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Antioxidant status and its association with elevated depressive symptoms among US adults: National Health and Nutrition Examination Surveys 2005–06

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Abstract

We examined the relationship of elevated depressive symptoms with antioxidant status. Cross-sectional data from the National Health and Nutrition Examination Surveys 2005–06 on US adults aged 20–85 years were analyzed. Depressive symptoms were measured using the Patient Health Questionnaire with a score cutpoint of 10 to define “elevated depressive symptoms”. Serum antioxidant status was measured by serum levels of carotenoids, retinol (free and retinyl esters), vitamin C and vitamin E. The main analyses consisted of multiple logistic and zero-inflated poisson regression models, taking into account sampling design complexity. The final sample consisted of 1,798 US adults with complete data. Higher total carotenoid serum level was associated with lower likelihood of elevated depressive symptoms with a reduction in the odds by 37% overall with each SD increase in exposure, and by 34% among women ($p < 0.05$). A dose-response relationship was observed when serum total carotenoids were expressed as quartiles [Q_4 (1.62–10.1 $\mu\text{mol/L}$) vs. Q_1 (0.06–0.86 $\mu\text{mol/L}$): OR=0.41; 95% CI: 0.23–0.76, $P < 0.001$; p -value for trend=0.035], though no significant associations were found with other antioxidant levels. Among carotenoids, β -carotene (men and women combined) and lutein+zeaxanthins (women only, after control for dietary lutein+zeaxanthin intake and supplement use) had an independent inverse association with elevated depressive symptoms among US adults. None of the other serum antioxidants had a significant association with depressive symptoms, independently of total carotenoids and other covariates. In conclusion, total carotenoids (mainly β -carotene and lutein+zeaxanthins) in serum were associated with reduced levels of depressive symptoms among community-dwelling US adults.

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Keywords

Depressive symptoms; antioxidants; carotenoids; adults

1. Introduction

Depressive symptoms are the most common outcomes of stress-induced reactions in humans (1) and have been studied in relation to a wide array of diseases and conditions including cardiovascular disease(2–3), cancer(4–5), type 2 diabetes mellitus(6–7) and allergic responses(8–9). Recent research has pointed to a link between oxidative stress and psychological stressors conducive to elevated depressive symptoms(10).

Dietary antioxidants include a number of micronutrients that were shown to reduce oxidative stress triggered by injury characterizing pathogenesis of many chronic diseases, including type 2 diabetes, CVD, rheumatological conditions, carcinogenesis(11). In fact, serum levels of antioxidants, reflected their dietary intakes based on several studies(12–13). One class of antioxidants, known as carotenoids are found primarily in fruits and vegetables (FV), while their secondary sources are bread, eggs, beverages, fats, and oils(14). Even though the human diet contains over 40 different types of carotenoids, only β -carotene, α -carotene, γ -cryptoxanthin, lycopene, lutein and zeaxanthin have detectable concentrations in human serum (14). Other micronutrients with well-established antioxidative properties include vitamin C, retinol (sparing pro-vitamin A) and vitamin E(15–17).

Evidence from basic experimental and epidemiological studies have triggered a number of hypotheses regarding the possible direction of association between serum antioxidant levels and depressive symptoms in humans as follows: (1) Depression is the outcome of low serum antioxidant status; (2) Antioxidant status is the outcome of poor diet resulting from depression; (3) Both depression and antioxidant status are markers of a third factor (e.g. a genetic variant), which makes them non-causally related. However, assuming the first scenario is true, one possible hypothesis (**Hypothesis A**) is that depressive symptoms caused by external stressors trigger oxidative stress which in turn causes reduced concentrations of certain antioxidants in serum, independently of dietary intake of those antioxidants(18–22). Another hypothesis (**Hypothesis B**) is that increased intake of antioxidants in the diet reduces oxidative stress by increasing serum antioxidant level(12–13), thereby reducing the incidence of elevated depressive symptoms by reducing lipid peroxidation in the brain(23–25). While both hypotheses are possible, **Hypothesis B** is more amenable to dietary intervention. In order to test **Hypothesis B**, it is important to allow dietary intakes of the specific antioxidant to vary while adjusting for other potentially confounding covariates. To test **Hypothesis A**, additional adjustment for dietary intake of antioxidants is required.

To date, however, few epidemiological studies have directly assessed the association between antioxidant status (or dietary intakes of antioxidants) and elevated depressive symptoms among adults(22, 26–32) and none so far have used nationally representative US data.

Our present study uses national data on US adults to examine the relationship of elevated depressive symptoms with antioxidant status. We hypothesize that there is an inverse association between serum antioxidant status and the number and severity of depressive symptoms among US adult men and women, that is partially accounted for by dietary intake of those antioxidants (**Hypotheses A and B**).

2. Materials and methods

2.1 Database and study subjects

The National Health and Nutrition Examination Surveys (NHANES) include a series of cross-sectional surveys providing nationally representative data on the nutrition and health status of the U.S. civilian population. The National Center for Health Statistics (NCHS), Centers for Disease Control (CDC), conducted three earlier waves of NHANES surveys (NHANES I, II, and III) in 1971–1975, 1976–1980, and 1988–1994, respectively. Since 1999, NHANES became a continuous survey. Using a stratified, multistage probability cluster sampling design, the NHANES survey consists of an in-home interview for demographic and basic health information and a health examination in a mobile examination center (MEC). Household interviews were conducted by trained staff and the MEC consists of physicians, medical and health technicians, and dietary and health interviewers. Detailed descriptions of the sample design, interview procedures, and physical examinations conducted were published elsewhere (33). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board of the CDC, NCHS. Written, or verbal informed consent was obtained from all subjects/patients. Verbal consent was witnessed and formally recorded. (34).

We analyzed NHANES data from adults aged 20–85 years from 2005–2006. Among a sample of 4,979 adult subjects (2,387 men and 2,592 women) with complete basic demographic data (**Sample 1**), 3,097 had complete serum antioxidant status and dietary data. Among those, complete data on other covariates of interest including physical activity (PA), smoking status, supplement use, weight and height, serum 25(OH)D, folate, vitamin B-12, homocysteine and total cholesterol were available for 2,859 participants (**Sample 2**). Among those in **Sample 2**, complete data on depressive symptoms was available for 1,798 participants (i.e. study sample: **Sample 3**). **Sample 3** differed from **Sample 1** on some basic demographic variables. Specifically, **Sample 3** participants were younger, more likely to be women, Non-Hispanic black or Mexican-Americans, with >High School level of education and to be current or former smokers compared to those selected only in **Sample 1**.

2.2. Outcome assessment

The questionnaire section of NHANES 2005–06 included the Patient Health Questionnaire (PHQ). This set of 10 questions reflects self-reported depressive signs and symptoms that are derived from the diagnostic and statistical manual (DSM) IV criteria. There are nine signs and symptoms in DSM IV that were scored between zero (not at all) and 3 (nearly every day), and an additional follow-up question to assess overall impairment ascribed to depressive symptoms. PHQ was validated in primary care settings and shown as a reliable tool for depression diagnosis (35–36). Summing scores on 10 PHQ items potentially yields a total score between 0 and 30. In our sample, the 90th percentile on total PHQ score corresponded to a value of 10. This cutoff point was similarly used elsewhere and had a sensitivity of 88% and a specificity of 88% for major depression.(35–36)

2.3. Exposure assessment

Using high performance liquid chromatography with photodiode array detection, serum concentrations of key antioxidants were measured. In this study, retinol and retinyl esters (defined as the sum of retinyl palmitate and retinyl stearate) were of interest separately and as their sum. Additionally, carotenoids grouped as α -carotene, β -carotene (cis+trans), γ -cryptoxanthin, lutein+zeaxanthin, total lycopene and total carotenoids were considered as exposures. Vitamin E was defined as the sum of α - and γ -tocopherol. Vitamin C was also

measured with high performance liquid chromatography with electrochemical detection.(37–38)

