.12)	0.72	−0.00 (0.17)	0.99	−0.07 (0.17)	0.67
			n-*3 HUFA*:n-*6 HUFA Ratio*[c]			
Fixed effect						
Intercept	16.00 (1.19)	<0.001	15.39 (1.70)	<0.001	15.71 (1.63)	<0.001
Time	1.14 (0.34)	0.001	1.27 (0.48)	0.009	0.88 (0.47)	0.06
Baseline age	−0.06 (0.02)	0.003	−0.06 (0.03)	0.023	−0.06 (0.03)	0.038
Baseline age × time	0.01 (0.01)	0.11	0.01 (0.01)	0.16	0.01 (0.01)	0.23
Sex (men vs. women)	−0.73 (0.41)	0.07				
Sex × time	−0.13 (0.12)	0.28				
n-3 HUFA:*n*-6 HUFA ratio	0.05 (0.04)	0.21	−0.01 (0.07)	0.94	0.07 (0.06)	0.22
n-3 HUFA:*n*-6 HUFA ratio × time	−0.01 (0.01)	0.31	0.00 (0.02)	0.89	−0.02 (0.01)	0.19

Table continues

did men (Table 1). Among men, those who had elevated depressive symptoms had lower mean intakes of total carotenoids than did those who did not (3.16 mg per 1,000 kcal vs. 3.82 mg per 1,000 kcal; $P < 0.05$). Among women, the same pattern of lower intakes among individuals with elevated depressive symptoms was noted for vitamin B6, folate, and vitamin C. Overall, vitamin E intakes were lower among persons with elevated depressive symptoms. Sex differences were found in energy-adjusted dietary intakes, with higher total carotenoids, vitamin C, vitamin E, and folate levels in women compared with men.

Using mixed-effects regression analyses (Table 2), we found that participants who were younger at baseline had higher CES-D scores than did older participants (overall

Table 2. Continued

Fatty Acid Exposure	Model 1: All Participants[a] (2,052 Total Participants and 3,336 Visits)		Model 2: Men Only[a] (883 Participants and 1,409 Visits)		Model 3: Women Only[a] (1,169 Participants and 1,927 Visits)	
	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value
			n-*3 HUFA*:n-*6 PUFA Ratio*			
Fixed effect						
Intercept	16.00 (1.18)	<0.001	15.32 (1.69)	<0.001	15.88 (1.63)	<0.001
Time	1.13 (0.34)	0.001	1.28 (0.48)	0.008	0.89 (0.47)	0.06
Baseline age	−0.06 (0.02)	0.004	−0.06 (0.03)	0.032	−0.06 (0.03)	0.036
Baseline age × time	0.01 (0.01)	0.12	0.01 (0.01)	0.16	0.01 (0.01)	0.26
Sex (men vs. women)	−0.76 (0.41)	0.06				
Sex × time	−0.12 (0.12)	0.32				
n-3 HUFA:*n*-6 PUFA ratio	1.06 (3.02)	0.73	−1.17 (3.20)	0.71	5.76 (6.09)	0.35
n-3 HUFA:*n*-6 PUFA ratio × time	−1.12 (0.74)	0.13	−0.04 (0.76)	0.95	−4.74 (1.68)	0.005[d]
			n-*3 PUFA*:n-*6 PUFA Ratio*			
Fixed effect						
Intercept	15.95 (1.23)	<0.001	15.36 (1.74)	<0.001	15.66 (1.71)	<0.001
Time	1.26 (0.35)	<0.001	1.30 (0.49)	0.009	1.22 (0.48)	0.012
Baseline age	−0.06 (0.02)	0.004	−0.06 (0.03)	0.033	−0.06 (0.03)	0.035
Baseline age × time	0.01 (0.01)	0.12	0.01 (0.01)	0.16	0.01 (0.01)	0.25
Sex (men vs. women)	−0.76 (0.41)	0.06				
Sex × time	−0.12 (0.11)	0.32				
n-3 PUFA:*n*-6 PUFA ratio	0.59 (2.62)	0.82	−0.62 (2.99)	0.84	2.28 (4.54)	0.62
n-3 PUFA:*n*-6 PUFA ratio × time	−1.17 (0.66)	0.08	−0.11 (0.73)	0.88	−3.40 (1.24)	0.006[d]

Abbreviations: HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SE, standard error.

[a] In addition to sex (in models 2 and 3), the models were adjusted for other covariates (main effects and interaction with time). See the Methods section for more details on covariate coding and model specifications. Time at baseline visit was set to zero. Baseline age was centered at 50 years, total energy intake at 2,000 kcal/day, total carotenoid intake at 3 mg/1,000 kcal/day, vitamin C intake at 30 mg/1,000 kcal/day, vitamin A intake at 300 retinol equivalents/1,000 kcal/day, vitamin E at 3 mg/1,000 kcal/day, vitamin B-6 at 0.8 mg/1,000 kcal/day, vitamin B12 at 3 μg/1,000 kcal/day, and folate at 170 μg/1,000 kcal/day. Random effects were comparable between models with the 5 fatty acid exposures.

[b] Estimated regression coefficients from the mixed-effects regression models.

[c] The sample sizes were the same for all exposures except for *n*-3 HUFA:*n*-6 HUFA ratio, which were as follows: model 1, 2,046 subjects and 3,328 visits; model 2, 881 subjects and 1,407 visits; and model 3, 1,165 subjects and 1,921 visits.

[d] In a separate model with interaction of *n*-3 exposure by time by sex, including all other terms in the current model, $P < 0.02$ for the null hypothesis that this interaction term is 0.

and among women; $P < 0.010$,). Overall and among men, there was an appreciable increase in the CES-D score over time ($P < 0.010$) that was independent of age at baseline (baseline age × time; $P > 0.02$). Nevertheless, the sex difference in the change of the CES-D score over time was not significant after controlling for all other covariates in the model (sex × time; $P > 0.02$). There were several key findings about the associations of the 5 *n*-3 fatty acid exposures with total baseline CES-D score and change in score over time. Among women but not men, higher *n*-3 HUFA:*n*-6 PUFA and *n*-3 PUFA:*n*-6 PUFA ratios were both associated with a slower pace of increase in CES-D scores over time (exposure × time; $P < 0.020$). These associations were not found for absolute *n*-3 exposures or *n*-3 HUFA:*n*-6 HUFA ratios. Significant findings for women are illustrated in Web Figures 1–4 (available at http://aje.oxfordjournals.org/), in which the trajectories of total CES-D scores over time are shown stratified by values of 2 relative exposures, the *n*-3 PUFA:*n*-6 PUFA and *n*-3 HUFA:*n*-6 PUFA ratios. A higher level of each of these exposures was associated with slower increases of CES-D scores over time among women but not men. Conversely, depressive trajectories were similar across various levels of *n*-3 HUFA:*n*-6 HUFA ratios for women (Web Figure 5) and men (Web Figure 6).

