

Antioxidant status and its association with elevated depressive symptoms among US adults: National Health and Nutrition Examination Surveys 2005–6

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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 2005–6 on US adults aged 20–85 years were analysed. Depressive symptoms were measured using the Patient Health Questionnaire with a score cut-off point 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 1798 US adults with complete data. A higher total serum carotenoid level was associated with a 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 total serum carotenoids were expressed as quartiles (Q_4 (1.62–10.1 $\mu\text{mol/l}$) *v.* Q_1 (0.06–0.86 $\mu\text{mol/l}$): OR 0.41; 95% CI 0.23, 0.76, $P < 0.001$; P for trend = 0.035), though no significant associations were found with the 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.

Key words: Depressive symptoms; Antioxidants; Carotenoids; Adults

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 CVD^(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 have been shown to reduce oxidative stress triggered by injury, characterising the pathogenesis of many chronic diseases, including type 2 diabetes, CVD, rheumatological conditions and 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 forty different types of carotenoids, only β -carotene, α -carotene, β -cryptoxanthin, lycopene, lutein and zeaxanthin have detectable concentrations in human serum⁽¹⁴⁾. Other micronutrients with well-established antioxidative properties include vitamin C, retinol (sparing provitamin A) and vitamin E^(15–17).

Evidence from basic experimental and epidemiological studies has triggered a number of hypotheses regarding the possible direction of the association between serum antioxidant levels and depressive symptoms in humans as follows:

Abbreviations: FV, fruits and vegetables; MEC, mobile examination centre; NHANES, National Health and Nutrition Examination Survey; PHQ, Patient Health Questionnaire; ZIP, zero-inflated Poisson.

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(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 the 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 levels^(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.

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

Materials and methods

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 US civilian population. The National Center for Health Statistics, Centers for Disease Control, conducted three earlier waves of NHANES (NHANES I, II and III) in 1971–5, 1976–80 and 1988–94, respectively. Since 1999, the NHANES became a continuous survey. Using a stratified, multistage probability cluster sampling design, the NHANES consists of an in-home interview for demographic and basic health information and a health examination in a mobile examination centre (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 have been published elsewhere⁽³³⁾. The present 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 Centers for Disease Control, National Center for Health Statistics. Written or verbal informed

consent was obtained from all subjects/patients. Verbal consent was witnessed and formally recorded⁽³⁴⁾.

We analysed NHANES data on adults aged 20–85 years from 2005 to 2006. Among a sample of 4979 adult subjects (2387 men and 2592 women) with complete basic demographic data (sample 1), 3097 had complete serum antioxidant status and dietary data. Among those, complete data on other covariates of interest including physical activity, smoking status, supplement use, weight and height, serum 25-hydroxyvitamin D, folate, vitamin B₁₂, homocysteine and total cholesterol were available for 2859 participants (sample 2). Among those in sample 2, complete data on depressive symptoms were available for 1798 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 an education level above high school and to be current or former smokers compared with those selected only in sample 1.

Outcome assessment

The questionnaire section of NHANES 2005–6 included the Patient Health Questionnaire (PHQ). This set of ten questions reflects self-reported depressive signs and symptoms that are derived from the diagnostic and statistical manual IV criteria. There are nine signs and symptoms in diagnostic and statistical manual IV that were scored between 0 (not at all) and 3 (nearly every day), and an additional follow-up question to assess overall impairment ascribed to depressive symptoms. The PHQ was validated in primary care settings and shown as a reliable tool for depression diagnosis^(35,36). Summing scores on ten PHQ items potentially yields a total score between 0 and 30. In our sample, the 90th percentile on the total PHQ score corresponded to a value of 10. This cut-off point was similarly used elsewhere and had a sensitivity of 88% and a specificity of 88% for major depression^(35,36).

Exposure assessment

Using HPLC with photodiode array detection, serum concentrations of key antioxidants were measured. In the present 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*), β -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 by HPLC with electrochemical detection^(37,38).

Covariates

Sociodemographic covariates included age, sex, race/ethnicity, education, marital status and family income, measured by the poverty:income ratio (<100% of the poverty line, 100–<200% and \geq 200%). An objective measure for physical activity was constructed based on individual leisure-time activities with an intensity score assessed by metabolic

equivalents multiplied by duration of the activity and frequency converted to per week unit. This metabolic equivalent \times h/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 categorised as '0–< 5', '5–10' and '> 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 h recalls in the 2005–6 wave. The first one was administered during MEC examinations and the second 3–10 d later by telephone interview. The average of the two 24 h 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. the sum of previously listed micronutrients in $\mu\text{g}/\text{d}$), vitamin C and vitamin E in mg/d) and *n*-3 PUFA (sum of DHA + EPA + *n*-3 docosapentaenoic acid ($\text{C} \geq 20$), 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 FV were estimated per individual and averaged out between the two 24 h recalls. Participants with only one 24 h recall were excluded in the final analysis. A general measure of dietary supplement use over the past 30 d was computed as follows: 0, non-users; 1, using one supplement; 2, using two supplements or more. Another measure of antidepressant use was obtained from the medication data file with the first-level category being '242' and the second-level category being '249', using Multum's therapeutic classification system⁽⁴⁷⁾. The final variable was coded as 'having used an antidepressant' (1 = yes; 0 = no) over the past 30 d.

Serum folate and vitamin B₁₂ were measured using the 'Quantaphase II Folate' radioassay kit (Bio-Rad Laboratories)^(48,49). Total homocysteine in serum was measured using the fully automated Abbott IMx Homocysteine assay method (Abbott Laboratories)⁽⁵⁰⁾. Serum 25-hydroxyvitamin D was measured by RIA (Diasorin, Inc.)⁽⁵¹⁾. Those nutritional biomarkers were associated with mood disorders and cognitive function in a number of previous studies^(52–68).

Moreover, because depressive symptoms are usually increased in number and severity in the presence of comorbid chronic conditions, we created three measures using self-reports for history of 'type 2 diabetes', 'CVD' (i.e. congestive heart failure, CVD, 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⁽⁶⁹⁾.

Statistical analysis

Using Stata (release 11.0; StataCorp LP), 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 the associations between log_e-transformed serum antioxidant variables, first, between each other 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 FV 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 2001–6 data⁽⁶⁹⁾. Third, multiple logistic regression models were conducted to test the associations between antioxidant status (standardised *z*-scores computed from the total NHANES sample aged 20–85 years 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⁽⁶⁸⁾. These associations were tested for depressive symptoms count score (range 0–30) as well using multiple zero-inflated Poisson (ZIP) regression models⁽⁷⁰⁾. The choice of the ZIP model over Poisson and negative binomial models was based on a previous similar analysis that compared the three models⁽⁷¹⁾. To test hypotheses A and B, two sets of models were carried out, 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⁽⁷²⁾, when dietary antioxidants and supplement use were entered into the model.