2.4. Covariates

Socio-demographic covariates included age, sex, race/ethnicity, education, marital status and family income, measured by the poverty income ratio (PIR<100% of the poverty line, 100%–<200%, 200%). An objective measure for physical activity was constructed based on individual leisure-time activities with an intensity score assessed by metabolic equivalent (MET) multiplied by duration of the activity and frequency converted to per week unit. This MET×hr/week value was summed for each subject depending on the number of leisure-time physical activities elicited, with participants not eliciting any activity being considered as sedentary (score=0)(39–41). This continuous score was categorized as “0–<5”; “5–10”; “>10”, in the main part of the analysis. Cigarette smoking status was defined as never, former or current smoker. All NHANES participants were eligible for two 24 hr recalls in the 2005–06 wave. The first one was administered during MEC exams and the second 3–10 days later by telephone interview. The average of two 24 hr recalls was examined from which nutrient intakes were estimated using a revised nutrient database(42). Total dietary intakes of alcohol, selected antioxidants (α -carotene, β -carotene, β -cryptoxanthin, lutein +zeaxanthin, lycopene, total carotenoids (i.e. sum of previously listed micronutrients in $\mu\text{g}/\text{d}$), vitamin C and vitamin E in mg/d) and *n*-3 polyunsaturated fatty acids (*n*-3 PUFA, sum of DHA+EPA+*n*-3 DPA; (C 20), in g/d) were considered as potential confounders, given their putative associations with elevated depressive symptoms(26, 43–45). Moreover, total energy intake was added into statistical models as a covariate to obtain energy-adjusted associations between nutrients and outcome. Using another database to obtain mypyramid equivalents (46) of each food group, cup equivalents of fruits and vegetables were estimated per individual and averaged out between the two 24-hr recalls. Participants with only one 24-hr recall were excluded in the final analysis. A general measure of dietary supplement use over the past 30 days was computed as follows: 0: non-users; 1: using one supplement; 2: using two supplements or more. Another measure of anti-depressant use was obtained from the medication data file with first level category being “242” and second-level category being “249”, using Multum’s therapeutic classification system(47). The final variable was coded as “having used an anti-depressant” (1=yes; 0=no) over the past 30 days.

Serum folate and vitamin B-12 were measured by Bio-Rad Laboratories “Quantaphase II Folate” radioassay kit(48–49). tHcy in serum was measured using “Abbott Homocysteine (HCY) assay”, a fully automated technique (50–51). Serum 25(OH)D was measured by radioimmunoassay (Diasorin Inc., Stillwater, Minnesota)(52). Those nutritional biomarkers were associated with mood disorders and cognitive function in a number of previous studies(53–69).

Moreover, because depressive symptoms are usually increased in number and severity in the presence of co-morbid chronic conditions, we created three measures using self-reports for history of “type 2 diabetes”, “cardiovascular disease” (i.e. congestive heart failure, coronary heart disease, angina, heart attack or stroke) and “cancer of any type”. Finally, many of the lipophilic vitamins are highly correlated with serum lipids and thus total cholesterol was also adjusted for in the analysis as was done in a previous study(70).

2.5. Statistical Analysis

Using Stata release 11.0(71), we first described NHANES 2005–06 study sample characteristics by sex and elevated depressive symptoms status. Differences in means across groups and associations between categorical variables were tested with *t*- and χ^2 tests, respectively. Second, we used Pearson’s correlations to examine associations between Log_e

transformed serum antioxidant variables, first between each others and second with selected dietary factors and the continuous PHQ score. Partial correlations were additionally conducted to examine the degree to which the association between serum antioxidants and the corresponding dietary antioxidant is explained by dietary intakes of fruits and vegetables and by dietary supplement use. A full multiple regression model of covariates and dietary factors as predictors of serum antioxidants has been published elsewhere using NHANES 2001–06 data(70). Third, multiple logistic regression models were conducted to test associations between antioxidant status (standardized z-scores computed from the total NHANES sample aged 20–85y with available antioxidant data) and “elevated depressive symptoms”, controlling for potential confounders (i.e. all covariates as described earlier, entered simultaneously) and stratifying by sex, as was done in a previous study(69). These associations were tested for depressive symptoms count score (range: 0–30) as well using multiple zero-inflated Poisson (ZIP) regression models (72). The choice of the ZIP model over poisson and negative binomial models was based on a previous similar analysis that compared the three models(73). Two sets of models were carried out to test **Hypotheses A** and **B**, as described earlier. One set controlled for all factors except for dietary antioxidants while another set, in addition to all factors, controlled for dietary antioxidants. Models were compared between **Hypotheses B** and **A**, in terms of change-in-estimate(74), when dietary antioxidants and supplement use were entered into the model.

To test dose-response relationships, quartiles of main antioxidants (Q_2 , Q_3 , Q_4 vs. Q_1) were entered into multiple logistic regression models and p-values for trend were computed. In all analyses, sampling design complexity was taken into account and adequate sampling weights, strata and primary sampling units were specified using Stata survey commands. Two-year MEC exam weights were used for most sample estimations and masked variance units were used to estimate variances using the Taylor series linearization method(75).

To additionally account for potential selection bias in all main analyses (logistic and zero-inflated poisson regression models), given missing data on the outcome variable compared to the MEC exam sample, we constructed a two-stage Heckman selection model(76), as was done previously in another study using a similar sample(69). To this end, a probit model was conducted at first in which the main selection variable (i.e. belonging to **Sample 3** vs. not, among those in **Sample 1**) were modeled against basic sociodemographic variables that were pseudo-complete for the total NHANES adult sample (i.e. **Sample 1**). These variables included age, sex, race/ethnicity, marital status, education, poverty income ratio and smoking status. A dummy variable for missing data on those variables was included. From the probit model, the conditional probability of being selected was predicted from which an inverse mills ratio, a function of that predicted probability, was computed and entered at a second stage as a covariate into the main statistical models(77).

In addition, confounding effects of various covariates included in the model were explored using a change-in-estimate type of analysis, in which all main exposures were retained in the model while other covariates were backward eliminated sequentially from each model. The effect of eliminating each covariate on the main effects of interest was assessed accordingly by examining the % change in the estimate of OR associated with each exposure when each covariate was eliminated(78). This analysis, however, did not take design complexity into account.

To test for sex differences in main associations, multiple logistic and ZIP regression models were re-ran in non-stratified model, adding a main effect for sex and an interaction term for each of the antioxidant exposure with sex. Sex difference in the association between each serum antioxidant and PHQ was deemed significant if the interaction term was significant at a level of 0.10, given the lower power of interaction terms compared to main effects (74).

All other p-values presented were 2-tailed with $p < 0.05$ was considered statistically significant and $p < 0.10$ considered as marginally significant, before correction for multiple testing. Multiple testing correction was done by considering a p -value < 0.001 as statistically significant when comparing one quartile of a predictor to the referent category (e.g. Q_4 vs. Q_1) or examining standardized predictor variables in relation to the outcome (i.e. per SD). The same cutoff was applied to the p -value for trend and p -value for interaction.

3. Results

3.1. Study sample characteristics

Table 1 presents the distribution of sample characteristics by sex and depressive symptoms status based on PHQ score. Mean PHQ score was significantly higher among women compared to men (4.5 ± 0.2 vs. 3.9 ± 0.2 , $p < 0.05$). Participants with elevated depressive symptoms were generally less educated, more likely to belong to $PIR < 100\%$ category, be unmarried, less physically active and had higher proportion with history of cancer. Women with elevated depressive symptoms were more likely to be current smokers and had a higher mean BMI. Men and women with elevated depressive symptoms had consistently lower serum levels of β -carotene and total carotenoids combined compared to their non-depressed counterparts and were more likely to use anti-depressants. Women with elevated depressive symptoms had lower serum levels of vitamin E and C as well as all carotenoids compared to their non-depressed counterparts. They additionally reported lower intakes of β -carotene, α -cryptoxanthin, lutein+zeaxanthin, total carotenoids, vitamin C, vitamin E, *n*-3 PUFA and lower consumption of fruits and vegetables. Moreover, they exhibited lower serum folate and 25-hydroxyvitamin D status. Men with elevated depressive symptoms had higher mean total homocysteine compared to their non-depressed counterparts. Gender differences in dietary intakes and other covariates were also noted, including in intakes of carotenoids, vitamin E, *n*-3 PUFA, anti-depressant use, dietary supplement use, consumption of fruits and vegetables as well as serum levels of folate and tHcy.

3.2. Correlation between key study variables

Pearson's correlation coefficients between Log_e transformed serum antioxidant status variables (**I.** between each others; **II.** With other dietary variables and PHQ continuous score) are presented in Table 2. For analysis **I.**, correlation coefficients between serum antioxidant variables ranged between < 0.10 and non-significant (e.g. Lycopene and retinol +retinyl esters), and > 0.70 (e.g. β -carotene and α -carotene). For analysis **II.**, most correlations were weak to moderate (< 0.10 – 0.40) with the strongest coefficients being 0.42 (supplement use vs. serum vitamin E). As expected, PHQ score was inversely related to most serum antioxidant variables. Partial correlations of serum antioxidants with their corresponding dietary intake variable, controlling for fruits and vegetable consumption and supplement use indicated that both sources were important in explaining those correlations, with partial correlation being attenuated by 14% (retinol) to 77% (vitamin E).