Table 3 shows the adjusted associations between the 3 *n*-3 fatty acid relative exposures and the 4 domains of the CES-D among women. Positive affect was the only domain that increased over time ($P < 0.001$), with a higher score indicating

Table 3. Analysis of Baseline *n*-3 Fatty Acid Relative Exposures and Longitudinal Changes in Center for Epidemiologic Studies-Depression Domain Scores Among Women Using Mixed-Effects Linear Regression Analysis, Healthy Aging in Neighborhoods of Diversity Across the Life Span Study, Baltimore, Maryland, 2004–2013

Outcome Variable	*n*-3 HUFA:*n*-6 HUFA Ratio[a] (1,165 Participants and 1,923 Visits)		*n*-3 HUFA:*n*-6 PUFA Ratio[a] (1,169 Participants and 1,929 Visits)		*n*-3 PUFA:*n*-6 PUFA Ratio[a] (1,169 Participants and 1,929 Visits)	
	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value	γ[b] (SE)	*P* Value
CES-D Domain 1: Somatic Complaints						
Fixed effect						
Intercept	9.26 (0.86)	<0.001	9.35 (0.86)	<0.001	9.15 (0.90)	<0.001
Time	1.30 (0.21)	<0.001	1.28 (0.21)	<0.001	1.38 (0.22)	<0.001
Baseline age	−0.03 (0.02)	0.18	−0.02 (0.02)	0.17	−0.02 (0.02)	0.17
Baseline age × time	0.00 (0.00)	0.23	0.00 (0.00)	0.23	0.00 (0.00)	0.22
n-3 exposure	0.04 (0.03)	0.21	3.47 (3.20)	0.28	2.04 (2.39)	0.39
n-3 exposure × time	−0.01 (0.01)	0.06	−1.47 (0.79)	0.06	−0.97 (0.58)	0.10
Random effects						
Level 1 residuals	3.26 (0.08)	<0.001	3.26 (0.08)	<0.001	3.27 (0.08)	<0.001
Level 2 residuals						
Intercept	2.90 (0.12)	<0.001	2.90 (0.12)	<0.001	2.90 (0.12)	<0.001
Linear slope	0.00 (0.00)	<0.001	0.00 (0.00)	<0.001	0.00 (0.00)	<0.001
CES-D Domain 2: Depressed Affect[c]						
Fixed effect						
Intercept	6.22 (0.85)	<0.001	6.37 (0.85)	<0.001	6.35 (0.89)	<0.001
Time	1.07 (0.20)	<0.001	1.06 (0.20)	<0.001	1.15 (0.21)	<0.001
Baseline age	−0.02 (0.01)	0.19	−0.02 (0.01)	0.21	−0.02 (0.01)	0.21
Baseline age × time	0.01 (0.00)	0.11	0.01 (0.00)	0.13	0.01 (0.00)	0.13
n-3 exposure	0.07 (0.03)	0.016	2.68 (3.16)	0.47	0.38 (2.36)	0.87
n-3 exposure × time	−0.015 (0.007)	0.022	−1.58 (0.75)	0.036	−1.00 (0.55)	0.07
Random effects						
Level 1 residuals	2.98 (0.11)	<0.001	2.98 (0.12)	<0.001	2.98 (0.11)	<0.001
Level 2 residuals						
Intercept	3.12 (0.12)	<0.001	3.12 (0.12)	<0.001	3.13 (0.12)	<0.001
Linear slope	0.25 (0.09)	<0.001	0.24 (0.09)	<0.001	0.24 (0.09)	<0.001

Table continues

fewer depressive symptoms. Importantly, higher *n*-3 HUFA:*n*-6 PUFA and *n*-3 PUFA:*n*-6 PUFA ratios were associated with a faster increase ($P < 0.020$). Three-way interaction term analysis (time × exposure × sex) indicated that the associations of those fatty acid ratios with the trajectories of CES-D score and positive affect were significantly different between men and women (Tables 3 and 4).

Among men, a higher *n*-3 HUFA:*n*-6 HUFA ratio was related to a slower rate of increase in somatic complaints (domain 1) ($P < 0.020$), although there was no significant difference by sex based on a 3-way interaction between exposure, sex, and time ($P < 0.020$) (Table 4). Overall, *n*-3 PUFA (percent of energy) and *n*-3 HUFA (percent of energy) intakes were not associated with longitudinal changes in the 4 CES-D domains (Web Table 1). When use of antidepressants (12.9% of the study subjects) was entered as a potential confounder in the mixed-effects regression models, the results remained unaltered. Antidepressant use was positively associated with the presence of depressive symptoms at baseline, although it did not have a significant relationship with the rate of change in depressive symptoms over time (data not shown). The associations of *n*-3 PUFA:*n*-6 PUFA ratio and consumption of fish high in *n*-3 fatty acids with intake of discretionary oils is presented in Web Figure 7, using fractional polynomials with 95% confidence interval bands. That Figure shows that the *n*-3 PUFA:*n*-6 PUFA is positively associated with intake of fish high in *n*-3 fatty acids and inversely related to discretionary oil intake.

DISCUSSION

The present study is one of a few population-based cohort studies in which a significant relationship between the *n*-3*:n*-6 fatty acid ratio and a slower increase in self-reported depressive symptoms (and selected subdomains) over time has been reported. We hypothesized that all associations would

Table 3. Continued

Outcome Variable	*n*-3 HUFA:*n*-6 HUFA Ratio[a] (1,165 Participants and 1,923 Visits)		*n*-3 HUFA:*n*-6 PUFA Ratio[a] (1,169 Participants and 1,929 Visits)		*n*-3 PUFA:*n*-6 PUFA Ratio[a] (1,169 Participants and 1,929 Visits)	
	γ^b (SE)	*P* Value	γ^b (SE)	*P* Value	γ^b (SE)	*P* Value
CES-D Domain 3: Positive Affect						
Fixed effect						
Intercept	8.67 (0.52)	<0.001	8.63 (0.52)	<0.001	8.67 (0.54)	<0.001
Time	0.68 (0.12)	<0.001	0.67 (0.12)	<0.001	0.59 (0.13)	<0.001
Baseline age	0.02 (0.01)	0.028	0.02 (0.01)	0.022	0.02 (0.01)	0.022
Baseline age × time	−0.00 (0.00)	0.65	−0.00 (0.00)	0.69	−0.00 (0.00)	0.66
n-3 exposure	−0.01 (0.02)	0.47	−1.83 (1.93)	0.34	−0.42 (1.44)	0.77
n-3 exposure × time	0.00 (0.00)	0.61[d]	1.34 (0.47)	0.005[d]	0.83 (0.35)	0.018[d]
Random effects						
Level 1 residuals	1.96 (0.07)	<0.001	1.94 (0.07)	<0.001	1.95 (0.07)	<0.001
Level 2 residuals						
Intercept	1.77 (0.07)	<0.001	1.79 (0.07)	<0.001	1.78 (0.07)	<0.001
Linear slope	0.05 (0.16)	<0.001	0.05 (0.15)	<0.001	0.04 (0.21)	<0.001
CES-D Domain 4: Interpersonal Problems						
Fixed effect						
Intercept	1.12 (0.25)	<0.001	1.14 (0.26)	<0.001	1.23 (0.27)	<0.001
Time	0.35 (0.07)	<0.001	0.35 (0.07)	<0.001	0.36 (0.07)	<0.001
Baseline age	−0.00 (0.00)	0.35	−0.00 (0.00)	0.37	−0.00 (0.00)	0.37
Baseline age × time	0.00 (0.00)	0.008	0.00 (0.00)	0.008	0.00 (0.00)	0.008
n-3 exposure	0.00 (0.01)	0.67	−0.95 (0.96)	0.32	−0.80 (0.72)	0.26
n-3 exposure × time	−0.00 (0.00)	0.48	0.02 (0.25)	0.94	0.08 (0.19)	0.68
Random effects						
Level 1 residuals	0.96 (0.04)	<0.001	0.97 (0.04)	<0.001	0.97 (0.04)	<0.001
Level 2 residuals						
Intercept	0.88 (0.04)	<0.001	0.88 (0.04)	<0.001	0.88 (0.02)	<0.001
Linear slope	0.12 (0.02)	<0.001	0.12 (0.02)	<0.001	0.12 (0.02)	<0.001

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression scale; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acids; SE, standard error.