To test dose–response relationships, quartiles of main antioxidants (Q₂, Q₃, Q₄ *v.* Q₁) 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. For most sample estimations, 2-year MEC examination weights were used, and masked variance units were used to estimate variances using the Taylor series linearisation method⁽⁷³⁾.

To additionally account for potential selection bias in all main analyses (logistic and ZIP regression models), given missing data on the outcome variable compared with the MEC examination sample, we constructed a two-stage Heckman selection model⁽⁷⁴⁾, as was done previously in another study using a similar sample⁽⁶⁸⁾. To this end, a probit model was conducted at first in which the main selection variable (i.e. belonging to sample 3 *v.* not, among those in sample 1) was modelled 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⁽⁷⁵⁾.

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 percentage change in the estimate of OR associated with each exposure when each covariate was eliminated⁽⁷⁶⁾. 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 a 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 the PHQ was deemed significant if the interaction term was significant at a level of 0.10, given the lower power of interaction terms compared with the main effects⁽⁷²⁾. All other *P* values presented were two-tailed with *P* < 0.05 being considered as 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 with the referent category (e.g. Q_4 *v.* Q_1) or examining standardised predictor variables in relation to the outcome (i.e. per SD). The same cut-off was applied to the *P* value for trend and the *P* value for interaction.

Results

Study sample characteristics

Table 1 presents the distribution of sample characteristics by sex and depressive symptoms status based on the PHQ score. The mean PHQ score was significantly higher among women compared with men (4.5 (SEM 0.2) *v.* 3.9 (SEM 0.2), *P* < 0.05). Participants with elevated depressive symptoms were generally less educated, more likely to belong to the poverty:income ratio < 100% category, be unmarried, less physically active and had a 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 with their non-depressed counterparts and were more likely to use antidepressants. Women with elevated depressive symptoms had lower serum levels of vitamins E and C as well as all carotenoids compared with their non-depressed counterparts. They additionally reported lower intakes of β -carotene, β -cryptoxanthin, lutein + zeaxanthin, total carotenoids, vitamin C, vitamin E, *n*-3 PUFA and a lower consumption of FV. Moreover, they exhibited lower serum folate and 25-hydroxyvitamin D status. Men with elevated depressive symptoms had higher mean total homocysteine compared with their

non-depressed counterparts. Sex differences in dietary intakes and other covariates were also observed, including intakes of carotenoids, vitamin E, *n*-3 PUFA, antidepressant use, dietary supplement use, consumption of FV as well as serum levels of folate and total homocysteine.

Correlation between key study variables

Pearson's correlation coefficients between the log_e-transformed serum antioxidant status variables (between each other (analysis I); with other dietary variables and the PHQ continuous score (analysis II)) are presented in Table 2. For analysis I, correlation coefficients between the serum antioxidant variables ranged between < 0.10 and non-significant (e.g. lycopene and retinol + retinyl esters), and > 0.70 (e.g. α -carotene and β -carotene). For analysis II, most correlations were weak to moderate (< 0.10–0.40), with the strongest coefficients being 0.42 (supplement use *v.* serum vitamin E). As expected, the PHQ score was inversely related to most serum antioxidant variables.

Partial correlations of serum antioxidants with their corresponding dietary intake variable, controlling for FV consumption and supplement use, indicated that both sources were important in explaining those correlations, with the partial correlation being attenuated by 14% (retinol) to 77% (vitamin E).

Antioxidant status and depressive symptoms

The findings from a series of multiple logistic and ZIP models examining the associations between the selected serum antioxidant status variables and depressive symptoms (measured by the PHQ) are presented in Table 3. In all the 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 the PHQ count (model 1) was the outcome in the series of ZIP models, though only the inverse relationship between retinyl esters and the 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

Table 1. Selected baseline characteristics of National Health and Nutrition Examination Survey 2005–6 participants by sex and ‘elevated depressive symptoms’ status*
(Mean values with their standard errors; percentages and standard errors of the proportion (SEP), n 1798)

	Men						Women						P		
	PHQ score < 10 (n 675)		PHQ score ≥ 10 (n 75)		All men (n 750)		PHQ score < 10 (n 928)		PHQ score ≥ 10 (n 120)		All women (n 1048)				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Men v. women	PHQ ≥ 10 v. < 10 (men)	PHQ ≥ 10 v. < 10 (women)
Sociodemographic, lifestyle factors															
Age (years)	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	0.269														
Non-Hispanic white	0.458														
%	74.9		68.3		74.3		73.0		65.7		72.2				
SEP	3.1		5.7		3.1		2.8		4.9		2.8				
Non-Hispanic black	0.270														
%	8.6		13.9		9.0		11.0		14.5		11.4				
SEP	1.5		3.2		1.5		2.1		3.8		2.1				
Mexican-American	0.019														
%	8.3		7.5		8.2		6.9		8.4		7.1				
SEP	1.4		3.2		1.4		0.8		1.9		0.8				
Other ethnicity	0.024														
%	8.1		10.3		8.3		9.1		11.4		9.3				
SEP	1.8		4.4		1.6		1.8		3.1		1.8				
Married	0.067														
%	61.8		46.9		60.6		57.2		45.5		56.0				
SEP	2.2		6.4		2.3		2.6		4.3		2.5				
Education	0.140														
< High school	0.043														
%	6.0		7.6		6.1		4.7		7.3		4.9				
SEP	0.8		2.6		0.7		0.7		1.7		0.7				
High school	< 0.001														
%	34.8		47.5		35.9		31.3		50.7		33.4				
SEP	2.3		5.3		2.3		2.2		4.5		2.2				
> High school	0.043														
%	59.2		44.9		58.0		64.1		41.9		61.6				
SEP	2.4		4.7		2.4		2.6		4.4		2.5				
Poverty:income ratio	0.043														
< 100%	< 0.001														
%	8.7		16.1		9.3		10.0		26.3		11.8				
SEP	1.4		4.4		1.4		1.1		4.7		1.2				
100– < 200%	0.043														
%	18.6		24.3		19.1		20.7		29.6		21.7				
SEP	2.3		4.8		2.3		1.8		5.2		1.9				
≥ 200%	0.043														
%	72.7		59.7		71.6		69.3		44.1		66.6				
SEP	2.7		6.7		2.8		2.7		5.6		2.7				
Smoking status	0.043														
Never smoker	0.271														
%	42.0		27.9		40.8		57.1		45.4		55.9				
SEP	2.7		10.6		2.9		2.5		5.6		2.4				