3.3. Antioxidant status and depressive symptoms

Findings from a series of multiple logistic and ZIP models examining associations between selected serum antioxidant status variables and depressive symptoms (measured by PHQ) are presented in Table 3. In all presented models, dietary antioxidants and supplement use were not among the covariates that were adjusted for. In a first series of models (**Model 1**), serum carotenoids, retinol, retinyl esters, vitamins C and E were entered separately as predictors for depressive symptoms along with potential confounding covariates. Significant inverse associations were found between elevated depressive symptoms and three different carotenoids (β -carotene (all, women), lutein+zeaxanthin (all, women) and lycopene (all)) and with retinyl esters in women only. Among those, only β -carotene remained significantly

inversely related to elevated depressive symptoms after correction for multiple testing (all: OR=0.52, 95% CI: 0.39–0.70 $p<0.001$).

Similar patterns of association were found when PHQ count (**Model 1**) was the outcome in the series of ZIP models, though only the inverse relationship between retinyl esters and PHQ count was deemed significant after correction for multiple testing, in both the total population and among women.

In **model 2**, when all antioxidants were entered into the same model simultaneously along with potential confounding covariates, some of the previously observed inverse associations remained significant (though slightly attenuated) but not others. In particular, while the association between lycopene and elevated depressive symptoms became non-significant, the significance of the inverse association was retained for β -carotene (all, women), while β -cryptoxanthin became associated with elevated depressive symptoms in the total population (OR=1.31; 95% CI: 1.10–1.56). In ZIP models, an inverse relationship between lutein +zeaxanthin and PHQ count score was found only among women. In addition, those models indicated that retinyl esters were inversely related to the PHQ count score among both men and women. None of these associations were deemed statistically significant when correcting for multiple testing ($p>0.001$).

In **model 3**, all antioxidants were similarly entered simultaneously while combining total carotenoids and retinol+retinyl esters together as two main exposures and adjusting for the same covariates as in **model 1**. In logistic regression models, among both sexes combined and in women, there was an inverse association between total carotenoid level and elevated depressive symptoms with a reduction in the odds by 38% overall and 32% in women ($p<0.001$). In ZIP models, this inverse association was observed only in both sexes combined, but did not reach significant after correction for multiple testing ($p>0.001$). None of the other antioxidants had a significant association with depressive symptoms independently of total carotenoids and other covariates. Although some of the associations were found to be significant in women and not in men, it is worth noting that sex differences as tested in a separate model with interaction terms were not statistically significant at a 0.10 level.

In Table 4 models, when additional control was made on dietary antioxidants and supplement use, most of the findings remained consistent with those of Table 3 corresponding models, even after correction for multiple testing.

In order to further examine dose-response relationships between antioxidant status and elevated depressive symptoms, quartiles of main exposures were used and p-value for trend was obtained (Figure 1) for men and women combined. This particular analysis was uncontrolled for dietary antioxidants or supplement use. Among all serum antioxidant exposures entered into the model, only total carotenoids showed an apparent linear dose-response relationship with a p-value for trend of 0.035 and the OR for elevated depressive symptoms and quartiles of carotenoids was only significant when comparing Q₄ (1.62–10.1 $\mu\text{mol/L}$) with Q₁ (0.06–0.86 $\mu\text{mol/L}$) [OR=0.41; 95% CI: 0.23–0.76; $p<0.001$], suggesting a possible threshold of 1.62 $\mu\text{mol/L}$ before a significant association can be detected between serum carotenoids and elevated depressive symptoms. When dietary antioxidants and supplement use were introduced into the same model (Figure 2), the results remained similar, with a p-value for trend of 0.041 for serum total carotenoids expressed as quartiles. However, linear dose-response was no longer significant in both Figures 1 and 2 when taking multiple testing into account ($p\text{-value for trend}>0.001$). A sensitivity analysis that additionally controlled for employment status and medical insurance coverage resulted in a slight attenuation of effects though the OR for Q₄ vs. Q₁ for total carotenoids in relation

to elevated depressive symptoms remained statistically significant in both Figures 1 and 2 analyses ($p=0.02$). Another sensitivity analysis in which subjects with inadequate dietary intakes of antioxidants were excluded did not alter our findings appreciably.

Potential confounding by various covariates entered into the main statistical models, particularly logistic regression model of Figure 2, was assessed using a change-in-estimate analysis, which did not take into account sampling design complexity (data not shown). Our findings suggest that when covariates aside from main antioxidant exposures were backward eliminated (Figure 2 model, with antioxidant quartiles entered as an ordinal variable to assess dose-response), change-in-estimate of the OR did not exceed 10% (for serum total carotenoids as a covariate, when main exposure was serum vitamin C), suggesting weak confounding effect exerted by each of the other antioxidants and the remaining covariates in the model. More notably, dietary antioxidants did not act as significant confounders in the association between serum antioxidants and elevated depressive symptoms.

4. Discussion

Our present study used national data on US adults to examine the relationship of elevated depressive symptoms with antioxidant status as measured by serum levels of carotenoids, retinol (free and retinyl esters), vitamin C and vitamin E. There were several key findings. First, we found an inverse association between serum total carotenoid level and elevated depressive symptoms with a reduction in the odds by 38% overall with each SD increase in exposure, and by 32% among women ($p<0.05$). Second, we found a dose-response relationship when total carotenoids were expressed as quartiles [$Q_4(1.62-10.1 \mu\text{mol/L})$ vs. $Q_1(0.06-0.86 \mu\text{mol/L})$: OR=0.41; 95% CI: 0.23–0.76, $P<0.001$; p -value for trend=0.035], though no significant associations were found with other antioxidant levels. Third, among carotenoids, β -carotene (both sexes combined) and lutein+zeaxanthins (among women, after control for dietary intake and supplement use) were associated inversely with elevated depressive symptoms among US adults.

To date, only a handful of studies have evaluated the role played by antioxidants in depressive symptoms (22, 26, 30, 32) or depression (22, 26–28, 30–31). The majority of those studies focused on elderly populations (26, 30–32) and the most frequently studied antioxidant was vitamin E (26–28, 30–31). Moreover, one study (26) examined carotene and vitamin C in relation to depressive symptoms, another focused on total serum carotenoids(32), while a third study (22) tested several antioxidants simultaneously. In a case-control study, Maes *et al.* (27) found significantly lower serum levels of vitamin E among 42 patients with major depressive disorder compared to 26 healthy volunteers. Owen *et al.* measured both serum and dietary levels of vitamin E among depressed adults based on Beck Depression Inventory. Their results suggested no significant association between dietary level of vitamin E and major depression, but a significantly lower serum vitamin E level among depressed adults compared to the general population (28). Shibata *et al.* used the short version of the Geriatric Depression Scale to assess cross-sectional and longitudinal effects of blood levels of vitamin E on depressive status among 504 elderly residents of a rural community. While cross-sectional analysis revealed no significant findings, longitudinal analysis showed a protective effect of vitamin E on depressive status among men only, after adjustment for age, education and baseline GDS score (31). In the Rotterdam study, Tiemeir *et al.* investigated the association of blood vitamin E level with depressive symptoms as determined by the Center for Epidemiologic Studies Depression (CES-D) scale, comparing 262 cases to 459 randomly selected controls. After adjustment for biological and behavioral factors, no significant associations were noted between blood vitamin E level and depressive symptoms or depression among elderly men and women (30). Oishi *et al.* conducted a cross-sectional study of 279 community-dwelling Japanese

elderly persons to evaluate dietary factors, including several antioxidants, in relation to depressive symptoms measured using the CES-D. Among males, the observed odds ratios (the 95% confidence intervals) for the depressive state were 0.36 (95% CI: 0.13–0.98) in the highest tertile of carotene intake, 0.33 (95% CI: 0.12–0.93) in the highest tertile of vitamin C intake and 0.33 (95% CI: 0.12–0.92) in the medium tertile of vitamin E intake (26). Among females, similar results were observed, but these results were not statistically significant (26). Recently, a longitudinal study using InChianti data found a clear inverse relationship between serum total carotenoids at baseline and incidence of elevated depressive symptoms after a 6yr follow-up period (OR=0.72, 95% CI=0.52–0.99, P = 0.04), adjusting for important confounders. This association was shown to be partially mediated by inflammatory markers such as interleukin-1 receptor antagonist(32).