[a] Models were adjusted for other covariates (main effects and interaction with time). See the Methods section for more details on covariate coding and model specifications. Time at baseline visit was set to zero. Baseline age was centered at 50 years, total energy intake at 2,000 kcal/day, total carotenoid intake at 3 mg/1,000 kcal/day, vitamin C intake at 30 mg/1,000 kcal/day, vitamin A intake at 300 retinol equivalents/1,000 kcal/day, vitamin E at 3 mg/1,000 kcal/day, vitamin B6 at 0.8 mg/1,000 kcal/day, vitamin B12 at 3 μg/1,000 kcal/day, and folate at 170 μg/1,000 kcal/day.

[b] Estimated regression coefficients from the mixed-effects regression models.

[c] The sample sizes were the same for all exposures except for depressed affect which were as follows: *n*-3 HUFA:*n*-6 HUFA ratio, 1,164 subjects and 1,922 visits; *n*-3 HUFA:*n*-6 PUFA ratio, 1,168 subjects and 1,928 visits; and *n*-3 PUFA:*n*-6 PUFA ratio, 1,168 subjects and 1,928 visits.

[d] In a separate model with interaction of *n*-3 exposure by time by sex, including all other terms in the current model, $P < 0.02$ for the null hypothesis that this interaction term is 0.

be in this direction (i.e., higher intakes would lead to slower increases); however, after controlling for sociodemographic, lifestyle, and health-related potential confounders, it was only significant among women for depressive symptoms overall and positive affect (reverse coded) in relation to *n*-3 HUFA:*n*-6 PUFA and *n*-3 PUFA:*n*-6 PUFA ratios and was only significant among men for somatic complaints in relation to *n*-3 HUFA:*n*-6 HUFA ratio.

In a cohort study, Colangelo et al. (21) found a moderating effect of sex similar to the one that we found, whereby higher intakes of EPA, DHA, and a combination of EPA and DHA were associated with lower levels of depressive symptoms after a 10-year follow-up in women only after controlling for antidepressant use. In another study of nondepressed women who were 50–77 years of age at baseline (follow-up period = 10 years), Lucas et al. (19) found that depression was positively linked

Table 4. Analysis of Baseline *n*-3 Fatty Acid Relative Exposures and Longitudinal Changes in Center for Epidemiologic Study-Depression Domain Scores Among Men Using Mixed-Effects Linear Regression Analysis, Healthy Aging in Neighborhoods of Diversity Across the Life Span Study, Baltimore, Maryland, 2004–2013

Outcome Variable	*n*-3 HUFA:*n*-6 HUFA Ratio[a,b]		*n*-3 HUFA:*n*-6 PUFA Ratio[a,c]		*n*-3 PUFA:*n*-6 PUFA Ratio[a,d]	
	γ^e (SE)	*P* Value	γ^e (SE)	*P* Value	γ^e (SE)	*P* Value
CES-D Domain 1: Somatic Complaints						
Fixed effect						
Intercept	8.31 (0.94)	<0.001	8.33 (0.93)	<0.001	8.22 (0.96)	<0.001
Time	1.49 (0.23)	<0.001	1.41 (0.22)	<0.001	1.45 (0.23)	<0.001
Baseline age	−0.03 (0.02)	0.07	−0.03 (0.02)	0.08	−0.03 (0.02)	0.08
Baseline age × time	0.00 (0.00)	0.32	0.00 (0.00)	0.36	0.00 (0.00)	0.35
n-3 exposure	0.03 (0.04)	0.40	0.19 (1.76)	0.91	0.74 (1.64)	0.66
n-3 exposure × time	−0.02 (0.01)	0.012	−0.32 (0.33)	0.33	−0.34 (0.32)	0.28
Random effects						
Level 1 residuals	2.50 (0.13)	<0.001	2.49 (0.13)	<0.001	2.49 (0.13)	<0.001
Level 2 residuals						
Intercept	2.86 (0.12)	<0.001	2.88 (0.12)	<0.001	2.88 (0.12)	<0.001
Linear slope	0.28 (0.08)	<0.001	0.29 (0.07)	<0.001	0.29 (0.07)	<0.001
CES-D Domain 2: Depressed Affect						
Fixed effect						
Intercept	6.91 (0.94)	<0.001	6.96 (0.93)	<0.001	7.00 (0.96)	<0.001
Time	1.01 (0.23)	<0.001	1.08 (0.22)	<0.001	1.10 (0.23)	<0.001
Baseline age	−0.01 (0.01)	0.53	−0.01 (0.02)	0.61	−0.01 (0.02)	0.62
Baseline age × time	0.00 (0.00)	0.28	0.00 (0.00)	0.52	0.00 (0.00)	0.51
n-3 exposure	0.06 (0.04)	0.13	−0.19 (1.75)	0.92	−0.29 (1.64)	0.86
n-3 exposure × time	−0.02 (0.01)	0.12	−0.19 (0.33)	0.56	−0.15 (0.32)	0.65
Random effects						
Level 1 residuals	2.50 (0.13)	<0.001	2.51 (0.13)	<0.001	2.52 (0.13)	<0.001
Level 2 residuals						
Intercept	2.81 (0.12)	<0.001	2.82 (0.12)	<0.001	2.81 (0.12)	<0.001
Linear slope	0.28 (0.07)	<0.001	0.29 (0.07)	<0.001	0.29 (0.07)	<0.001
CES-D Domain 3: Positive Affect						
Fixed effect						
Intercept	8.44 (0.58)	<0.001	8.52 (0.57)	<0.001	8.42 (0.60)	<0.001
Time	0.60 (0.14)	<0.001	0.57 (0.15)	<0.001	0.58 (0.15)	<0.001
Baseline age	0.02 (0.01)	0.044	0.02 (0.01)	0.06	0.02 (0.01)	0.06
Baseline age × time	−0.00 (0.00)	0.07	−0.00 (0.00)	0.06	−0.00 (0.00)	0.06
n-3 exposure	0.03 (0.02)	0.17	0.65 (1.08)	0.55	0.85 (1.01)	0.40
n-3 exposure × time	−0.02 (0.01)	0.026	−0.11 (0.22)	0.61	−0.12 (0.21)	0.56
Random effects						
Level 1 residuals	1.63 (0.08)	<0.001	1.64 (0.08)	<0.001	1.64 (0.08)	<0.001
Level 2 residuals						
Intercept	1.66 (0.08)	<0.001	1.66 (0.08)	<0.001	1.66 (0.08)	<0.001
Linear slope	0.19 (0.04)	<0.001	0.19 (0.04)	<0.001	0.19 (0.05)	<0.001

Table continues

to LA intake and *n*-6:*n*-3 fatty acid ratios and inversely associated with ALA:LA ratios. However, they found no relationship between depression and absolute intakes of ALA or EPA and DHA combined. Our cross-sectional analysis of HANDLS Study data indicated that elevated depressive symptoms at wave 1 and CES-D domain scores suggestive of depression were inversely related to several *n*-3 absolute and relative exposures (expressed as tertiles of intake) among women only (15).