Table 1. Continued

	Men						Women						P		
	PHQ score < 10 (n 675)		PHQ score ≥ 10 (n 75)		All men (n 750)		PHQ score < 10 (n 928)		PHQ score ≥ 10 (n 120)		All women (n 1048)				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Men v. women	PHQ ≥ 10 v. < 10 (men)	PHQ ≥ 10 v. < 10 (women)
Former smoker															
%	30.0		32.9		30.3		22.4		19.1		22.1				
SEP	1.5		7.1		1.7		1.9		5.3		1.9				
Current smoker															
%	27.9		39.2		28.9		20.4		35.4		22.1				
SEP	2.4		9.8		2.6		1.8		4.5		1.9				
Physical activity (MET × h/week)	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
BMI (kg/m ²)	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													0.613	0.154	0.656
%	7.4		12.6		7.9		8.6		9.7		8.8				
SEP	1.1		3.5		1.0		1.1		2.5		1.1				
CVD													0.548	0.434	0.125
%	7.4		9.7		7.6		7.9		12.1		8.4				
SEP	0.8		3.0		0.7		1.2		3.6		1.3				
Cancer													0.002	0.004	0.582
%	4.7		16.7		5.8		9.5		11.8		9.7				
SEP	1.0		4.3		0.9		1.1		3.9		0.9				
Antioxidant status															
Retinol (μmol/l)	2.23	0.03	2.40	0.08	2.24	0.03	2.00	0.04	1.90	0.08	1.99	0.03	<0.001	<0.001	0.835
Retinyl esters (μmol/l)	0.121	0.003	0.106	0.009	0.120	0.003	0.116	0.004	0.080	0.004	0.112	0.003	0.075	0.531	<0.001
Total retinol + retinyl esters (μmol/l)	2.35	0.03	2.51	0.08	2.36	0.03	2.12	0.04	1.98	0.04	2.10	0.04	<0.001	0.001	0.500
α-Carotene (μmol/l)	0.07	0.01	0.04	0.00	0.07	0.01	0.11	0.09	0.06	0.00	0.11	0.01	<0.001	0.065	0.001
β-Carotene (μmol/l)	0.30	0.01	0.19	0.02	0.29	0.01	0.51	0.03	0.24	0.02	0.48	0.01	<0.001	0.003	<0.001
β-Cryptoxanthin (μmol/l)	0.17	0.01	0.15	0.02	0.17	0.01	0.19	0.01	0.14	0.02	0.18	0.01	0.001	0.135	0.045
Lutein + zeaxanthin (μmol/l)	0.28	0.01	0.24	0.02	0.28	0.01	0.24	0.02	0.31	0.01	0.30	0.01	0.091	0.067	<0.001
Lycopene (μmol/l)	0.48	0.02	0.41	0.02	0.47	0.01	0.44	0.01	0.39	0.02	0.43	0.01	0.04	0.063	0.009
Total carotenoids (μmol/l)	1.30	0.03	1.03	0.07	1.28	0.03	1.56	0.05	1.05	0.04	1.50	0.04	<0.001	0.006	<0.001
Vitamin E (μmol/l)	28.9	0.4	28.4	2.0	28.8	0.4	30.4	0.7	26.3	1.5	30.0	0.6	0.059	0.742	0.005
Vitamin C (μmol/l)	48.3	1.1	46.1	5.2	48.2	1.15	60.3	1.7	42.9	3.8	58.4	1.6	<0.001	0.413	<0.001
Dietary intake															
Total energy intake (MJ/d)	11.74	0.28	11.44	0.82	11.71	0.30	8.03	0.11	6.94	0.30	7.54	0.11	<0.001	0.849	0.073
Alcohol intake (g/d)	15.3	1.7	20.6	7.0	15.8	1.5	5.6	0.9	3.7	1.1	5.4	0.8	<0.001	0.535	0.535
α-Carotene (μg/d)	442.9	52.7	296.0	70.7	430.3	47.1	443.9	31.0	266.2	45.8	424.3	29.1	0.813	0.830	0.207
β-Carotene (μg/d)	2190.2	160.2	1535.7	156.9	2134.2	141.8	2364.5	120.7	1368.4	258.1	2254.8	117.5	0.715	0.577	0.004
β-Cryptoxanthin (μg/d)	137.9	10.2	156.0	43.1	139.4	9.8	123.9	5.3	77.8	10.6	118.8	4.9	0.335	0.979	0.008
Lutein + zeaxanthin (μg/d)	1414.7	139.4	1203.3	145.6	1396.0	135.1	1603.4	88.4	1001.2	158.3	1537.1	80.8	0.350	0.724	0.031
Lycopene (μg/d)	6520.8	474.9	6180.8	1349.0	6491.7	453.6	5039.9	320.6	4253.6	1134.0	4953.3	311.0	<0.001	0.596	0.628
Total carotenoids (μg/d)	12 453	727	10 611	1471	12 296	625	11 496	538	8069	1592	11 118	553	0.017	0.471	0.013
Vitamin C (mg/d)	91.6	4.0	101.4	13.7	92.4	3.4	82.7	2.6	67.1	7.6	81.0	2.6	0.070	0.353	0.039
Vitamin E (mg/d)	8.6	0.3	6.9	0.6	8.4	0.3	6.9	0.1	5.5	0.4	6.7	0.1	<0.001	0.147	0.002
n-3 PUFA (g/d)	0.19	0.01	0.14	0.03	0.19	0.01	0.13	0.01	0.06	0.01	0.13	0.01	<0.001	0.366	0.031

Antioxidant status and depression in adults

Table 1. Continued

	Men						Women						P		
	PHQ score < 10 (n 675)		PHQ score ≥ 10 (n 75)		All men (n 750)		PHQ score < 10 (n 928)		PHQ score ≥ 10 (n 120)		All women (n 1048)		Men v. wom- en	PHQ ≥ 10 v. < 10 (men)	PHQ ≥ 10 v. < 10 (women)
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Dietary supplement use, past 30 d													<0.001	0.326	0.147
None															
%	43.7		37.9		43.2		32.1		41.5		33.1				
SEP	1.9		6.6		2.1		2.3		5.7		2.4				
One															
%	30.0		28.8		29.9		28.3		25.8		28.0				
SEP	2.1		5.6		1.9		1.0		4.1		1.1				
Two or more															
%	26.3		33.3		26.9		39.6		32.7		38.8				
SEP	1.9		3.4		1.9		2.6		5.6		2.6				
Fruit and vegetable intake (cup equivalents/d)	2.78	0.09	2.63	0.22	2.77	0.09	2.5	0.1	1.88	0.13	2.43	0.10	0.003	0.502	< 0.001
Antidepressant medication use													<0.001	0.005	< 0.001
% yes	6.8		18.0		7.7		15.9		38.6		18.4				
SEP	1.2		4.4		1.2		0.8		6.4		1.0				
Folate, Vitamin B ₁₂ , tHcy and 25-hydroxyvitamin D in serum															
Folate (nmol/l)	27.7	0.7	26.2	2.1	27.6	0.7	33.9	1.0	25.5	1.6	33.0	0.9	<0.001	0.505	< 0.001
Vitamin B ₁₂ (pmol/l)	376.4	10.9	356.9	31.3	374.8	10.8	393.7	9.6	377.4	24.7	391.9	8.6	0.137	0.384	0.804
tHcy (μmol/l)	8.9	0.2	10.1	0.5	8.9	0.1	7.6	0.2	8.3	0.5	7.7	0.2	<0.001	0.030	0.106
25-Hydroxyvitamin D (ng/ml)	21.8	0.5	20.9	0.9	21.2	0.5	21.8	0.5	18.3	0.9	21.4	0.4	0.269	0.792	< 0.001

PHQ, Patient Health Questionnaire; MET, metabolic equivalents; tHcy, total homocysteine.