Our findings of an association between serum total carotenoids, α -carotene and lutein +zeaxanthin and elevated depressive symptoms are similar to that study(32), and unlike the study by Oishi *et al.* (26), we did not observe a strong association between depressive symptoms and dietary carotenoids. In addition, while our unadjusted analyses indicated that depressed women had lower levels of vitamins C and E compared to non-depressed women, this association became null after adjustment for potentially confounding factors and other serum antioxidants. These findings indicate that only total carotenoids are associated with a reduced risk of depression especially after reaching a threshold corresponding to the uppermost quartile in our sample ($>1.62 \mu\text{mol/L}$). Since this effect was not attenuated by dietary or supplemental intake, this suggests that **hypothesis A** is more likely than **hypothesis B**. In other words, depressive symptoms caused by external stressors (10) trigger oxidative stress which in turn causes reduced concentrations of total carotenoids in serum, independently of dietary intake of this class of antioxidants. The differential effect of total carotenoids as opposed to vitamins C and E should be studied further. Nevertheless, reverse causation whereby lower carotenoid levels among depressed individuals is due to poor diet can only be ruled out in a longitudinal study.

The biological mechanisms behind the putative causal association between antioxidant status and depression in general can be outlined as follows: the brain is considered particularly vulnerable to oxidative stress due to its high oxygen consumption, its modest antioxidant defenses, and its lipid-rich constitution (79–80). The brain is also susceptible to secondary and self-perpetuating damage from oxidative cellular injury via the neurotoxic effects of released excitatory amines (mainly glutamate) and iron, and the activated inflammatory response(79). Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to scavenge those species with various antioxidants, has been implicated in the pathogenesis of many chronic diseases, including type 2 diabetes mellitus, cardiovascular disease, rheumatologic disorders and carcinogenesis(11). Oxidative stress induces damage to DNA and cell membranes in both animal experiments(17) and human epidemiological studies(81). Many studies found evidence of an increase in ROS with age and their deleterious effects on lipids, especially polyunsaturated fatty acids. The increase in lipid peroxidation affects the oxidation of structurally important proteins disrupting transmembrane ion movements and cellular metabolic processes(82–83), the most notable one of which is brain synaptic function.

Furthermore, oxidative damage may cause an autoimmune response by changing the chemical structures of otherwise ubiquitous molecules to generate a variety of new, highly immunogenic epitopes (84). Overall, these oxidative insults lead to a decrease in membrane fluidity, an inactivation of enzymes, ion channels and receptors, and as a result, alterations of neurotransmission, neuronal function and general brain activity(82–83).

In animal studies, adding immobilization stress on rats triggered increased levels of lipid peroxidation and a weakened endogenous antioxidant system in plasma (18–19). In mice, adding another form of stressor, a communication box paradigm with electric stress, was shown to increase lipid peroxidation activity in the brain (20). Those stress-induced changes in lipid peroxidation and levels of antioxidants have also been shown recently in humans(21–22).

Our study has notable strengths, which include its selection of a large nationally representative sample, the collection of antioxidant status biomarker data and use of a validated questionnaire for assessment of depressive symptoms. However, our study also suffers from some limitations. First, although use of dietary supplements over a 30-days period was considered a proxy or crude measure for individual micronutrient supplementation, it was previously shown to be directly associated with each of the serum antioxidant status measures, with a clear dose-response relationship, independently of socio-demographic, lifestyle and health-related factors(70). Second, because of the study's cross-sectional design, it was not possible to make causal inference through ascertaining temporality of associations. Hence, we were not able to ascertain the temporality of the associations between serum antioxidants, depressive symptoms and oxidative stress, though it was clear that serum carotenoids were a direct reflection of an increased consumption of fruits and vegetables as well as dietary supplement use. Lower intake of fruits, vegetables and fibers may mediate the relationship between lower carotenoid levels and depression. Indeed, depression is directly associated with reduction in dietary quality, thus reduction in the consumption of antioxidants. (70) Finally, residual confounding by unmeasured covariates cannot be totally discounted.

In conclusion, we found a relation between carotenoids and depressive symptoms with some dose-response. Further investigation is needed through RCTs and longitudinal studies to examine the nature of the temporality of the relation. Currently, there is limited evidence to support the role of antioxidants in general and carotenoids in particular for the prevention of depression or depressive symptoms. Thus, additional intervention research is needed before making any prescriptive or policy recommendations for antioxidants in prevention of depression.

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ABBREVIATIONS

CES-D	Center for Epidemiologic Studies Depression
CVD	cardiovascular disease
DEP	Depressive symptoms
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DSM	Diagnostic and Statistical Manual
EPA	eicosapentaenoic acid
GSH	plasma-reduced glutathione
GSSG	glutathione disulfide

MEC	Mobile examination center
n-3 PUFA	n-3 polyunsaturated fatty acids
NHANES	National Health and Nutrition Examination Survey
PHQ	Patient Health Questionnaire
ROS	Reactive oxygen species
SEM	Standard error of the mean
SEP	Standard Error of the Proportion
tHcy	Total homocysteine

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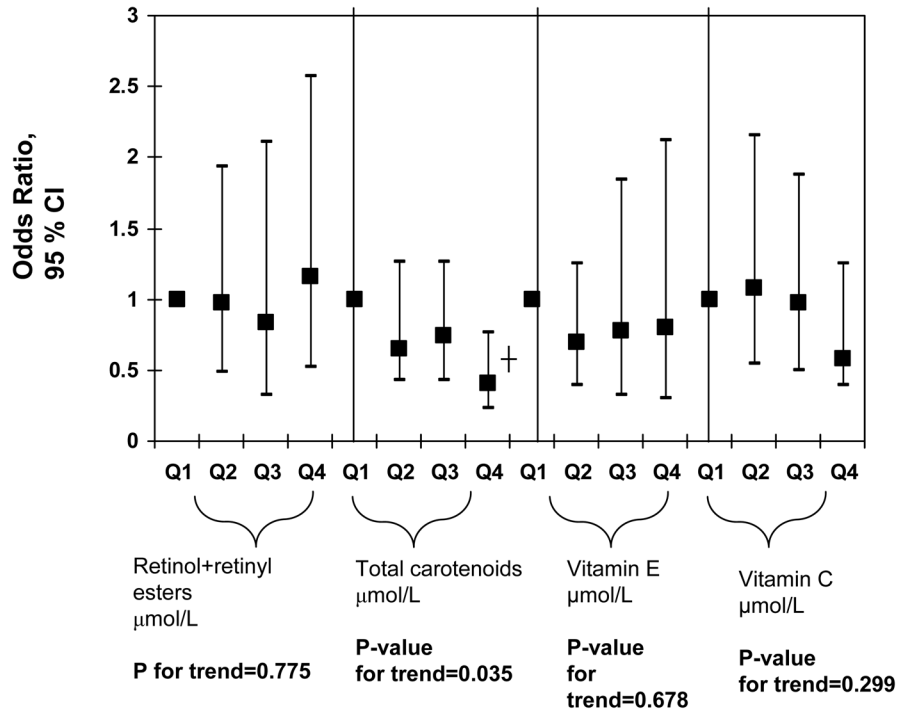


FIGURE 1. Adjusted odds ratios (with 95% CI) of major serum antioxidant level (expressed as quartiles, Q₂, Q₃, Q₄ vs. Q₁) and elevated depressive symptoms among US adults, uncontrolled for dietary antioxidant intakes or supplement use; NHANES 2005–06

Notes: CI=Confidence Interval. Ranges for each antioxidant quartile is as follows in µmol/L: Retinol+retinyl esters (Q₁: 0.07–1.7; Q₂: 1.7–2.1; Q₃: 2.1–2.5; Q₄: 2.5–8.9); Total carotenoids (Q₁: 0.06–0.86; Q₂: 0.86–1.18; Q₃: 1.18–1.62; Q₄: 1.62–10.1); Vitamin E (Q₁: 0.2–26.7; Q₂: 21.7–27.3; Q₃: 27.4–35.9; Q₄: 35.9–303.8); Vitamin C (Q₁: 0.6–34.6; Q₂: 35.2–54.5; Q₃: 55.1–70.4; Q₄: 71.0–274.2). Analyses were based on multiple logistic regression models that included all antioxidant exposures simultaneously adjusted for socio-demographic factors: Lifestyle and health-related factors (smoking status, BMI, physical activity: Mets.hr.wk⁻¹, recoded as “0–<5”; “5–10”; “>10”, history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)), anti-depressant use and dietary intakes (total energy intake, alcohol, *n*-3 PUFA), serum levels folate, total homocysteine, vitamin B-12, 25-hydroxyvitamin D and serum total cholesterol, anti-depressant use, and the inverse mills ratio, 2-stage Heckman selection model.