Table 4. Continued

Outcome Variable	*n*-3 HUFA:*n*-6 HUFA Ratio[a,b]		*n*-3 HUFA:*n*-6 PUFA Ratio[a,c]		*n*-3 PUFA:*n*-6 PUFA Ratio[a,d]	
	γ^e (SE)	*P* Value	γ^e (SE)	*P* Value	γ^e (SE)	*P* Value
	CES-D Domain 4: Interpersonal Problems					
Fixed effect						
Intercept	1.95 (0.34)	<0.001	2.00 (0.33)	<0.001	2.00 (0.35)	<0.001
Time	0.40 (0.09)	<0.001	0.38 (0.09)	<0.001	0.38 (0.09)	<0.001
Baseline age	−0.01 (0.01)	0.028	−0.01 (0.01)	0.025	−0.01 (0.01)	0.024
Baseline age × time	0.00 (0.00)	0.14	0.00 (0.00)	0.12	0.00 (0.00)	0.11
n-3 exposure	0.01 (0.01)	0.50	0.07 (0.62)	0.91	−0.02 (0.58)	0.98
n-3 exposure × time	−0.00 (0.00)	0.50	−0.10 (0.13)	0.41	−0.05 (0.12)	0.67
Random effects						
Level 1 residuals	1.04 (0.04)	<0.001	1.04 (0.04)	<0.001	1.04 (0.04)	<0.001
Level 2 residuals						
Intercept	0.85 (0.05)	<0.001	0.85 (0.05)	<0.001	0.85 (0.05)	<0.001
Linear slope	0.05 (0.05)	<0.001	0.05 (0.05)	<0.001	0.05 (0.05)	<0.001

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression scale; HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SE, standard error.

[a] Models were adjusted for other covariates (main effects and interaction with time). See the Methods section for more details on covariate coding and model specifications. Time at baseline visit was set to zero. Baseline age was centered at 50 years, total energy intake at 2,000 kcal/day, total carotenoid intake at 3 mg/1,000 kcal/day, vitamin C intake at 30 mg/1,000 kcal/day, vitamin A intake at 300 retinol equivalents/1,000 kcal/day, vitamin E at 3 mg/1,000 kcal/day, vitamin B6 at 0.8 mg/1,000 kcal/day, vitamin B12 at 3 μg/1,000 kcal/day, and folate at 170 μg/1,000 kcal/day.

[b] The sample sizes were as follows: somatic complaints, 881 participants and 1,408 visits; depressed affect, 879 participants and 1,406 visits; positive affect, 880 participants and 1,407 visits; and interpersonal problems, 880 participants and 1,409 visits.

[c] The sample sizes were as follows: somatic complaints, 883 participants and 1,411 visits; depressed affect, 881 participants and 1,409 visits; positive affect, 882 participants and 1,409 visits; and interpersonal problems, 880 participants and 1,409 visits.

[d] The sample sizes were as follows: somatic complaints, 883 participants and 1,411 visits; depressed affect, 881 participants and 1,409 visits; positive affect, 882 participants and 1,409 visits; and interpersonal problems, 882 participants and 1,421 visits.

[e] Estimated regression coefficients from the mixed-effects regression models.

Among many potential underlying mechanisms of this relationship, the phospholipid fatty acid composition of the neuronal cell membrane has been suggested to reflect dietary intake (73). *n*-3 HUFAs from fish and fish oil exert the most profound influence on brain fatty acid concentrations (74). The highly unsaturated nature of EPA and DHA provides them the ability to influence the membrane fluidity of many cell types (6). These fatty acids can regulate signal transduction by enhancing G-protein–mediated signal transduction (6, 75). Within brain membranes, PUFAs have also been shown to increase the activity of the enzymes adenylate cyclase and protein kinase A by driving the c-adenosine monophosphate messenger system used by serotonin, noradrenaline, and adrenaline (α_2- and β-adrenergic), as well as by dopamine receptors 1 and 2 (76–78). Moreover, PUFAs can affect serotonergic and α_1-adrenergic transmission by exerting their effect on phospholipase C and protein kinase C (79, 80). The membrane changing induced by *n*-3 HUFAs might affect different neurotransmissions, thereby altering the regulation of serotoninergic and catecholaminergic neurotransmission in depressed individuals (6).

Another explanation for these associations might be the roles of phospholipases D and A_2 in neurotransmission. Phospholipase A_2 is activated by dopamine receptor 2, serotonin, glutamate, and muscarinic acetylcholine receptors (73). Moreover, phospholipase A_2 can release AA, dihomo-γ-linolenic acid, and EPA from membrane phospholipids, but with markedly differing consequences. In fact, dihomo-γ-linolenic acid, AA, and EPA can be transformed into prostaglandins 1, 2, and 3, respectively. Although prostaglandin 2 is highly proinflammatory, prostaglandin 3 is antiinflammatory and prostaglandin 1 has intermediate properties. A highly reactive phospholipase A_2 is hypothesized to be related to various psychiatric disorders (81). This high reactivity, coupled with an elevated concentration of *n*-6 fatty acids in brain membranes, might aggravate inflammatory conditions and trigger neuronal dysfunction that manifests in psychiatric disorders (81). This condition can potentially be countered by increasing *n*-3 fatty acids concentrations in brain phospholipids.

DHA and EPA can also modulate calcium and sodium channels. They inhibit the enzyme calcium adenosine triphosphatase in neuronal membranes and synaptosomal sodium-potassium

adenosine triphosphatase (82), a mechanism that has been suggested to explain the dampening effect of *n*-3 fatty acids on neuronal activity. Finally, dietary intakes of *n*-3 fatty acids, especially DHA, might modulate brain energy and glucose metabolism by regulating the glucose transporter 1. Molecular mechanisms of DHA might implement transcriptional, post-transcriptional, and post-translational events of glucose transporter 1 expression. Because DHA is a potent endogenous ligand for transcriptional factors in neural cells, it might modulate gene transcription through activation of peroxisome proliferator-activated receptors (83).

Sex-specific differences in the association between intakes of *n*-3 PUFAs and depression can be explained by at least 2 mechanisms. First, on average, women have higher plasma DHA concentrations than do men independent of DHA intake, possibly because estrogen increases DHA levels, whereas testosterone decreases them (84, 85). Second, conversion from ALA to DHA occurs at a higher rate in women (86).

Our study has many strengths. First, to our knowledge, it is the only large prospective cohort study in which the longitudinal association between *n*-3 fatty acids and depressive symptoms has been assessed among young and middle-aged white and African-American subjects from the US population using the CES-D total score and scores on domains. Thus, it was possible to ascertain the temporality of associations, unlike in previous cross-sectional studies. Second, we considered a wide array of exposures of interest, including total *n*-3 PUFAs, *n*-3 HUFAs, and *n*-3:*n*-6 ratios, while stratifying the analysis by sex. Third, our study is among the few large US studies that included two 24-hour dietary recalls, thus limiting measurement error and enhancing the ability of dietary variables to reflect usual intake to a greater extent than a single recall.

However, our study has limitations. First, measurement errors in dietary exposures cannot be totally avoided by using multiple 24-hour recalls. However, for reasons listed in our previous cross-sectional study (15), taking the mean of two 24-hour recalls from the Automated Multiple-Pass Method is considered a good estimate of usual but not long-term intake. Second, data on baseline supplemental intakes of *n*-3 fatty acids were not available, which precluded assessment of total *n*-3 fatty acid intake. A biomarker measure of *n*-3 fatty acid concentration in adipose tissue, red blood cells, or serum is the gold standard for total intake assessment because it accounts for all sources and provides an unbiased measure of exposure. Finally, only 2 waves of data were used, which precluded testing for time-varying confounders that might alter the trajectory of the CES-D scores over time.