* Sampling design complexity is taken into account in all analyses. This analysis is done among participants with complete data for the PHQ and other key variables of interest (n 1798).

† P value was based on t test when the row variable was continuous and design-based χ^2 test when the row variable was categorical.

Table 2. Pearson's correlation coefficients between the log_e-transformed serum antioxidant status variables (analysis I) and the log_e-transformed serum antioxidant status variables v. selected dietary intake variables and the continuous Patient Health Questionnaire (PHQ) score (analysis II); National Health and Nutrition Examination Surveys 2005–6 (n 1798)

	Retinol + retinyl esters	Vitamin E	Vitamin C	Total carotenoids	α-Carotene	β-Carotene	β-Cryptoxanthin	Lutein + zeaxanthin	Lycopene
Analysis I									
Log _e -transformed serum antioxidants (μmol/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					
α-Carotene	0.10	0.32	0.41	0.76	1				
β-Carotene	0.12	0.40	0.45	0.86	0.79	1			
β-Cryptoxanthin	0.02*	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*	0.15	0.05	0.57	0.22	0.25	0.31	0.29	1
Analysis II									
Log _e -transformed dietary intake									
Retinol (μg/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 (μg/d)	0.08	0.10	0.18	0.29 (0.16)†	0.27	0.26	0.18	0.25	0.16
α-Carotene (μg/d)	0.11	0.15	0.18	0.22	0.36 (0.24)†	0.28	0.18	0.19	−0.05*
β-Carotene (μg/d)	0.12	0.16	0.21	0.30	0.37	0.34 (0.22)†	0.18	0.28	−0.01*
β-Cryptoxanthin (μg/d)	0.04*	0.12	0.31	0.27	0.22	0.21	0.39 (0.28)†	0.23	0.03*
Lutein + zeaxanthin (μg/d)	0.09	0.14	0.21	0.29	0.26	0.25	0.20	0.33 (0.19)†	0.04*
Lycopene (μg/d)	0.01	0.03*	0.05*	0.13	0.06	0.05	0.08	0.06	0.25 (0.24)†
Fruit and vegetable intake (cup equivalents/d)	0.08	0.14	0.28	0.30	0.31	0.26	0.29	0.26	0.09
Supplement use, past 30 d (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*

* $P > 0.05$ for null hypothesis that Pearson's correlation coefficient (r) = 0. All other correlations were statistically significant.

† Correlation coefficients in parentheses 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).

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); two-stage Heckman selection models; National Health and Nutrition Examination Surveys 2005–6† (Odds ratio and 95% confidence intervals or β coefficients with their standard errors of the estimate (SEE))

	PHQ score ≥ 10 v. < 10						PHQ count score					
	All		Men		Women		All		Men		Women	
	OR	95% CI	OR	95% CI	OR	95% CI	β	SEE	β	SEE	β	SEE
Model 1‡	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
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	-0.02	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	-0.18**	0.04
α -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	-0.05	0.03
β -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	-0.04	0.04
β -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	+0.01	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	-0.07*	0.02
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	-0.05	0.03
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	+0.04	0.04
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	-0.03	0.06
Model 2§	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
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	-0.03	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	-0.16*	0.06
α -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	-0.01	0.03
β -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	-0.01	0.05
β -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	+0.05	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	-0.06*	0.03
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	-0.00	0.03
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	+0.09	0.04
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	-0.01	0.05
Model 3 	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
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	-0.05	0.04
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

PHQ, Patient Health Questionnaire.

* $P < 0.05$; ** $P < 0.001$ for null hypothesis that $\beta = 0$ or $\log_e(\text{OR}) = 0$ based on the Wald test.

† Sampling design complexity is taken into account in all analyses.

‡ Model 1 included each antioxidant exposure separately and adjusted for sociodemographic 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: metabolic equivalents \times h/week, recoded as '0– < 5'; '5–10'; '> 10', history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)), antidepressant use and dietary intakes (total energy intake, alcohol, *n*-3 PUFA), serum folate, total homocysteine, vitamin B₁₂, 25-hydroxyvitamin D and total serum cholesterol levels, and the inverse Mills ratio, two-stage Heckman selection model.

§ Model 2 included all serum antioxidant exposures simultaneously, controlling for the same covariates as above.

|| 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, vitamin E and vitamin C.

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 the 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 was 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 the 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 significance 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 the 0.10 level.

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

In order to further examine the dose–response relationships between antioxidant status and elevated depressive symptoms, quartiles of main exposures were used and the P value for trend was obtained (Fig. 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 Q_4 (1.62–10.1 $\mu\text{mol/l}$) with Q_1 (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 (Fig. 2), the results remained similar, with a P value for trend of 0.041 for total serum carotenoids expressed as quartiles. However, the linear dose–response relationship was no longer significant in both Figs. 1 and 2 when taking multiple testing into account (P 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 Q_4 v. Q_1 for total carotenoids in relation to elevated depressive symptoms remained statistically significant in the analyses shown in both Figs. 1 and 2 ($P = 0.02$). Another sensitivity analysis in which subjects

with inadequate dietary intakes of antioxidants were excluded did not alter the present findings appreciably.

Potential confounding by various covariates entered into the main statistical models, particularly the logistic regression model of Fig. 2, was assessed using a change-in-estimate analysis, which did not take into account sampling design complexity (data not shown). The present findings suggest that when covariates aside from main antioxidant exposures were backward eliminated (model presented in Fig. 2, with antioxidant quartiles entered as an ordinal variable to assess the dose–response relationship), change-in-estimate of the OR did not exceed 10% (for total serum carotenoids as a covariate, when main exposure was serum vitamin C), suggesting a 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.