*P<0.05; P<0.001 for null hypothesis that Log_e(OR)=0.

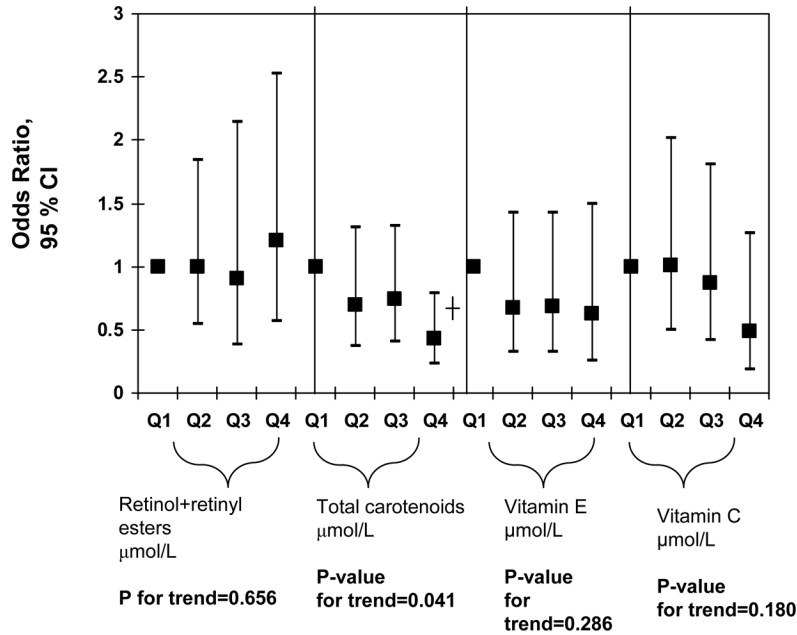


FIGURE 2.

Adjusted odds ratios (with 95% CI) of major serum antioxidant level (expressed as quartiles, Q₂, Q₃, Q₄ vs. Q₁) and elevated depressive symptoms among US adults, controlled for dietary antioxidant intakes and supplement use; NHANES 2005–06

Notes: CI=Confidence Interval. Ranges for each antioxidant quartile is as follows in µmol/L: Retinol+retinyl esters (Q₁: 0.07–1.7; Q₂: 1.7–2.1; Q₃: 2.1–2.5; Q₄: 2.5–8.9); Total carotenoids (Q₁: 0.06–0.86; Q₂: 0.86–1.18; Q₃: 1.18–1.62; Q₄: 1.62–10.1); Vitamin E (Q₁: 0.2–26.7; Q₂: 21.7–27.3; Q₃: 27.4–35.9; Q₄: 35.9–303.8); Vitamin C (Q₁: 0.6–34.6; Q₂: 35.2–54.5; Q₃: 55.1–70.4; Q₄: 71.0–274.2). Analyses were based on multiple logistic regression models that included all antioxidant exposures simultaneously adjusted for socio-demographic factors: age, sex, race/ethnicity, marital status, educational level and poverty income ratio, and other potential confounders: Lifestyle and health-related factors (smoking status, BMI, physical activity: Mets.hr.wk⁻¹, recoded as “0–<5”; “5–10”; “>10”, history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)) and dietary intakes (total energy intake, alcohol, dietary antioxidant (or group of antioxidants), n-3 PUFA, dietary supplement use), serum levels folate, total homocysteine, vitamin B-12, 25-hydroxyvitamin D and serum total cholesterol, anti-depressant use, and the inverse mills ratio, 2-stage Heckman selection model.

*P<0.05 P<0.001 for null hypothesis that Log_e(OR)=0.

TABLE 1

Selected baseline characteristics of NHANES 2005–06 participants by sex and “elevated depressive symptoms” status ($n=1,798$)^a

	Men						Women						<i>p</i> ^b		
	PHQ score <10 (<i>n</i> =675)		PHQ score 10 (<i>n</i> =75)		All men (<i>n</i> =750)		PHQ score <10 (<i>n</i> =928)		PHQ score 10 (<i>n</i> =120)		All Women (<i>n</i> =1,048)				Men vs. women
	Mean or %	SEM/SEP	Mean or %	SEM/SEP	Mean or %	SEM/SEP	Mean or %	SEM/SEP	Mean or %	SEM/SEP	Mean or %	SEM/SEP			
Socio-demographic, lifestyle factors															
Age (y)	45.5	1.0	46.3	2.6	45.5	1.0	47.3	1.0	45.7	1.8	47.1	1.0	0.011	0.759	0.447
Race/ethnicity, %															
Non-Hispanic White	74.9	3.1	68.3	5.7	74.3	73.1	73	2.8	65.7	4.9	72.2	2.8	0.269	0.458	0.27
Non-Hispanic black	8.6	1.5	13.9	3.2	9	1.5	11	2.1	14.5	3.8	11.4	2.1			
Mexican-American	8.3	1.4	7.5	3.2	8.2	1.4	6.9	0.8	8.4	1.9	7.1	0.8			
Other ethnicity	8.1	1.8	10.3	4.4	8.3	1.6	9.1	1.8	11.4	3.1	9.3	1.8			
Married, %	61.8	2.2	46.9	6.4	60.6	2.3	57.2	2.6	45.5	4.3	56	2.5	0.048	0.024	0.019
Education, %															
<High School	6.0	0.8	7.6	2.6	6.1	0.7	4.7	0.7	7.3	1.7	4.9	0.7	0.067	0.036	<0.001
High School	34.8	2.3	47.5	5.3	35.9	2.3	31.3	2.2	50.7	45.1	33.4	2.2			
>High School	59.2	2.4	44.9	4.7	58	2.4	64.1	2.6	41.9	4.4	61.6	2.5			
Poverty Income Ratio, %															
<100%	8.7	1.4	16.1	4.4	9.3	1.4	10	1.1	26.3	4.7	11.8	1.2	0.14	0.043	<0.001
100%–<200%	18.6	2.3	24.3	4.8	19.1	2.3	20.7	1.8	29.6	5.2	21.7	1.9			
200%	72.7	2.7	59.7	6.7	71.6	2.8	69.3	2.7	44.1	5.6	66.6	2.7			
Smoking status, %															
Never smoker	42	2.7	27.9	10.6	40.8	2.9	57.1	2.5	45.4	5.6	55.9	2.4	<0.001	0.271	0.018
Former smoker	30	1.5	32.9	7.1	30.3	1.7	22.4	1.9	19.1	5.3	22.1	1.9			
Current smoker	27.9	2.4	39.2	9.8	28.9	2.6	20.4	1.8	35.4	4.5	22.1	1.9			
Physical activity, <i>Mets.br.wk⁻¹</i>	7.8	0.6	4.0	0.7	7.5	0.6	6.1	0.5	4.2	0.9	5.9	0.4	0.001	0.033	0.045
Body mass index, <i>kg.m⁻²</i>	28.7	0.3	29.1	1.2	28.7	0.3	28.3	0.3	30.8	1.0	28.6	0.3	0.167	0.846	0.039
History of selected chronic conditions															
Type 2 diabetes, %	7.4	1.1	12.6	3.5	7.9	1.0	8.6	1.1	9.7	2.5	8.8	1.1	0.613	0.154	0.656
Cardiovascular disease, %	7.4	0.8	9.7	3.0	7.6	0.7	7.9	1.2	12.1	3.6	8.4	1.3	0.548	0.434	0.125
Cancer, %	4.7	1.0	16.7	4.3	5.8	0.9	9.5	1.1	11.8	3.9	9.7	0.9	0.002	0.004	0.582
Antioxidant status															
Retinol, <i>μmol/L</i>	2.23	0.03	2.4	0.08	2.24	0.03	2.00	0.04	1.9	0.08	1.99	0.03	<0.001	<0.001	0.835
Serum retinyl esters, <i>μmol/L</i>	0.121	0.003	0.106	0.009	0.120	0.003	0.116	0.004	0.08	0.004	0.112	0.003	0.075	0.531	<0.001
Total retinol+retinyl esters, <i>μmol/L</i>	2.35	0.03	2.51	0.08	2.36	0.03	2.12	0.04	1.98	0.04	2.1	0.04	<0.001	0.001	0.5