In summary, in this biracial sample of US adults, the *n*-3:*n*-6 dietary fatty acid ratio was associated with longitudinal changes in depressive symptoms, with a higher ratio linked to a slower increases in depressive symptoms over time, particularly among women. Future randomized controlled trials assessing the impact of the ratios of *n*-3:*n*-6 dietary fatty acids on health outcomes should be conducted to examine their long-term effects on depression.

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Web Material legends

Web Figure 1. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3:n-6 PUFA ratio, among women

Web Figure 2. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3:n-6 PUFA ratio, among men

Web Figure. 3. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3 HUFA:n-6 PUFA ratio, among women

Web Figure. 4. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3 HUFA:n-6 PUFA ratio, among men

Web Figure. 5. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3:n-6 HUFA ratio, among women

Web Figure. 6. Predicted CES-D score trajectory from mixed-effects regression model for five levels of n-3:n-6 HUFA ratio, among men

Web TABLE 1. Analysis of baseline *n-3* fatty acid absolute exposures and longitudinal change in CES-D component scores (both sexes combined), mixed-effects linear regression analysis, HANDLS study, Baltimore, MD, 2004-2013

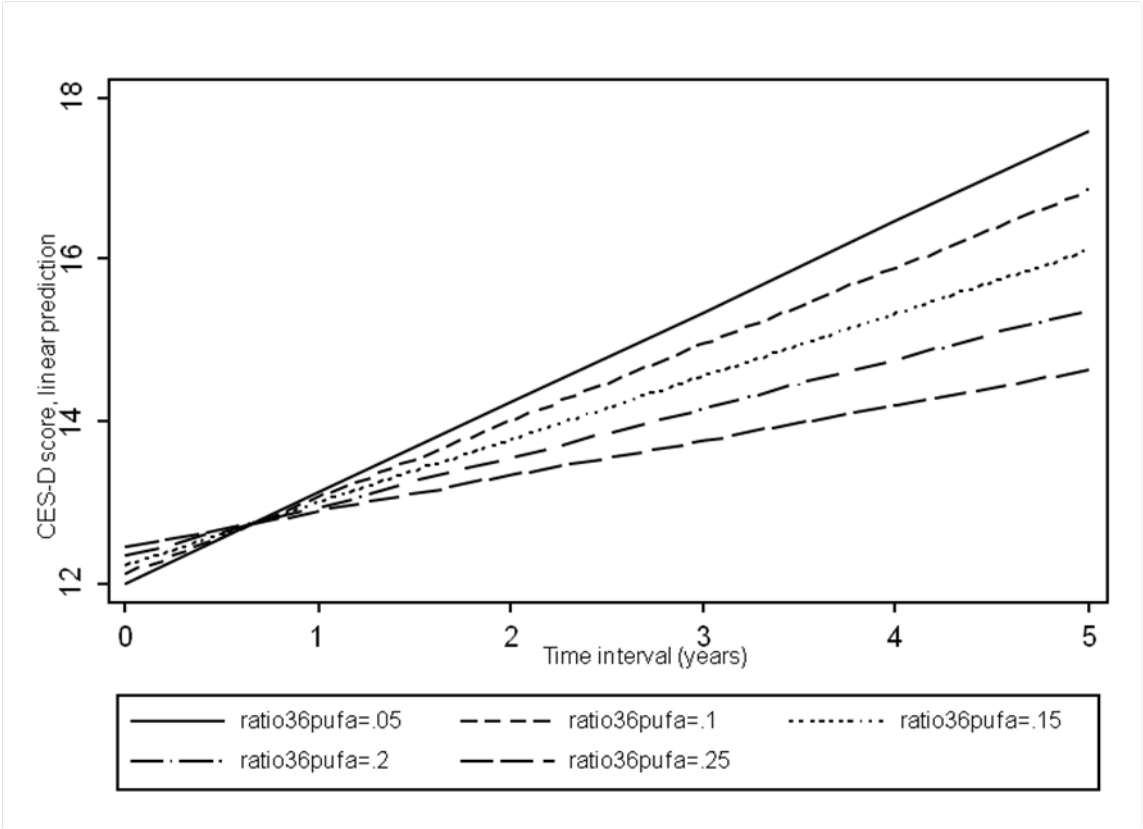
Web Figure. 7. n-3:n-6 PUFA vs. high omega-3 fish consumption and intake of oils

1 Oz cooked lean meat from fish, other seafood high in n-3 fatty acids.

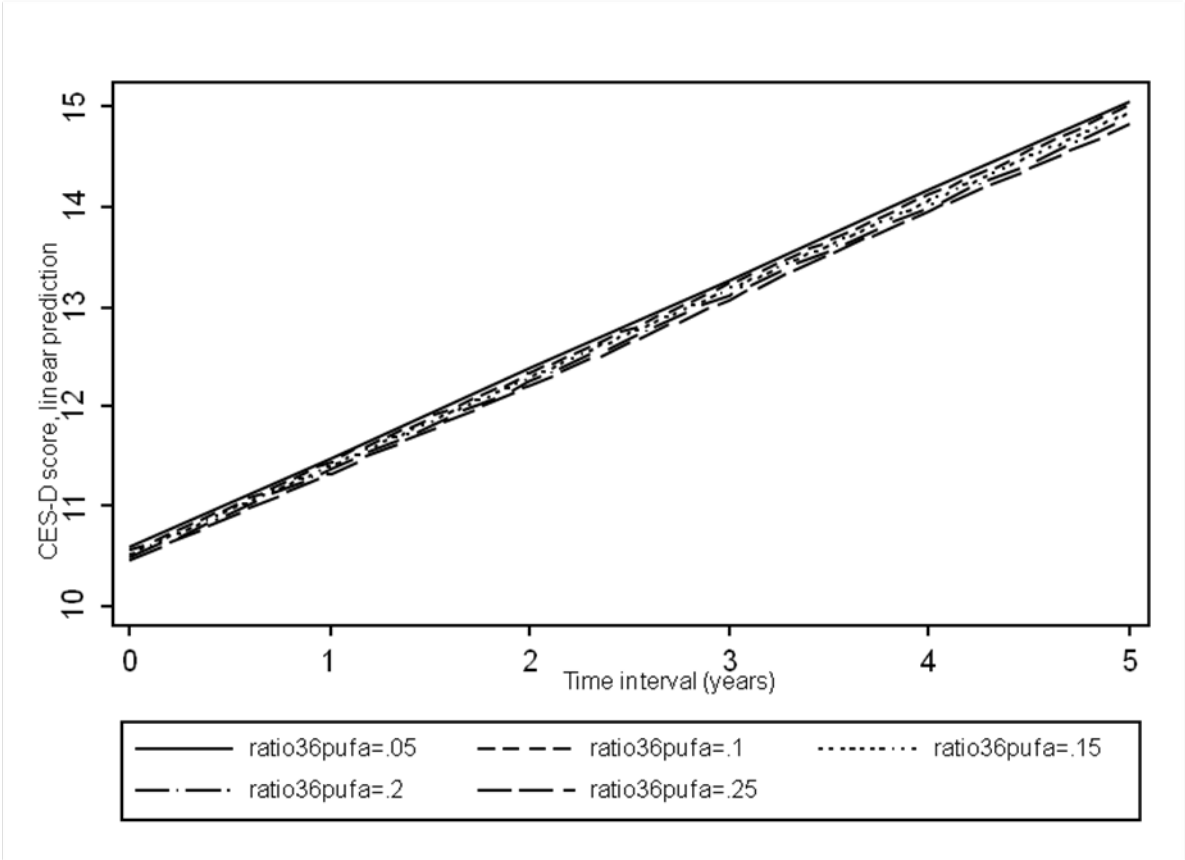
2 Grams of discretionary Oil.