Discussion

The 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 the total serum 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 $\mu\text{mol/l}$) v. Q_1 (0.06–0.86 $\mu\text{mol/l}$): OR 0.41, 95% CI 0.23, 0.76, $P < 0.001$; P for trend = 0.035), though no significant associations were found with the 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 forty-two patients with major depressive disorder compared with twenty-six healthy volunteers. Owen *et al.* measured both serum and dietary levels of vitamin E among depressed adults based on the Beck Depression Inventory. Their results suggested no significant association between the dietary level of vitamin E and major depression, but a significantly lower serum vitamin E level among depressed adults compared with the general population⁽²⁸⁾. Shibata *et al.* used the short version of the Geriatric Depression Scale to assess the cross-sectional and longitudinal effects of blood levels of 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); two-stage Heckman selection models; National Health and Nutrition Examination Surveys 2005–6† (Odds ratio and 95% confidence intervals or β coefficients with their standard errors of the estimate (SEE))

	PHQ score ≥ 10 v. < 10						PHQ count score					
	All		Men		Women		All		Men		Women	
	OR	95% CI	OR	95% CI	OR	95% CI	β	SEE	β	SEE	β	SEE
Model 1‡	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
Retinol (per 0.63 $\mu\text{mol/l}$)	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
Retinyl esters (per 0.09 $\mu\text{mol/l}$)	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
α -Carotene (per 0.11 $\mu\text{mol/l}$)	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
β -Carotene (per 0.41 $\mu\text{mol/l}$)	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
β -Cryptoxanthin (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
Lutein + zeaxanthin (per 0.16 $\mu\text{mol/l}$)	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
Lycopene (per 0.20 $\mu\text{mol/l}$)	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 E (per 14.6 $\mu\text{mol/l}$)	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
Vitamin C (per 28.5 $\mu\text{mol/l}$)	0.78	0.51, 1.22	0.87	0.50, 1.50	0.80	0.46, 1.39	- 0.06	0.05	- 0.05	0.07	- 0.04	0.06
Model 2§	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
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*	0.03	- 0.09	0.05	- 0.17*	0.06
α -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
β -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
β -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 	<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040		<i>n</i> 1776		<i>n</i> 736		<i>n</i> 1040	
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
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

PHQ, Patient Health Questionnaire.

* $P < 0.05$; ** $P < 0.01$ for null hypothesis that $\beta = 0$ or $\log_e(\text{OR}) = 0$ based on the Wald test.

† Sampling design complexity is taken into account in all analyses.

‡ Model 1 included each antioxidant exposure separately and adjusted for sociodemographic 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: metabolic equivalents \times h/week, 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 folate, total homocysteine, vitamin B₁₂, 25-hydroxyvitamin D and total serum cholesterol levels, antidepressant use, and the inverse Mills ratio, two-stage Heckman selection model.

§ Model 2 included all antioxidant exposures simultaneously, controlling for the same covariates as above.

|| 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, vitamin E and vitamin C.

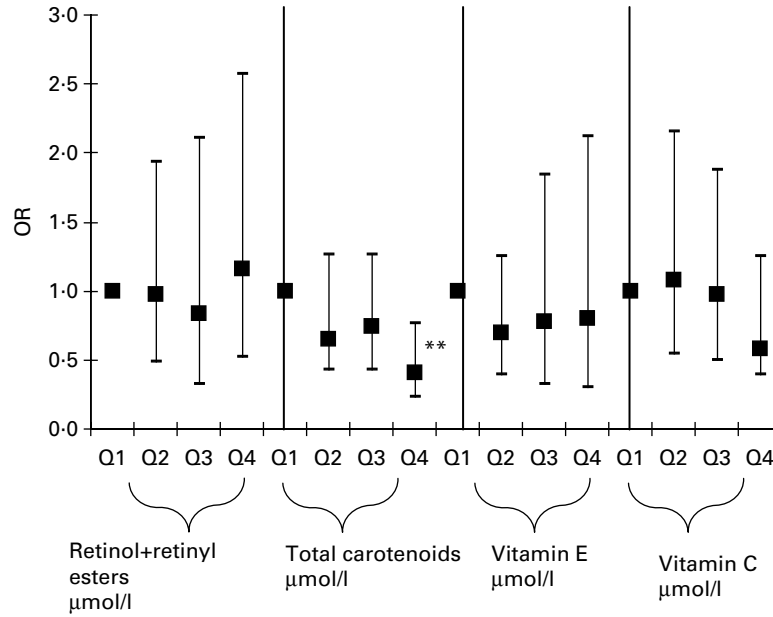


Fig. 1. Adjusted OR (with 95% CI) of the major serum antioxidant levels (expressed as quartiles, Q₂, Q₃, Q₄ v. Q₁) and elevated depressive symptoms among US adults, uncontrolled for dietary antioxidant intakes or supplement use; National Health and Nutrition Examination Surveys 2005–6. Ranges (in μmol/l) for each antioxidant quartile are as follows: retinol + retinyl esters (Q₁: 0.07–1.7; Q₂: 1.7–2.1; Q₃: 2.1–2.5; Q₄: 2.5–8.9; *P* for trend=0.775); total carotenoids (Q₁: 0.06–0.86; Q₂: 0.86–1.18; Q₃: 1.18–1.62; Q₄: 1.62–10.1; *P* for trend=0.035); vitamin E (Q₁: 0.2–26.7; Q₂: 21.7–27.3; Q₃: 27.4–35.9; Q₄: 35.9–303.8; *P* for trend=0.678); vitamin C (Q₁: 0.6–34.6; Q₂: 35.2–54.5; Q₃: 55.1–70.4; Q₄: 71.0–274.2; *P* for trend=0.299). Analyses were based on multiple logistic regression models that included all antioxidant exposures simultaneously adjusted for sociodemographic factors: lifestyle and health-related factors (smoking status, BMI, physical activity; metabolic equivalents h/week, recoded as '0–<5'; '5–10'; '> 10', history of selected chronic conditions (i.e. type 2 diabetes, CVD and cancer)), antidepressant use and dietary intakes (total energy intake, alcohol, *n*-3 PUFA), serum folate, total homocysteine, vitamin B₁₂, 25-hydroxyvitamin D and total serum cholesterol levels, antidepressant use, and the inverse Mills ratio, two-stage Heckman selection model. ** *P*<0.001 for null hypothesis that log_e(OR) = 0.

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 Geriatric Depression Scale 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 Scale, comparing 262 cases with 459 randomly selected controls. After adjustment for biological and behavioural factors, no significant associations were observed 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 Center for Epidemiologic Studies Depression Scale. Among males, the observed OR 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 total serum carotenoids at baseline and the incidence of elevated depressive symptoms after a 6-year follow-up period (OR 0.72, 95% CI 0.52, 0.99,

P=0.04), adjusting for important confounders. This association has been shown to be partially mediated by inflammatory markers such as IL-1 receptor antagonist⁽³²⁾.