	Men						Women						<i>p</i>	
	PHQ score <10 (n=675)			All men (n=750)			PHQ score <10 (n=928)			All Women (n=1,048)			Men vs. women	PHQ <10 vs. PHQ <10 (women)
	Mean or %	SEM/SEP	PHQ score 10 (n=75)	Mean or %	SEM/SEP	PHQ score <10 (n=928)	Mean or %	SEM/SEP	PHQ score 10 (n=120)	Mean or %	SEM/SEP	PHQ <10 vs. PHQ <10 (men)	PHQ <10 vs. PHQ <10 (women)	
-Carotene, $\mu\text{mol/L}$	0.07	0.01	0.04	0.00	0.07	0.11	0.09	0.06	0.00	0.11	0.01	<0.001	0.065	0.001
-Carotene, $\mu\text{mol/L}$	0.3	0.01	0.19	0.02	0.29	0.51	0.03	0.24	0.02	0.48	0.01	<0.001	0.003	<0.001
-Cryptoxanthin, $\mu\text{mol/L}$	0.17	0.01	0.15	0.02	0.17	0.19	0.01	0.14	0.02	0.18	0.01	0.001	0.135	0.045
Lutein+zeaxanthin, $\mu\text{mol/L}$	0.28	0.01	0.24	0.02	0.28	0.24	0.02	0.31	0.01	0.3	0.01	0.091	0.067	<0.001
Lycopene, $\mu\text{mol/L}$	0.48	0.02	0.41	0.02	0.47	0.44	0.01	0.39	0.02	0.43	0.01	0.04	0.063	0.009
Total carotenoids, $\mu\text{mol/L}$	1.3	0.03	1.03	0.07	1.28	1.56	0.05	1.05	0.04	1.5	0.04	<0.001	0.006	<0.001
Vitamin E, $\mu\text{mol/L}$	28.9	0.4	28.4	2.0	28.8	30.4	0.7	26.3	1.5	30	0.6	0.059	0.742	0.005
Vitamin C, $\mu\text{mol/L}$	48.3	1.1	46.1	5.2	48.2	60.3	1.7	42.9	3.8	58.4	1.6	<0.001	0.413	<0.001
Dietary Intake														
Total Energy Intake, <i>MJ/d</i>	11.74	0.28	11.44	0.82	11.71	8.03	0.11	6.94	0.3	7.54	0.11	<0.001	0.849	0.073
Alcohol intake, <i>g/d</i>	15.3	1.7	20.6	7.0	15.8	5.6	0.9	3.7	1.1	5.4	0.8	<0.001	0.535	0.535
-carotene, $\mu\text{g/d}$	442.9	52.7	296	70.7	430.3	443.9	31.0	266.2	45.8	424.3	29.1	0.813	0.83	0.207
-carotene, $\mu\text{g/d}$	2190.2	160.2	1535.7	156.9	2134.2	2364.5	120.7	1368.4	258.1	2254.8	117.5	0.715	0.577	0.004
-Cryptoxanthin, $\mu\text{g/d}$	137.9	10.2	156	43.1	139.4	123.9	5.3	77.8	10.6	118.8	4.9	0.335	0.979	0.008
Lutein+zeaxanthin, $\mu\text{g/d}$	1414.7	139.4	1203.3	145.6	1396	1603.4	88.4	1001.2	158.3	1537.1	80.8	0.35	0.724	0.031
Lycopene, $\mu\text{g/d}$	6520.8	474.9	6180.8	1349	6491.7	5039.9	320.6	4253.6	1134	4953.3	311	<0.001	0.596	0.628
Total carotenoids, $\mu\text{g/d}$	12453	727	10611	1471	12296	11496	538	8069	1592	11118	553	0.017	0.471	0.013
vitamin C, <i>mg/d</i>	91.6	4.0	101.4	13.7	92.4	82.7	2.6	67.1	7.6	81	2.6	0.07	0.353	0.039
vitamin E, <i>mg/d</i>	8.6	0.3	6.9	0.6	8.4	6.9	0.1	5.5	0.4	6.7	0.1	<0.001	0.147	0.002
n-3 PUFA, <i>g/d</i>	0.19	0.01	0.14	0.03	0.19	0.13	0.01	0.06	0.01	0.13	0.01	<0.001	0.366	0.031
Dietary supplement use, past 30 d														
None	43.7	1.9	37.9	6.6	43.2	32.1	2.3	41.5	5.7	33.1	2.4	<0.001	0.326	0.147
One	30	2.1	28.8	5.6	29.9	28.3	1.0	25.8	4.1	28	1.1			
Two or more	26.3	1.9	33.3	3.4	26.9	39.6	2.6	32.7	5.6	38.8	2.6			
Fruits and vegetable intake, cup equivalent/d	2.78	0.09	2.63	0.22	2.77	2.5	0.1	1.88	0.13	2.43±	0.1	0.003	0.502	<0.001
Anti-depressant medication use, % yes	6.8	1.2	18	4.4	7.7	15.9	0.8	38.6	6.4	18.4	1.0	<0.001	0.005	<0.001
Folate, B-12, tHcy and 25-hydroxyvitamin D in serum														
Folate, <i>nmol/L</i>	27.7	0.7	26.2	2.1	27.6	33.9	1.0	25.5	1.6	33	0.9	<0.001	0.505	<0.001
Vitamin B-12, <i>pmol/L</i>	376.4	10.9	356.9	31.3	374.8	393.7	9.6	377.4	24.7	391.9	8.6	0.137	0.384	0.804
Total homocysteine, $\mu\text{mol/L}$	8.9	0.2	10.1	0.5	8.9	7.6	0.2	8.3	0.5	7.7	0.2	<0.001	0.03	0.106
25-hydroxyvitamin D, <i>ng/mL</i>	21.8	0.5	20.9	0.9	21.2	21.8	0.5	18.3	0.9	21.4	0.4	0.269	0.792	<0.001

Abbreviations: DEP=Depressive symptoms; n-3 PUFA=n-3 highly unsaturated fatty acids; NHANES=National Health and Nutrition Examination Survey; PHQ=Patient Health Questionnaire; SEM=Standard error of the mean; SEP=Standard Error of the Proportion; tHcy=Total homocysteine.

^aValues are mean±SEM or percent±SEP. Sampling design complexity is taken into account in all analyses. This analysis is done among participants with complete data for PHQ and other key variables of interest (n=1,798).

^bP-value was based on t-test when row variable is continuous and design-based χ^2 test when row variable is categorical.

TABLE 2

Pearson's correlation coefficients between: (I) Log_e transformed serum antioxidant status variables and (II) Log_e transformed serum antioxidant status variables vs. selected dietary intake variables and continuous PHQ score; NHANES 2005–06 (*n*=1,798)

I. Correlations between Log _e transformed serum antioxidant status variables									
	Retinol+retinyl esters	Vitamin E	Vitamin C	Total carotenoids	-carotene	-carotene	-cryptoxanthin	Lutein +zeaxanthin	Lycopene
Log _e transformed serum antioxidants, $\mu\text{mol/L}$									
Retinol+retinyl esters	1								
Vitamin E	0.38	1							
Vitamin C	0.10	0.28	1						
Total carotenoids	0.11	0.42	0.42	1					
-carotene	0.10	0.32	0.41	0.76	1				
-carotene	0.12	0.40	0.45	0.86	0.79	1			
-cryptoxanthin	0.02 ^b	0.27	0.45	0.74	0.59	0.59	1		
Lutein+zeaxanthin	0.09	0.40	0.35	0.75	0.54	0.56	0.60	1	
Lycopene	0.04 ^b	0.15	0.05	0.57	0.22	0.25	0.31	0.29	1

