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Association of serum levels of antioxidant micronutrients with mortality in US adults: National Health and Nutrition Examination Survey 1999–2002

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Abstract

Objective: To examine associations between serum antioxidant levels and mortality (all-cause, cancer and CVD) among US adults.
Design: We examined the risk of death from all-cause and cause-specific mortality associated with serum antioxidant (vitamin E and carotenoids) and vitamin A levels using Cox regression models to estimate hazards ratios (HR) and 95 % CI.
Setting: The National Health and Nutrition Examination Survey (NHANES) 1999–2002 was followed up through 31 December 2015.
Participants: The NHANES 1999–2002 cohort included 8758 participants aged ≥ 20 years. Serum carotenoid levels were only assessed for the 1999–2000 cycle. Therefore, sample size for each assessed antioxidant ranged from 4633 to 8758.
Results: Serum vitamin E level was positively associated with all-cause mortality (HR = 1·22, 95 % CI 1·04, 1·43, highest *v.* lowest quartile). No other antioxidants were associated with mortality in overall analysis. In race/ethnicity-specific analyses, high vitamin E and α-tocopherol levels were associated with increased risk of all-cause mortality among non-Hispanic Whites. Among non-Hispanic Blacks, serum α-tocopherol level was associated with decreased risk of cancer mortality (HR = 0·30, 95 % CI 0·12, 0·75, third *v.* first quartile) and total carotenoid levels with reduced risk of CVD mortality (HR = 0·26; 95 % CI 0·07, 0·97, second *v.* lowest quartile). Hispanics with high β-carotene levels had reduced risk of CVD mortality.
Conclusions: Serum antioxidant levels may be related to mortality; these associations may differ by race/ethnicity and appeared to be non-linear for all-cause and cause-specific mortality. Further studies are needed to confirm our results.

Keywords
Antioxidant micronutrients
CVD
Cancer
All-cause
Mortality
Race/Ethnicity

Oxidative stress is a shift in the balance of oxidants and antioxidants in favour of the former, and an increase in the intracellular levels of reactive oxidative species or inadequate levels of antioxidants can cause damage to lipids, proteins and DNA[1,2]. Strong evidence supports the role of oxidative stress and chronic inflammation in the development of chronic diseases[3] including CVD[4] and cancer[5], as well as increased risk of all-cause and cause-specific mortality[6]. Dietary factors with anti-inflammatory and antioxidant properties may aid in lowering the risk of chronic diseases and mortality.

Lipid-soluble micronutrients such as vitamin A (retinol), vitamin E (tocopherols) and carotenoids are potent antioxidants with anti-inflammatory properties, with broad effects on immune function providing protection against oxidative damage[7]. Several observational studies have shown that higher serum levels and/or dietary intake of carotenoids, α-tocopherol (the predominate form of vitamin E) and β-carotene were associated with reduced risk of chronic disease as well as cause-specific and all-cause mortality[8–10]. However, clinical trials report no benefit of vitamins A, E or β-carotene against incidence of chronic disease or mortality[11]. A comprehensive systematic review found that higher levels of antioxidants, including β-carotene, and vitamins E and A may increase the risk of mortality[12], suggesting the presence of non-linear relationships with a threshold of efficacy and toxicity on disease or mortality risk. To date, optimal levels of serum antioxidants for protection against

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disease incidence and mortality have not been fully elucidated.

The burden of chronic diseases varies by race/ethnicity in the USA, with minority populations having a generally greater burden than non-Hispanic White population, although trends are not universal[13]. As such, minority racial/ethnic groups have shorter life expectancies, higher incidence of chronic diseases and less favourable mortality outcomes than their non-Hispanic White counterparts[13]. Differential chronic inflammation and oxidative stress may contribute to these disparities. One study reported that chronic inflammatory markers such as C-reactive protein (CRP) and IL-6 were significantly higher in African Americans[14], while adiponectin levels were lower when compared with their White counterparts[14], suggesting increased exposure to oxidative stress and chronic inflammation in African American populations. Furthermore, ethnicity has been independently correlated with dietary micronutrient intake and serum levels, including key antioxidants in the US population[15]. One study found that dietary intake of vitamin C, carotenes, vitamin E, folate and iron differed by race/ethnicity, with lower intakes of vitamin E, folate and iron in non-Hispanic Blacks than in non-Hispanic Whites, and higher vitamin C and carotene intake in Mexican Americans than in non-Hispanic Whites[15]. A recent study reported differential use of dietary supplements across racial/ethnic groups, among non-Hispanic Whites (61 %), non-Hispanic Blacks (40 %) and Hispanics (36 %)[16]. Differences in chronic inflammation and oxidative stress[14], dietary intake of micronutrients[17] and use of dietary supplements across racial/ethnic groups[16] may contribute to the observed racial/ethnic disparity in mortality in the USA. Little research, however, has been conducted to assess the non-linear effects of serum lipid-soluble antioxidant levels on mortality, and even less has been done to assess the associations across racial/ethnic groups.