The present findings of an association between total serum carotenoids, β-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 with 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 μmol/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.

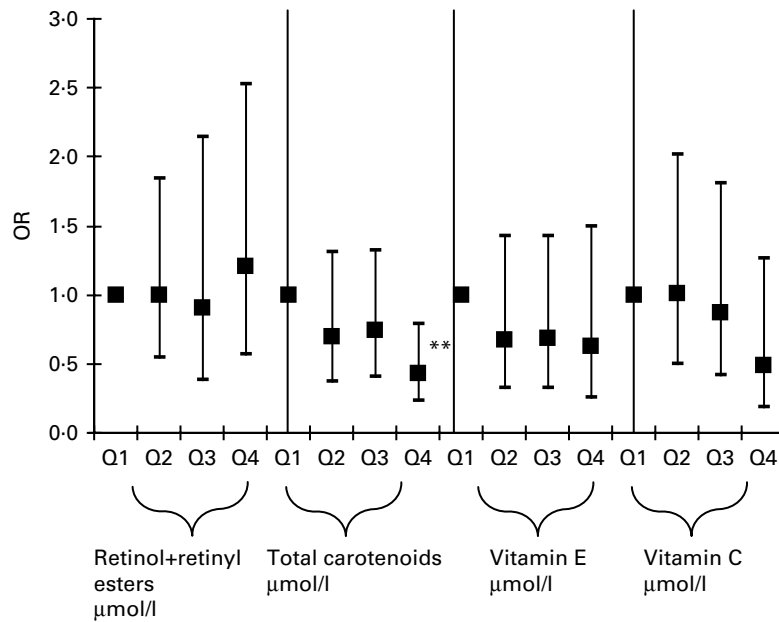


Fig. 2. Adjusted OR (with 95% CI) of the major serum antioxidant levels (expressed as quartiles, Q₂, Q₃, Q₄ v. Q₁) and elevated depressive symptoms among US adults, controlled for dietary antioxidant intakes and supplement use; National Health and Nutrition Examination Surveys 2005–6. Ranges (in µmol/l) for each antioxidant quartile are as follows: retinol + retinyl esters (Q₁: 0.07–1.7; Q₂: 1.7–2.1; Q₃: 2.1–2.5; Q₄: 2.5–8.9; *P* for trend=0.656); total carotenoids (Q₁: 0.06–0.86; Q₂: 0.86–1.18; Q₃: 1.18–1.62; Q₄: 1.62–10.1; *P* for trend=0.041); vitamin E (Q₁: 0.2–26.7; Q₂: 21.7–27.3; Q₃: 27.4–35.9; Q₄: 35.9–303.8; *P* for trend=0.286); vitamin C (Q₁: 0.6–34.6; Q₂: 35.2–54.5; Q₃: 55.1–70.4; Q₄: 71.0–274.2; *P* for trend=0.180). Analyses were based on multiple logistic regression models that included all antioxidant exposures simultaneously adjusted for sociodemographic 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: metabolic equivalents h/week, 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 folate, total homocysteine, vitamin B₁₂, 25-hydroxyvitamin D and total serum cholesterol levels, antidepressant use, and the inverse Mills ratio, two-stage Heckman selection model. ** *P*<0.001 for null hypothesis that log_e(OR) = 0.

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 defences and its lipid-rich constitution^(77,78). 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 Fe, and the activated inflammatory response⁽⁷⁷⁾. Oxidative stress, an imbalance between the production of reactive oxygen species 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, CVD, rheumatological disorders and carcinogenesis⁽¹¹⁾. Oxidative stress induces damage to DNA and cell membranes in both animal experiments⁽¹⁷⁾ and human epidemiological studies⁽⁷⁹⁾. Many studies have found evidence of an increase in reactive oxygen species with age and their deleterious effects on lipids, especially PUFA. The increase in lipid peroxidation affects the oxidation of structurally important proteins disrupting transmembrane ion movements and cellular metabolic processes^(80,81), 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⁽⁸²⁾. 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^(80,81).

In animal studies, adding immobilisation 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, has been shown to increase lipid peroxidation activity in the brain⁽²⁰⁾. Those stress-induced changes in lipid peroxidation and levels of antioxidants have also been recently shown in human subjects^(21,22).

The present 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, the present study also suffers from some limitations. First, although use of dietary supplements over a 30 d 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 sociodemographic, lifestyle and health-related factors⁽⁶⁹⁾. 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 FV as well as dietary supplement use. Lower intake of fruits, vegetables and fibres may mediate the relationship between lower carotenoid levels and depression. Indeed, depression is directly associated with a reduction in dietary quality, thus a reduction in the consumption of antioxidants⁽⁶⁹⁾. Finally, residual confounding by unmeasured covariates cannot be totally discounted.

In conclusion, we found a relationship between carotenoids and depressive symptoms with some dose–response. Further investigation is needed through randomised controlled trials and longitudinal studies to examine the nature of the temporality of the relationship. 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 the prevention of depression.