URL for MEPED 2.0: http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/mped/mped2_doc.pdf

Web Figure. 1.


CES-D score, linear prediction
12
14
16
18
0
1
2
3
4
5
Time interval (years)
ratio36pufa=.05
ratio36pufa=.1
ratio36pufa=.15
ratio36pufa=.2
ratio36pufa=.25

Web Figure 2


CES-D score, linear prediction
10
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15
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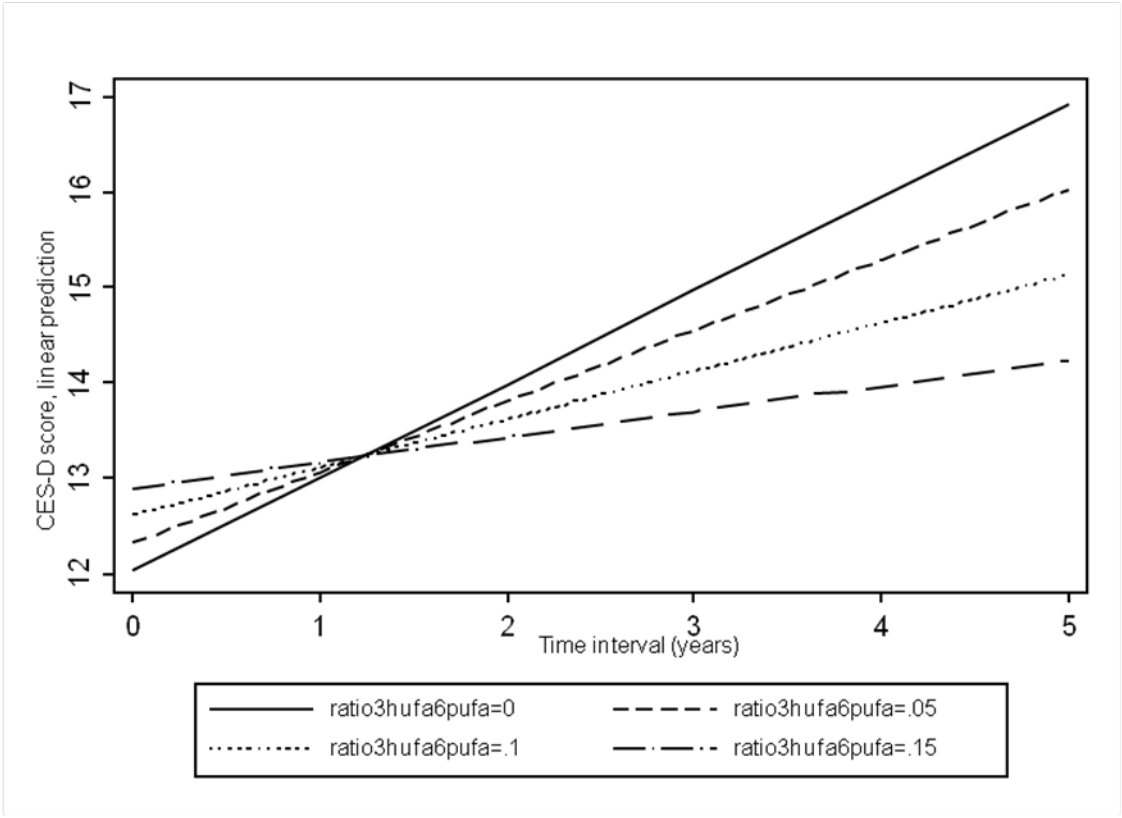
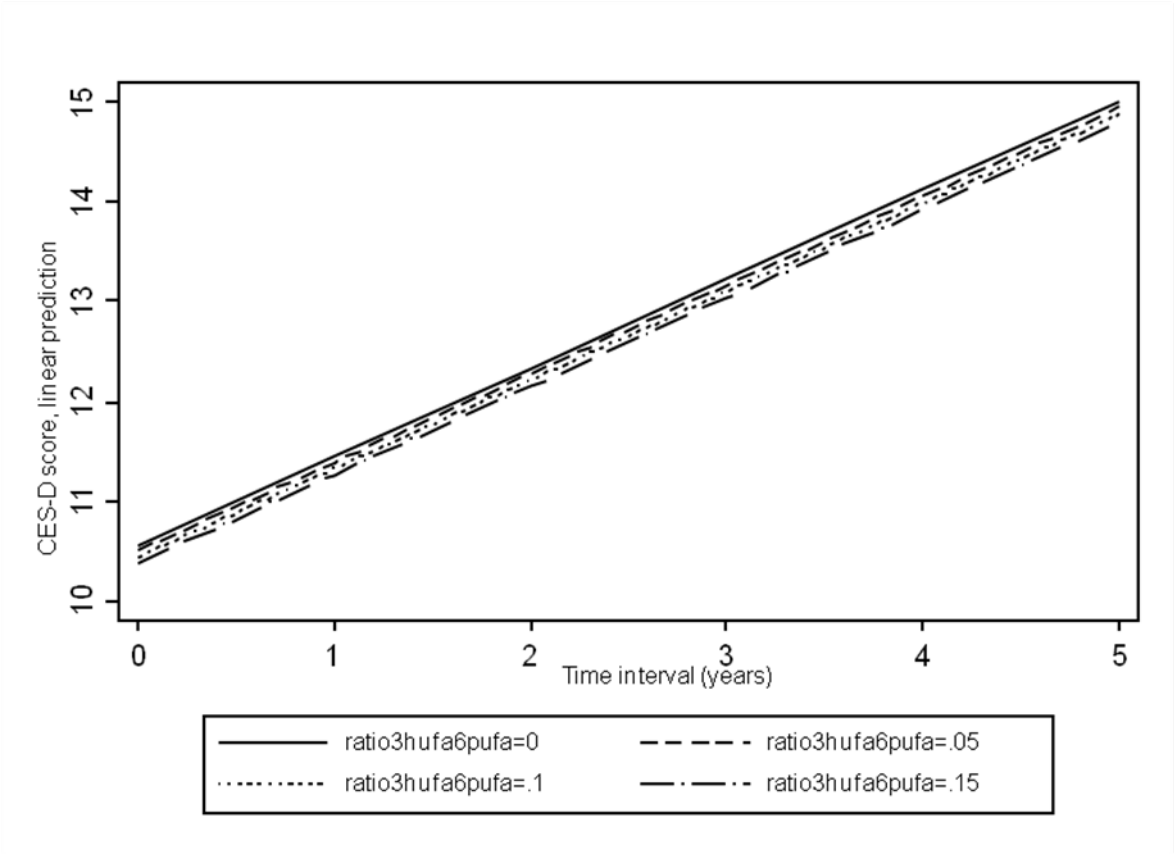
5
Time interval (years)
ratio36pufa=.05
ratio36pufa=.1
ratio36pufa=.15
ratio36pufa=.2
ratio36pufa=.25

Web Figure. 3.


CES-D score, linear prediction
12
13
14
15
16
17
0
1
2
3
4
5
Time interval (years)
ratio3hufa6pufa=0
ratio3hufa6pufa=.05
ratio3hufa6pufa=.1
ratio3hufa6pufa=.15

Web Figure. 4.