Using data from the National Health and Nutrition Examination Survey (NHANES) 1999–2002, we examined the hypothesis that high serum levels of lipid-soluble antioxidants may be non-linearly associated with lower risk of all-cause, cancer and CVD mortality, and the associations may vary across racial/ethnic groups.

Materials and methods

Study population and outcomes

The current study utilised data from two continuous cycles of the NHANES between 1999 and 2002, in which serum antioxidant levels were analysed. The NHANES is a cross-sectional survey under the purview of the Centers for Disease Control and Prevention, designed to assess health and nutritional status in a nationally representative sample of the non-institutionalised US population, described previously in detail[18]. In total, 10 282 adults were aged 20 years or older at the time of blood collection. Of these, 1524 participants were excluded due to pregnancy or lactation at baseline, as well as missing serum antioxidant levels. Finally, a total of 8758 participants were included in the current analysis (Fig. 1). All participants provided written informed consent, and the Institutional Review Board of the National Center for Health Statistics approved the study protocol[19].

Participants were followed for mortality status from the date of Mobile Examination Center (MEC) visit until 31 December 2015. Mortality outcomes were determined using matching methods between NHANES and the National Death Index death certificate records described previously[20]. Additionally, Social Security Administration, Centers for Medicare and Medicaid Services or death certificate review were used for confirmation of mortality status[20]. The ninth and tenth revision of the International Statistical Classifications of Diseases, Injuries and Causes of Death (ICD-9 and ICD-10) were utilised to classify deaths from CVD (I00-I78) and cancer (C00-C97)[21]. All-cause mortality was classified as death from any cause.

Assessment of serum lipid-soluble antioxidants/micronutrients

Blood was drawn from participants in the MEC by phlebotomists. Serum levels of vitamin A (measured here as retinol), vitamin E (measured here as α-tocopherol and γ-tocopherol) and the six major carotenoids (α-carotene, trans-β-carotene, cis β-carotene, β-cryptoxanthin, combined lutein/zeaxanthin and trans-lycopene) were assessed by isocratic HPLC, and spectrophotometric methods were used for quantitative analysis[22]. Total carotenoid levels were calculated by summing values for the six major carotenoids as described in a previous report[23]. β-Carotene was calculated by summing levels of the two major components of the nutrient: cis and trans β-carotene. α-Tocopherol was calculated by subtracting γ-tocopherol from total vitamin E levels. Isoforms of the tocopherols (α and γ) were assessed independently due to their different physiological properties[24]. Serum carotenoid levels were only assessed for the 1999–2000 cycle. Therefore, sample size for each assessed antioxidant ranged from 4633 to 8758. All serum antioxidant nutrient levels were captured in microgram per deciliter (μg/dl).

Statistical analysis

Due to the complex nature of the NHANES multistage clustered probability sampling, the 'Survey' procedure in SAS 9.4 software (SAS Institute) was used to estimate variance with incorporation of sampling weights in the study design. Hazards ratios (HR) and 95 % CI for the associations between serum antioxidant levels and all-cause, cancer or cardiovascular mortality were estimated from Cox proportional hazards regression models. Participants were followed until an event (death) or the end of the study

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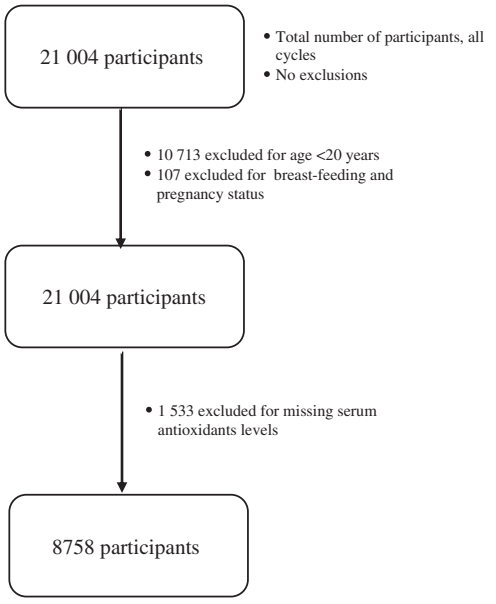

Fig. 1 Study participants flow chart

period. Those without an event were censored at the conclusion of the study period (31 December 2015). Serum antioxidant nutrient levels were categorised into quartiles based upon their concentrations in the study population, using the lowest category as the reference for all antioxidants except vitamin A. Because previous studies have reported normal range of vitamin A for normal dark adaptation[25] and a possible threshold effect of vitamin A on mortality[26], we utilised the second quartile as the reference in our analyses.