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References

- Leonard BE (2001) Stress, norepinephrine and depression. *J Psychiatry Neurosci* **26**, S11–S16.
- Lesperance F & Frasere-Smith N (2000) Depression in patients with cardiac disease: a practical review. *J Psychosom Res* **48**, 379–391.
- Ketterer MW, Mahr G & Goldberg AD (2000) Psychological factors affecting a medical condition: ischemic coronary heart disease. *J Psychosom Res* **48**, 357–367.
- Newport DJ & Nemeroff CB (1998) Assessment and treatment of depression in the cancer patient. *J Psychosom Res* **45**, 215–237.
- Spiegel D (1996) Cancer and depression. *Br J Psychiatry Suppl* **168**, Suppl. 30, 109–116.
- Talbot F & Nouwen A (2000) A review of the relationship between depression and diabetes in adults: is there a link? *Diabetes Care* **23**, 1556–1562.
- Kawakami N, Takatsuka N, Shimizu H, *et al.* (1999) Depressive symptoms and occurrence of type 2 diabetes among Japanese men. *Diabetes Care* **22**, 1071–1076.
- Cuffel B, Wamboldt M, Borish L, *et al.* (1999) Economic consequences of comorbid depression, anxiety, and allergic rhinitis. *Psychosomatics* **40**, 491–496.
- Bell IR, Jasnoski ML, Kagan J, *et al.* (1991) Depression and allergies: survey of a nonclinical population. *Psychother Psychosom* **55**, 24–31.
- Moller P, Wallin H & Knudsen LE (1996) Oxidative stress associated with exercise, psychological stress and life-style factors. *Chem Biol Interact* **102**, 17–36.
- Soory M (2009) Relevance of nutritional antioxidants in metabolic syndrome, ageing and cancer: potential for therapeutic targeting. *Infect Disord Drug Targets* **9**, 400–414.
- Campbell DR, Gross MD, Martini MC, *et al.* (1994) Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol Biomarkers Prev* **3**, 493–500.
- Stryker WS, Kaplan LA, Stein EA, *et al.* (1988) The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* **127**, 283–296.
- Rao AV & Rao LG (2007) Carotenoids and human health. *Pharmacol Res* **55**, 207–216.
- Paolisso G, D'Amore A, Balbi V, *et al.* (1994) Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. *Am J Physiol Endocrinol Metab* **266**, E261–E268.
- Ford ES, Mokdad AH, Giles WH, *et al.* (2003) The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey. *Diabetes* **52**, 2346–2352.
- Sies H & Stahl W (1995) Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr* **62**, Suppl. 6, 1315S–1321S.
- Liu J, Wang X & Mori A (1994) Immobilization stress-induced antioxidant defense changes in rat plasma: effect of treatment with reduced glutathione. *Int J Biochem* **26**, 511–517.
- Davydov VV & Shvets VN (2001) Lipid peroxidation in the heart of adult and old rats during immobilization stress. *Exp Gerontol* **36**, 1155–1160.
- Matsumoto K, Yobimoto K, Huong NT, *et al.* (1999) Psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide systems and its modulation by anxiolytic and anxiogenic drugs in mice. *Brain Res* **839**, 74–84.
- Cernak I, Savic V, Kotur J, *et al.* (2000) Alterations in magnesium and oxidative status during chronic emotional stress. *Magnes Res* **13**, 29–36.
- Tsuboi H, Shimoi K, Kinane N, *et al.* (2004) Depressive symptoms are independently correlated with lipid peroxidation in a female population: comparison with vitamins and carotenoids. *J Psychosom Res* **56**, 53–58.
- Irwin M (1999) Immune correlates of depression. *Adv Exp Med Biol* **461**, 1–24.
- Maddock C & Pariante CM (2001) How does stress affect you? An overview of stress, immunity, depression and disease. *Epidemiol Psychiatr Soc* **10**, 153–162.
- Craft NE, Haitema TB, Garnett KM, *et al.* (2004) Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *J Nutr Health Aging* **8**, 156–162.
- Oishi J, Doi H & Kawakami N (2009) Nutrition and depressive symptoms in community-dwelling elderly persons in Japan. *Acta Med Okayama* **63**, 9–17.
- Maes M, De Vos N, Pioli R, *et al.* (2000) Lower serum vitamin E concentrations in major depression. Another

- marker of lowered antioxidant defenses in that illness. *J Affect Disord* **58**, 241–246.
28. Owen AJ, Batterham MJ, Probst YC, *et al.* (2005) Low plasma vitamin E levels in major depression: diet or disease? *Eur J Clin Nutr* **59**, 304–306.
 29. Sher L (2008) Depression and suicidal behavior in alcohol abusing adolescents: possible role of selenium deficiency. *Minerva Pediatr* **60**, 201–209.
 30. Tiemeier H, Hofman A, Kiliaan AJ, *et al.* (2002) Vitamin E and depressive symptoms are not related. The Rotterdam Study. *J Affect Disord* **72**, 79–83.
 31. Shibata H, Kumagai S, Watanabe S, *et al.* (1999) Relationship of serum cholesterol and vitamin E to depressive status in the elderly. *J Epidemiol* **9**, 261–267.
 32. Milaneschi Y, Bandinelli S, Penninx BW, *et al.* (2011) The relationship between plasma carotenoids and depressive symptoms in older persons. *World J Biol Psychiatry* (Epublication ahead of print version 20 September 2011).
 33. Ogden CL, Carroll MD, Curtin LR, *et al.* (2006) Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA* **295**, 1549–1555.
 34. Center for Disease Control and Prevention (CDC) (2006) National Health and Nutrition Examination Survey. <http://www.cdc.gov/nchs/nhanes.htm> (accessed 25 September 2006, updated 2006, cited 25 September 2006).
 35. Kroenke K, Spitzer RL & Williams JB (2001) The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* **16**, 606–613.
 36. Spitzer RL, Kroenke K & Williams JB (1999) Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *JAMA* **282**, 1737–1744.
 37. Centers for Disease Control and Prevention (CDC) (2009) *National Health and Nutrition Examination Surveys (NHANES 2005–06): Laboratory Procedure Manual: Fat Soluble Micronutrients (Vitamins A, E and Carotenoids) – UV-Visible Detection*. Hyatsville, MD: Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/vitae_c_d_met_aecar.pdf (updated 2009, cited).
 38. Centers for Disease Control and Prevention (CDC) (2009) *National Health and Nutrition Examination Surveys (NHANES 2005–06): Laboratory Procedure Manual: Vitamin C*. Hyatsville, MD: Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/vic_d_met.pdf (updated 2009, cited).
 39. Cheng YJ, Gregg EW, De Rekeneire N, *et al.* (2007) Muscle-strengthening activity and its association with insulin sensitivity. *Diabetes Care* **30**, 2264–2270.
 40. Lagerros YT & Lagiou P (2007) Assessment of physical activity and energy expenditure in epidemiological research of chronic diseases. *Eur J Epidemiol* **22**, 353–362.
 41. McCullough ML, Feskanich D, Rimm EB, *et al.* (2000) Adherence to the dietary guidelines for Americans and risk of major chronic disease in men. *Am J Clin Nutr* **72**, 1223–1231.
 42. United States Department of Agriculture (USDA) (2008) *Agriculture Research Service FSRG. Food and Nutrient Database for Dietary Studies, 3.0*. Beltsville, MD: USDA. <http://www.ars.usda.gov/Services/docs.htm?docid=17,031> (accessed March 2008, updated 2008, cited).
 43. Silvers KM & Scott KM (2002) Fish consumption and self-reported physical and mental health status. *Public Health Nutr* **5**, 427–431.
 44. Hasin DS & Glick H (1993) Depressive symptoms and DSM-III-R alcohol dependence: general population results. *Addiction* **88**, 1431–1436.
 45. Raimo EB & Schuckit MA (1998) Alcohol dependence and mood disorders. *Addict Behav* **23**, 933–946.
 46. United States Department of Agriculture (USDA) (2007) *Agriculture Research Service. MyPyramid Equivalents Database for USDA Survey Food Codes version 1.0*. Beltsville, MD: USDA. <http://www.ars.usda.gov/Services/docs.htm?docid=8503> (accessed July 2007, updated 2007, cited).
 47. Center for Disease Control and Prevention (CDC) (2010) National Health and Nutrition Examination Survey 1988–2008 Data Documentation, Codebook, and Frequencies. http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/RXQ_RX_D.htm (updated 2010, cited 25 April 2011).
 48. Centers for Disease Control and Prevention (CDC) (2008) *National Health and Nutrition Examination Surveys (NHANES 2005–06): Description of Laboratory Methodology: Vitamin B-12*. Hyatsville, MD: Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/B12_D.htm#Description_of_Laboratory_Methodology (updated 2008, cited).
 49. Centers for Disease Control and Prevention (CDC) (2008) *National Health and Nutrition Examination Surveys (NHANES 2005–06): Description of Laboratory Methodology: Folate*. Hyatsville, MD: Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/FOLATE_D.htm#Description_of_Laboratory_Methodology (updated 2008, cited).
 50. Pernet P, Lasnier E & Vaubourdoille M (2000) Evaluation of the AxSYM homocysteine assay and comparison with the IMx homocysteine assay. *Clin Chem* **46**, 1440–1441.
 51. Centers for Disease Control and Prevention (CDC) (2008) *National Health and Nutrition Examination Surveys (NHANES 2005–06): Description of Laboratory Methodology: Vitamin D*. Hyatsville, MD: Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/VID_D.htm#Description_of_Laboratory_Methodology (updated 2008, cited).
 52. Wilkins CH, Sheline YI, Roe CM, *et al.* (2006) Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriatr Psychiatry* **14**, 1032–1040.
 53. Forti P, Rietti E, Pisacane N, *et al.* (2010) Blood homocysteine and risk of depression in the elderly. *Arch Gerontol Geriatr* **51**, 21–25.
 54. Almeida OP, McCaul K, Hankey GJ, *et al.* (2008) Homocysteine and depression in later life. *Arch Gen Psychiatry* **65**, 1286–1294.
 55. Tiemeier H, van Tuijl HR, Hofman A, *et al.* (2002) Vitamin B₁₂, folate, and homocysteine in depression: the Rotterdam Study. *Am J Psychiatry* **159**, 2099–2101.
 56. Bottiglieri T, Laundry M, Crellin R, *et al.* (2000) Homocysteine, folate, methylation, and monoamine metabolism in depression. *J Neurol Neurosurg Psychiatry* **69**, 228–232.
 57. Dimopoulos N, Piperi C, Salonicoti A, *et al.* (2007) Correlation of folate, vitamin B₁₂ and homocysteine plasma levels with depression in an elderly Greek population. *Clin Biochem* **40**, 604–608.
 58. Kim JM, Stewart R, Kim SW, *et al.* (2008) Predictive value of folate, vitamin B₁₂ and homocysteine levels in late-life depression. *Br J Psychiatry* **192**, 268–274.
 59. Nanri A, Mizoue T, Matsushita Y, *et al.* (2010) Serum folate and homocysteine and depressive symptoms among Japanese men and women. *Eur J Clin Nutr* **64**, 289–296.
 60. Kendrick T, Dunn N, Robinson S, *et al.* (2008) A longitudinal study of blood folate levels and depressive symptoms among young women in the Southampton Women's Survey. *J Epidemiol Community Health* **62**, 966–972.