CES-D score, linear prediction
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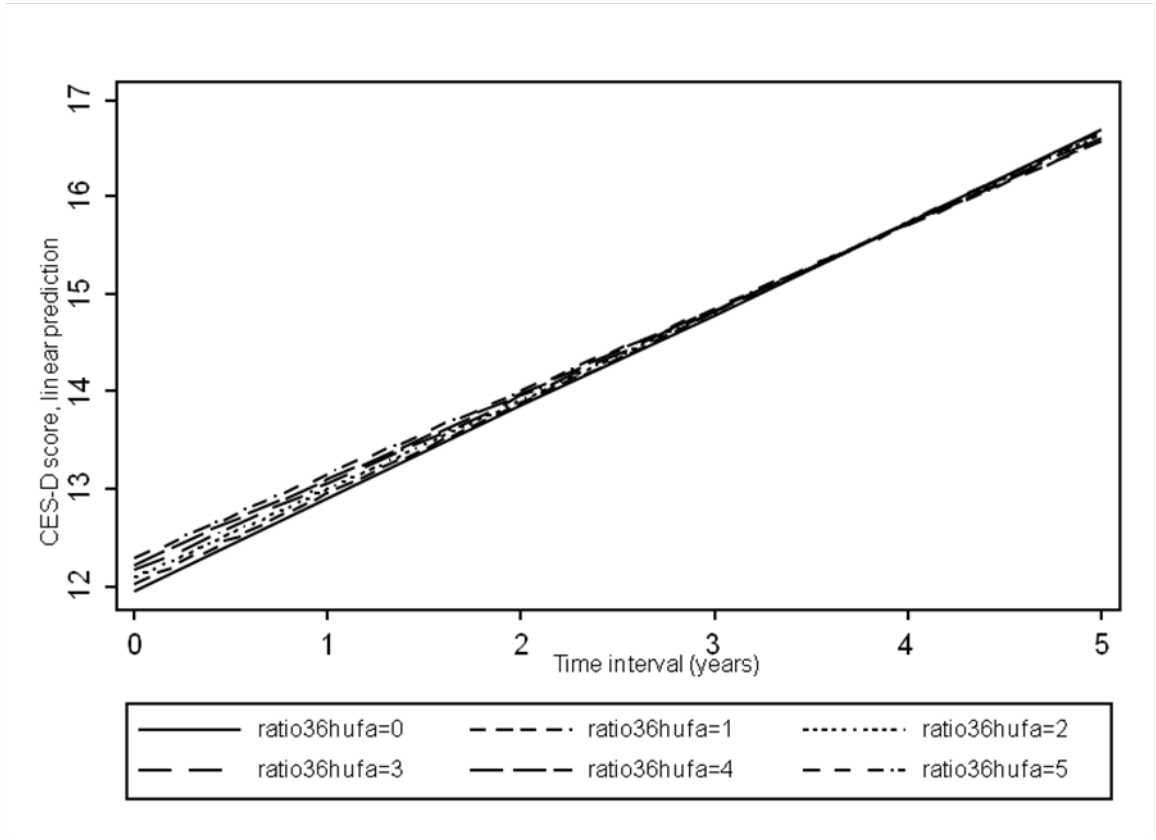
Time interval (years)
ratio3hufa6pufa=0
ratio3hufa6pufa=.05
ratio3hufa6pufa=.1
ratio3hufa6pufa=.15

Web Figure 5.


CES-D score, linear prediction
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Time interval (years)
ratio36hufa=0
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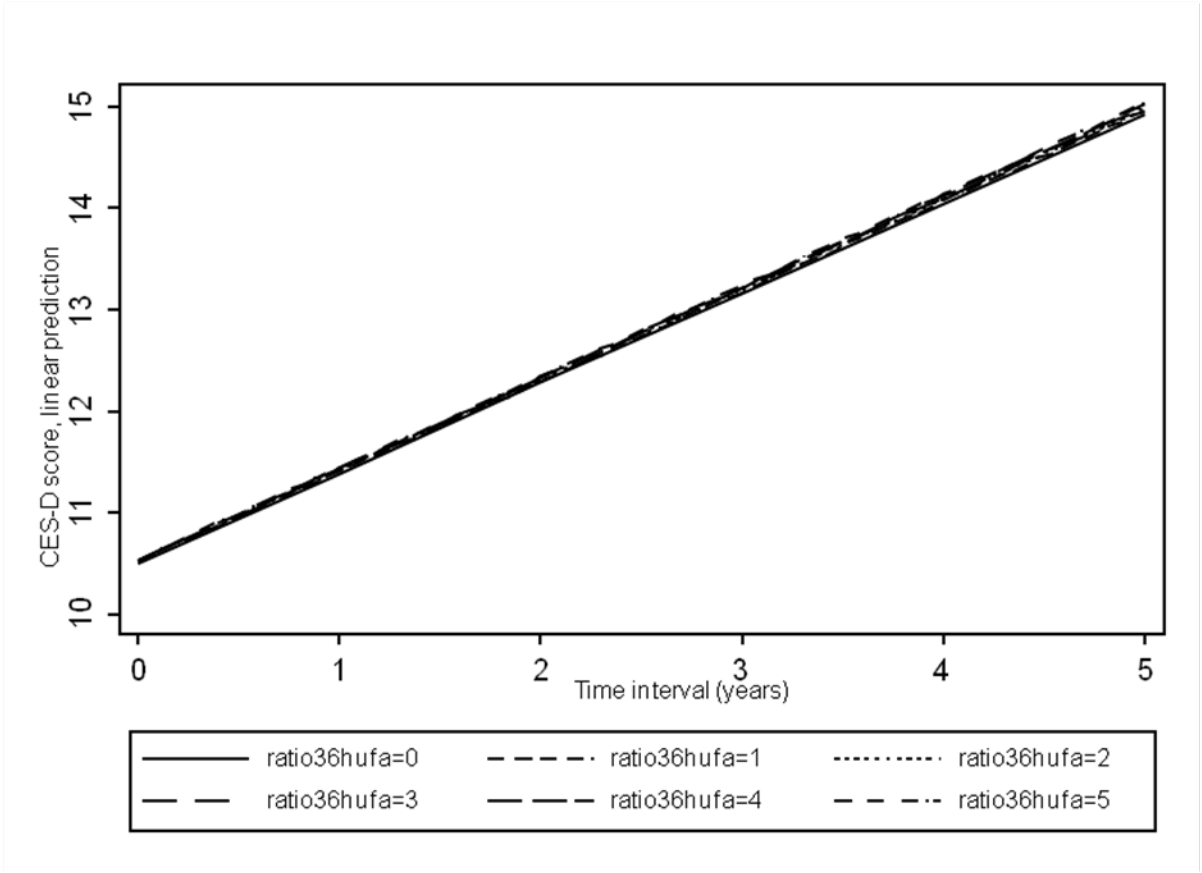
ratio36hufa=3
ratio36hufa=4
ratio36hufa=5

Web Figure 6.