II. Correlations of Log _e transformed serum antioxidants with selected dietary intake variables and PHQ score									
	Retinol+retinyl esters	Vitamin E	Vitamin C	Total carotenoids	-carotene	-carotene	-cryptoxanthin	Lutein +zeaxanthin	Lycopene
Log _e transformed dietary intake									
Retinol, $\mu\text{g/d}$	0.14;0.12	0.10	0.08	-0.00*	0.05	0.05	-0.02*	-0.00*	-0.01*
vitamin E, mg/d	0.10	0.13;0.03*	0.13	0.17	0.16	0.14	0.09	0.17	0.14
vitamin C, mg/d	0.02*	0.11	0.36;0.26	0.27	0.26	0.21	0.35	0.24	0.03*
Total carotenoids, $\mu\text{g/d}$	0.08	0.10	0.18	0.29;0.16	0.27	0.26	0.18	0.25	0.16
-carotene, $\mu\text{g/d}$	0.11	0.15	0.18	0.22	0.36;0.24	0.28	0.18	0.19	-0.05*
-carotene, $\mu\text{g/d}$	0.12	0.16	0.21	0.30	0.37	0.34;0.22	0.18	0.28	-0.01*
-Cryptoxanthin, $\mu\text{g/d}$	0.04*	0.12	0.31	0.27	0.22	0.21	0.39;0.28	0.23	0.03*
Lutein+zeaxanthin, $\mu\text{g/d}$	0.09	0.14	0.21	0.29	0.26	0.25	0.20	0.33;0.19	0.04*
Lycopene, $\mu\text{g/d}$	0.01	0.03*	0.05*	0.13	0.06	0.05	0.08	0.06	0.25;0.24

I. Correlations between Log_e transformed serum antioxidant status variables

	Retinol+retinyl esters	Vitamin E	Vitamin C	Total carotenoids	-carotene	-carotene	-cryptoxanthin	Lutein +zeaxanthin	Lycopene
Fruits and vegetable intake, cup equivalent/d	0.08	0.14	0.28	0.30	0.31	0.26	0.29	0.26	0.09
Supplement use, past 30 days (0, 1, 2)	0.16	0.42	0.29	0.19	0.21	0.28	0.06	0.14	-0.00
Log _e transformed (PHQ score)	-0.06	-0.10	-0.07	-0.07	-0.08	-0.09	-0.09	-0.09	-0.04*

Abbreviations: NHANES=National Health and Nutrition Examination Survey; PHQ=Patient Health Questionnaire; SEE=Standard error of the estimate.

^a Values are Pearson's correlation coefficients. Italicized correlation coefficients are partial correlations between serum antioxidant and its corresponding dietary antioxidant (both Log_e transformed), controlling for Log_e transformed fruit and vegetable intake and supplement use (0, 1, 2 or more).

* P>0.05 for null hypothesis that r=0; All other correlations were statistically significant.

TABLE 3

Associations between selected serum antioxidant status (per 1 SD increase) and depressive symptoms: Multiple logistic and zero-inflated poisson regression models, uncontrolled for dietary antioxidant intakes or supplement use (Hypothesis B): 2-stage Heckman selection models; NHANES 2005–06^a

	PHQ score 10 Vs. PHQ score <10				PHQ count score			
	All	Men	Women	Women	All	Men	Women	Women
	OR	95% CI	OR	95% CI	±SEE	±SEE	±SEE	±SEE
MODEL 1 ^b		(n=1,776)	(n=736)	(n=1,040)	(n=1,776)	(n=736)	(n=1,040)	(n=1,040)
Retinol, per 0.63 $\mu\text{mol/L}$	1.05	(0.82;1.35)	1.20	(0.90;1.60)	0.97	(0.60;1.56)	+0.01±0.02	+0.04±0.04
Retinyl esters, per 0.09 $\mu\text{mol/L}$	0.63	(0.37;1.08)	0.87	(0.54;1.40)	0.46*	(0.21;0.99)	-0.15±0.03	-0.11±0.05*
-Carotene, per 0.11 $\mu\text{mol/L}$	0.70*	(0.50;0.97)	0.57	(0.26;1.24)	0.79	(0.61;1.03)	-0.05±0.02*	-0.05±0.03
-Carotene, per 0.41 $\mu\text{mol/L}$	0.52	(0.39;0.70)	0.57	(0.26;1.23)	0.58*	(0.42;0.79)	-0.06±0.04	-0.09±0.06
-Cryptoxanthin, per 0.16 $\mu\text{mol/L}$	0.95	(0.76;1.19)	0.99	(0.72;1.37)	1.00	(0.70;1.42)	-0.02±0.02	-0.07±0.03
Lutein+zeaxanthin, per 0.16 $\mu\text{mol/L}$	0.67*	(0.49;0.91)	0.79	(0.49;1.28)	0.65*	(0.44;0.97)	-0.07±0.02*	-0.04±0.05
Lycopene, per 0.20 $\mu\text{mol/L}$	0.81*	(0.70;0.94)	0.79	(0.60;1.04)	0.84	(0.68;1.04)	-0.08±0.03*	-0.09±0.05
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.83	(0.55;1.23)	1.16	(0.53;2.53)	0.85	(0.45;1.60)	-0.00±0.04	-0.00±0.08
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.85	(0.58;1.26)	1.04	(0.67;1.62)	0.82	(0.50;1.36)	-0.03±0.04	-0.01±0.06
MODEL 2 ^c		(n=1,776)	(n=736)	(n=1,040)	(n=1,776)	(n=736)	(n=1,040)	(n=1,040)
Retinol, per 0.63 $\mu\text{mol/L}$	1.07	(0.83;1.39)	1.18	(0.85;1.63)	0.99	(0.58;1.68)	+0.01±0.02	+0.04±0.04
Retinyl esters, per 0.09 $\mu\text{mol/L}$	0.81	(0.48;1.39)	0.88	(0.44;1.78)	0.60	(0.28;1.29)	-0.13±0.04*	-0.10±0.05*
-Carotene, per 0.11 $\mu\text{mol/L}$	1.02	(0.63;1.65)	0.66	(0.26;1.69)	1.16	(0.72;1.87)	-0.01±0.02	-0.01±0.04
-Carotene, per 0.41 $\mu\text{mol/L}$	0.58*	(0.36;0.94)	0.78	(0.30;1.65)	0.60*	(0.39;0.92)	-0.02±0.04	-0.03±0.08
-Cryptoxanthin, per 0.16 $\mu\text{mol/L}$	1.31*	(1.10;1.56)	1.27	(0.97;1.65)	1.36	(1.00;1.85)	+0.03±0.02	-0.04±0.03
Lutein+zeaxanthin, per 0.16 $\mu\text{mol/L}$	0.75	(0.54;1.04)	0.80	(0.50;1.28)	0.72	(0.50;1.04)	-0.04±0.03	+0.00±0.05
Lycopene, per 0.20 $\mu\text{mol/L}$	0.92	(0.76;1.10)	0.89	(0.62;1.26)	0.99	(0.77;1.28)	-0.04±0.02	-0.06±0.05
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.97	(0.62;1.39)	1.19	(0.67;1.80)	1.04	(0.56;1.94)	+0.04±0.04	+0.04±0.08
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.97	(0.67;1.39)	1.10	(0.67;1.80)	0.93	(0.61;1.41)	-0.01±0.05	+0.01±0.08
MODEL 3 ^d		(n=1,776)	(n=736)	(n=1,040)	(n=1,776)	(n=736)	(n=1,040)	(n=1,040)
Total retinol+retinyl esters, per 0.66 $\mu\text{mol/L}$	1.06	(0.80; 1.42)	1.15	(0.80;1.65)	0.95	(0.55;1.65)	-0.01±0.02	+0.02±0.05

	PHQ score 10 Vs. PHQ score<10				PHQ count score				
	All	Men	Women	All	Men	Women	All	Men	Women
	OR	95% CI	OR	95% CI	OR	95% CI	±SEE	±SEE	±SEE
Total carotenoids, per 0.73 $\mu\text{mol/L}$	0.62	(0.51; 0.76)	0.62	(0.37; 1.03)	0.68	(0.55; 0.83)	-0.08±0.03*	-0.12±0.06	-0.06±0.03
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.89	(0.60; 1.32)	1.12	(0.48; 2.60)	0.95	(0.51; 1.74)	+0.01±0.04	-0.00±0.08	+0.07±0.04
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.96	(0.66; 1.40)	1.10	(0.67; 1.83)	0.90	(0.59; 1.40)	-0.01±0.05	+0.01±0.07	-0.02±0.06

Abbreviations. BMI=Body Mass Index; CI=confidence interval; Met=Metabolic Equivalent; NHANES=National Health and Nutrition Examination Survey; OR=odds ratio; PHQ=Patient Health Questionnaire; SEE=Standard error of the estimate.