61. Beydoun MA, Fanelli Kuczumarski MT, Beydoun HA, *et al.* (2010) The sex-specific role of plasma folate in mediating the association of dietary quality with depressive symptoms. *J Nutr* **140**, 338–347.
62. Sachdev PS, Parslow RA, Lux O, *et al.* (2005) Relationship of homocysteine, folic acid and vitamin B₁₂ with depression in a middle-aged community sample. *Psychol Med* **35**, 529–538.
63. Bjelland I, Tell GS, Vollset SE, *et al.* (2003) Folate, vitamin B₁₂, homocysteine, and the MTHFR 677C → T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. *Arch Gen Psychiatry* **60**, 618–626.
64. Kim JM, Kim SW, Shin IS, *et al.* (2008) Folate, vitamin B(12), and homocysteine as risk factors for cognitive decline in the elderly. *Psychiatry Investig* **5**, 36–40.
65. Astorg P, Couthouis A, de Courcy GP, *et al.* (2008) Association of folate intake with the occurrence of depressive episodes in middle-aged French men and women. *Br J Nutr* **100**, 183–187.
66. Sanchez-Villegas A, Doreste J, Schlatter J, *et al.* (2009) Association between folate, vitamin B(6) and vitamin B(12) intake and depression in the SUN cohort study. *J Hum Nutr Diet* **22**, 122–133.
67. Tolmunen T, Hintikka J, Ruusunen A, *et al.* (2004) Dietary folate and the risk of depression in Finnish middle-aged men. A prospective follow-up study. *Psychother Psychosom* **73**, 334–339.
68. Beydoun MA, Shroff MR, Beydoun HA, *et al.* (2010) Serum folate, vitamin B-12, and homocysteine and their association with depressive symptoms among U.S. adults. *Psychosom Med* **72**, 862–873.
69. Beydoun MA, Shroff MR, Chen X, *et al.* (2011) Serum antioxidant status is associated with metabolic syndrome among U.S. adults in recent national surveys. *J Nutr* **141**, 903–913.
70. Long JS (1997) *Regression Models for Categorical and Limited Dependent Variables*. Thousand Oaks, CA: Sage.
71. Bandiera FC, Arheart KL, Caban-Martinez AJ, *et al.* (2010) Secondhand smoke exposure and depressive symptoms. *Psychosom Med* **72**, 68–72.
72. Selvin S (2004) *Statistical Analysis of Epidemiologic Data*, 3rd ed. New York: Oxford University Press.
73. Lohr SL (2010) *Sampling: Design and Analysis*, 2nd ed. Pacific Grove, CA: Brooks/Cole.
74. Heckman JJ (1979) Sample selection bias as a specification error. *Econometrica* **47**, 153–161.
75. Puhani P (2000) The Heckman correction for sample selection and its critique. *J Econ Surveys* **14**, 53–68.
76. Wang Z (2007) Two postestimation commands for assessing confounding effects in epidemiological studies. *Stata J* **7**, 183–196.
77. Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? *J Neurochem* **97**, 1634–1658.
78. Valko M, Leibfritz D, Moncol J, *et al.* (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44–84.
79. Florence TM (1995) The role of free radicals in disease. *Aust N Z J Ophthalmol* **23**, 3–7.
80. Mason RP, Walter MF & Mason PE (1997) Effect of oxidative stress on membrane structure: small-angle X-ray diffraction analysis. *Free Radic Biol Med* **23**, 419–425.
81. Tirosh O, Kohen R, Katzhendler J, *et al.* (1997) Oxidative stress effect on the integrity of lipid bilayers is modulated by cholesterol level of bilayers. *Chem Phys Lipids* **87**, 17–22.
82. Maes M, Galecki P, Chang YS, *et al.* (2011) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 676–692.