CES-D score, linear prediction
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Time interval (years)
ratio36hufa=0
ratio36hufa=1
ratio36hufa=2
ratio36hufa=3
ratio36hufa=4
ratio36hufa=5

Web TABLE 1.

	n-3 HUFA (% energy) [a,b] γ±SEE	p-value	**n-3 PUFA (% energy)** [a,b] γ±SEE	p-value
CES-D component 1: Somatic complaints	**N=2,053**	**N'=3,341**	**N=2,052**	**N'=3,340**
Fixed effect				
Intercept	+9.02±0.64	<0.001	+9.15±0.66	<0.001
Time	+1.37±0.15	<0.001	+1.39±0.16	<0.001
Age_{base}	-0.02±0.01	0.031	-0.02±0.01	0.035
Age_{base} ×Time	+0.00±0.00	0.14	+0.00±0.00	0.14
n-3 exposure	+0.37±0.43	0.40	-0.14±0.24	0.56
n-3 exposure×Time	-0.12±0.09	0.19	-0.04±0.06	0.51
Random effects				
Level 1 residuals	+3.03±0.08	<0.001	+3.03±0.08	<0.001
Level 2 residuals				
Intercept	+2.89±0.09	<0.001	+2.89±0.09	<0.001
Linear slope	+0.11±0.14	<0.001	+0.12±0.13	<0.001
CES-D component 2: Depressed affect	**N=2,049**	**N'=3,337**	**N=2,049**	**N'=3,337**
Fixed effect				
Intercept	+6.90±0.63	<0.001	+7.12±0.65	<0.001
Time	+1.08±0.15	<0.001	+1.05±0.16	<0.001
Age_{base}	-0.02±0.01	0.19	-0.01±0.01	0.21
Age_{base} ×Time	+0.00±0.00	0.15	+0.00±0.00	0.16
n-3 exposure	+0.24±0.43	0.58	-0.29±0.24	0.27
n-3 exposure×Time	-0.08±0.09	0.41	-0.04±0.06	0.51
Random effects				
Level 1 residuals	+2.81±0.09	<0.001	+2.81±0.09	<0.001
Level 2 residuals				
Intercept	+3.02±0.09	<0.001	+3.01±0.09	<0.001
Linear slope	+0.28±0.09	<0.001	+0.28±0.06	<0.001
CES-D component 3: Positive affect	**N=2,051**	**N'=3,338**	**N=2,051**	**N'=3,338**
Fixed effect				

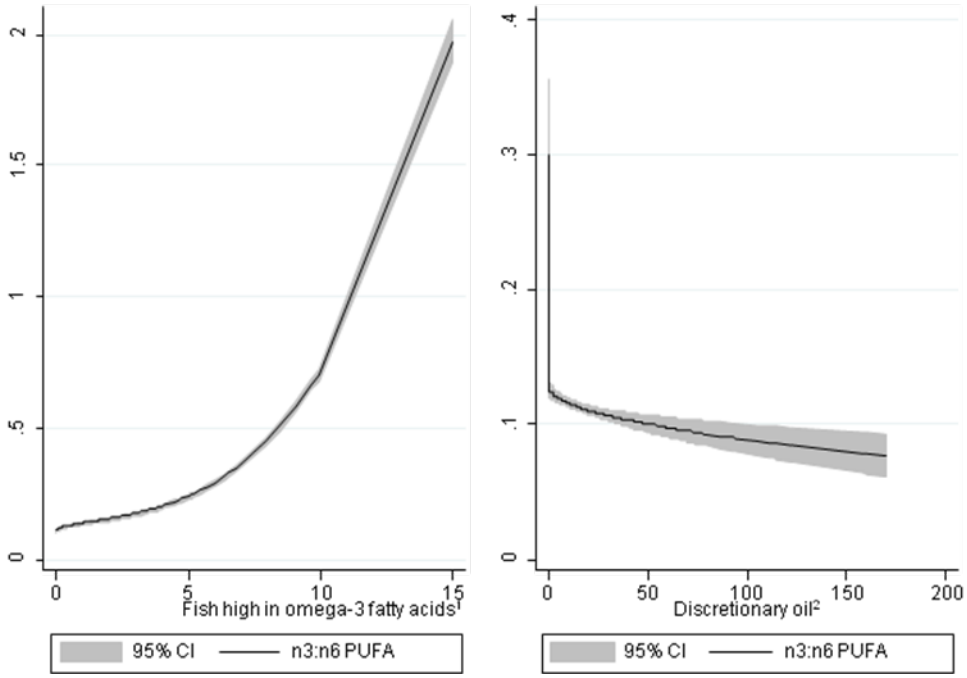
Intercept	+8.53±0.39	<0.001	+8.34±0.40	<0.001
Time	+0.63±0.10	<0.001	+0.63±0.10	<0.001
Age_{base}	+0.02±0.01	0.004	+0.02±0.01	0.006
Age_{base} ×Time	-0.00±0.00	0.22	-0.00±0.00	0.22
n-3 exposure	+0.14±0.26	0.61	+0.28±0.15	0.05
n-3 exposure ×Time	+0.02±0.06	0.75	-0.01±0.03	0.73
Random effects				
Level 1 residuals	+1.84±0.05	<0.001	+1.84±0.05	<0.001
Level 2 residuals				
Intercept	+1.74±0.06	<0.001	+1.74±0.06	<0.001
Linear slope	+0.13±0.05	<0.001	+0.13±0.04	<0.001
CES-D component 4: Interpersonal problems	**N=2,051**	**N'=3,344**	**N=2,051**	**N'=3,344**
Fixed effect				
Intercept	+1.39±0.20	<0.001	+1.41±0.21	<0.001
Time	+0.38±0.05	<0.001	+0.39±0.05	<0.001
Age_{base}	-0.01±0.00	0.033	-0.01±0.00	0.035
Age_{base} ×Time	+0.00±0.00	0.003	+0.00±0.00	0.003
n-3 exposure	+0.06±0.13	0.64	-0.02±0.08	0.83
n-3 exposure ×Time	-0.02±0.03	0.51	-0.01±0.02	0.64
Random effects				
Level 1 residuals	+0.99±0.03	<0.001	+1.00±0.03	<0.001
Level 2 residuals				
Intercept	+0.88±0.03	<0.001	+0.88±0.03	<0.001
Linear slope	+0.10±0.02	<0.001	+0.10±0.02	<0.001

Abbreviations: AA=Arachidonic acid; ALA=α-linolenic acid; CES-D=Center for Epidemiologic Studies-Depression scale; DHA=Docosahexaenoic acid; DPA=Docosapentaenoic acid EPA=Eicosapentaenoic acid; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Lifespan; HS=High School; HUFA=highly unsaturated fatty acids; LA=Linoleic acid; *n-3*=omega-3; *n-6*=omega-6; PIR=Poverty Income Ratio; PUFA=polyunsaturated fatty acids; SEM=standard error of the mean.

[a] Models were further adjusted for other covariates (main effects and interaction with time). See methods section for more details on covariate coding and model specifications. Time at baseline visit was set to zero. Baseline age was centered at 50y, total energy intake at 2000kcal/d, total

carotenoid intake at 3mg/1,000kcal/d, vitamin C intake at 30mg/1,000kcal/d, vitamin A intake at 300 RE/1,000 kcal/d, vitamin E at 3 mg/1,000 kcal/d, vitamin B-6 at 0.8 mg/1,000 kcal/d, vitamin B-12 at 3 µg/1,000 kcal/d, folate at 170 µg/1,000 kcal/d. [b] N=number of participants in the analysis; N'=total number of visits included in the analysis.

Web Figure 7.


0
.5
1
1.5
2
0
5
10
15
Fish high in omega-3 fatty acids[1]
95% CI
n3:n6 PUFA
0
.1
.2
.3
.4
0
50
100
150
200
Discretionary oil[2]
95% CI
n3:n6 PUFA