^a Values are odds ratios with 95% confidence intervals or ±SEE. Sampling design complexity is taken into account in all analyses.

* P<0.05;

P<0.001 for null hypothesis that =0 or Loge(OR)=0 based on Wald test.

^b Model 1 included each antioxidant exposure separately and adjusted for socio-demographic factors: age, sex, race/ethnicity, marital status, educational level and poverty income ratio, and other potential confounders: Lifestyle and health-related factors (smoking status, BMI, physical activity: Mets.hr.wk⁻¹, recoded as “0–<5”; “5–10”; “>10”; history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)), anti-depressant use and dietary intakes (total energy intake, alcohol, n-3 PUFA), serum levels folate, total homocysteine, vitamin B-12, 25-hydroxyvitamin D and serum total cholesterol, anti-depressant use, and the inverse mills ratio, 2-stage Heckman selection model.

^c Model 2 included all serum antioxidant exposures simultaneously, controlling for the same covariate as above.

^d Model 3 is model 2 (i.e. controlling for the same covariates as above) but with main exposures being the total retinol+retinyl esters, total carotenoids and vitamin E.

TABLE 4

Associations between selected serum antioxidant status (per 1 SD increase) and depressive symptoms: Multiple logistic and zero-inflated poisson regression models, controlled for dietary antioxidant intakes and supplement use (**Hypothesis A**): 2-stage Heckman selection models; NHANES 2005–06^a

	PHQ score 10 Vs. PHQ score <10				PHQ count score				
	All	Men	Women	All	Men	Women	All	Men	Women
MODEL 1 ^b									
Retinol, per 0.63 $\mu\text{mol/L}$	OR (p=1,776)	OR 95% CI (p=736)	OR 95% CI (p=1,040)	±SEE (p=1,776)	±SEE (p=736)	±SEE (p=1,040)			
Retinyl esters, per 0.09 $\mu\text{mol/L}$	1.06 (0.81;1.37)	1.18 (0.87;1.63)	0.97 (0.61;1.56)	0.00±0.02	+0.03±0.05	-0.03±0.04			
-Carotene, per 0.11 $\mu\text{mol/L}$	0.60 (0.34;1.06)	0.82 (0.49;1.37)	0.42* (0.19;0.90)	-0.17±0.03	-0.13±0.05*	-0.20±0.04			
-Carotene, per 0.41 $\mu\text{mol/L}$	0.71* (0.52;0.97)	0.59 (0.26;1.33)	0.79 (0.62;1.00)	-0.06±0.02*	-0.05±0.04	-0.05±0.03			
-Cryptoxanthin, per 0.16 $\mu\text{mol/L}$	0.52 (0.37;0.74)	0.55 (0.25;1.21)	0.58* (0.37;0.90)	-0.06±0.04	-0.10±0.06	-0.04±0.05			
Lutein+zeaxanthin, per 0.16 $\mu\text{mol/L}$	0.94 (0.73;1.21)	0.86 (0.55;1.33)	1.07 (0.78;1.47)	-0.02±0.02	-0.10±0.05	0.02±0.03			
Lycopene, per 0.20 $\mu\text{mol/L}$	0.66* (0.48;0.92)	0.74 (0.45;1.24)	0.65* (0.43;0.99)	-0.07±0.02*	-0.06±0.05	-0.08±0.03*			
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.80* (0.70;0.92)	0.79 (0.60;1.04)	0.83 (0.64;1.07)	-0.08±0.02*	-0.09±0.05	-0.06±0.03			
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.75 (0.47;1.18)	0.98 (0.41;2.34)	0.78 (0.38;1.61)	-0.03±0.04	-0.05±0.08	0.01±0.05			
MODEL 2 ^c									
Retinol, per 0.63 $\mu\text{mol/L}$	1.08 (0.82;1.43)	1.12 (0.80;1.58)	1.02 (0.59;1.74)	0.01±0.02	0.04±0.05	-0.03±0.04			
Retinyl esters, per 0.09 $\mu\text{mol/L}$	0.83 (0.47;1.47)	0.91 (0.42;1.97)	0.54 (0.24;1.21)	-0.13±0.05*	-0.09±0.05	-0.17±0.06*			
-Carotene, per 0.11 $\mu\text{mol/L}$	1.08 (0.68;1.72)	0.77 (0.29;2.01)	1.19 (0.75;1.90)	-0.01±0.02	+0.00±0.04	-0.01±0.03			
-Carotene, per 0.41 $\mu\text{mol/L}$	0.56* (0.32;0.97)	0.80 (0.35;1.86)	0.60* (0.38;0.93)	-0.03±0.04	-0.04±0.08	-0.02±0.05			
-Cryptoxanthin, per 0.16 $\mu\text{mol/L}$	1.37* (1.07;1.75)	1.15 (0.79;1.68)	1.54* (1.11;2.13)	+0.04±0.02*	-0.06±0.04	+0.08±0.03*			
Lutein+zeaxanthin, per 0.16 $\mu\text{mol/L}$	0.74 (0.54;1.03)	0.86 (0.54;1.37)	0.67* (0.46;0.98)	-0.04±0.02	+0.01±0.05	-0.06±0.03*			
Lycopene, per 0.20 $\mu\text{mol/L}$	0.91 (0.79;1.06)	0.84 (0.60;1.18)	0.99 (0.74;1.34)	-0.04±0.02	-0.06±0.05	-0.01±0.03			
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.84 (0.52;1.34)	0.99 (0.31;3.13)	0.86 (0.46;1.62)	0.01±0.04	-0.02±0.08	0.06±0.05			
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.89 (0.58;1.35)	0.91 (0.46;1.82)	0.93 (0.60;1.43)	-0.03±0.05	-0.02±0.08	-0.02±0.06			
MODEL 3 ^d									
Total retinol+retinyl esters, per 0.66 $\mu\text{mol/L}$	1.07 (0.81;1.42)	1.13 (0.79;1.63)	0.97 (0.58;1.60)	-0.00±0.02	+0.02±0.05	-0.05±0.04			

	PHQ score 10 Vs. PHQ score <10						PHQ count score		
	All		Men		Women		All	Men	Women
	OR	95% CI	OR	95% CI	OR	95% CI	±SEE	±SEE	±SEE
Total carotenoids, per 0.73 $\mu\text{mol/L}$	0.62	(0.51;0.76)	0.63	(0.36;1.08)	0.65	(0.47;0.89)	-0.09±0.03*	-0.12±0.07	-0.07±0.04
Vitamin E, per 14.6 $\mu\text{mol/L}$	0.79	(0.51;1.22)	0.97	(0.39;2.42)	0.84	(0.46;1.55)	-0.01±0.04	-0.05±0.08	+0.04±0.05
Vitamin C, per 28.5 $\mu\text{mol/L}$	0.88	(0.57;1.35)	0.92	(0.47;1.80)	0.88	(0.55;1.42)	-0.03±0.05	-0.02±0.07	-0.03±0.06

Abbreviations. BMI=Body Mass Index; CI=confidence interval; Met=Metabolic Equivalent; NHANES=National Health and Nutrition Examination Survey; OR=odds ratio; PHQ=Patient Health Questionnaire; SEE=Standard error of the estimate.

^a Values are odds ratios with 95% confidence intervals or ±SEE. Sampling design complexity is taken into account in all analyses.

* P<0.05;

P<0.01 for null hypothesis that =0 or Log_e(OR)=0 based on Wald test.

^b Model 1 included each antioxidant exposure separately and adjusted for socio-demographic factors: age, sex, race/ethnicity, marital status, educational level and poverty income ratio, and other potential confounders: Lifestyle and health-related factors (smoking status, BMI, physical activity: Mets.hr.wk^{-1} , recoded as “0–<5”; “5–10”; “>10”; history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)) and dietary intakes (total energy intake, alcohol, dietary antioxidant (or group of antioxidants), n-3 PUFA, dietary supplement use), serum levels folate, total homocysteine, vitamin B-12, 25-hydroxyvitamin D and serum total cholesterol, anti-depressant use, and the inverse mills ratio, 2-stage Heckman selection model.

^c Model 2 included all antioxidant exposures simultaneously, controlling for the same covariate as above.

^d Model 3 is model 2 (i.e. controlling for the same covariates as above) but with main exposures being the total retinol+retinyl esters, total carotenoids and vitamin E.