Potential confounders included in multivariable modelling were age, sex and race/ethnicity, BMI, history of CVD, cancer and diabetes, as well as alcohol use, smoking status and physical activity. Alcohol use was defined dichotomously as used within the past 12 months from time of MEC exam, yes or no. Physical activity was categorised into two strata: no and low level, and moderate and high level, defined as no vigorous or moderate physical activity in the past 30 d, and at least 10 min of sustained moderate or vigorous physical activity, respectively. Diabetes status was defined as having Hb A1C $\geq$ 6·5 % at the time of the MEC visit, as per the WHO guidelines[27]. Prior studies showed a relationship between CRP and mortality[28], and γ-tocopherol has been associated with CRP[29]. We examined associations with adjustment for log-transformed CRP (since it was not normally distributed in the current study); results were similar to those without adjustment for CRP. Therefore, log-transformed CRP was ultimately not included in final models. Because α-tocopherol and γ-tocopherol are lipophilic compounds transported in lipoprotein fractions in the blood, their concentration depends on total lipid serum levels[30]. Therefore, total cholesterol to total tocopherol ratio was calculated and categorised into quartiles. Similar associations were observed when examining total cholesterol to total tocopherol ratio (data not shown) and total tocopherol levels (Vitamin E) with cause-specific and all-cause mortality; thus, the ratio was not included in final analysis. The temporal association between serum antioxidants and disease history (including CVD, cancer, liver problems and diabetes) cannot be determined due to the cross-sectional nature of the survey. In order to assess whether disease history was a mediator of the association between antioxidant serum status and mortality, separate analyses were run excluding disease history. Results were not materially changed; therefore, the history of diseases was included in final models. Supplement use was defined as a participant reporting use of a multivitamin at the time of the MEC exam. In order to avoid over adjustment of the potential association of antioxidant serum status with mortality, and because inclusion of this covariate did not alter our findings, we did not include supplement use in our final models. Because total daily energetic intake and season of MEC visit did not alter our findings, we chose not to retain them in our final models. Serum antioxidant levels were compared with characteristics using the non-parametric Wilcoxon rank sum test. To test our hypothesis that the associations of serum antioxidants on mortality may vary by race/ethnicity, we analysed these associations within strata of race/ethnicity. Interaction between serum antioxidant nutrients and race/ethnicity was tested in the Cox regression model by evaluating a multiplicative term using the Wald test (data not shown). Sensitivity analyses were conducted after excluding participants who died within 2 years of blood collection; however, results did not change materially (data not shown). Tests for dose–response relationship were estimated by fitting models with exposure variables included as continuous variables for all serum antioxidants in the overall study population and stratified by race/ethnicity (data not shown). All reported *P*-values were two-tailed, and statistical significance was set at 0·05. R-Studio was used to visualise relationships of serum antioxidants and all-cause mortality stratified by race/ethnicity.

Results

Of the 8758 subjects included in the study, 2083 died (1151 men and 932 women) during a median follow-up time of 14·2 years. Three hundred and forty-five deaths were due to CVD, and 429 deaths were due to cancer. Table 1 presents the baseline serum antioxidants levels by demographic and selected lifestyle factors.

Associations of serum levels of lipid-soluble antioxidant micronutrient levels with all-cause, CVD and cancer mortality after adjustment for age at blood collection, race/ethnicity and other potential confounders are presented in Table 2. For total vitamin E, compared with participants in the lowest quartile, those in the highest quartile had an increased risk of all-cause mortality (HR = 1·22, 95 % CI 1·04, 1·43), and those

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Table 1 Serum antioxidant nutrient levels among adults ≥ 20 years, NHANES 1999–2002

		Vitamin A (μg/dl)		Vitamin E (μg/dl)		α-Tocopherol (μg/dl)		γ-Tocopherol (μg/dl)		Total carotenoids (μg/dl)		β-Carotene (μg/dl)	
Factors	Participants (*n*)	Median	SE	Median	SE	Median	SE	Median	SE	Median	SE	Median	SE
Sex													
Male	4171	63·12	0·62†	1134·17	16·76†	868·53	17·64†	219·98	4·86†	62·99	1·56†	11·51	0·33†
Female	4587	54·96	0·48†	1171·54	14·64†	909·18	14·45†	212·82	5·20†	64·33	1·59†	14·89	0·67†
Age													
20–39 years	3096	54·68	0·54†	960·98	9·58†	719·47	9·57†	218·69	4·69†	60·57	1·59*	10·19	0·37†
40–59 years	2664	60·55	0·50†	1249·96	13·77†	983·04	16·48†	224·81	5·87†	65·91	1·83*	13·73	0·52†
≥ 60 years	2998	64·45	0·51†	1477·18	18·58†	1243·42	20·47†	189·90	6·05†	68·09	2·82*	18·46	1·03†
Race/Ethnicity													
Non-Hispanic White	4306	61·43	0·61†	1211·00	12·16†	946·67	15·96†	212·97	5·16†	63·03	1·49*	12·99	0·56†
Hispanic	2569	53·09	0·78†	1070·45	16·24†	855·84	18·40†	205·47	5·00†	64·98	3·06*	13·07	0·82†
Non-Hispanic Black	1603	50·58	0·65†	964·18	12·33†	694·70	11·86†	248·52	6·77†	61·95	2·00*	11·29	0·56†
Supplement use													
Yes	4393	62·14	0·61†	1368·27	18·03†	1158·53	17·94†	170·92	4·48†	68·86	1·80†	16·80	0·71†
No	4355	55·55	0·45†	991·76	8·85†	716·16	11·82†	257·74	5·82†	58·70	1·44†	10·24	0·29†
BMI													
≤ 25	2717	58·04	0·61†	1088·32	16·10†	872·99	18·63†	177·61	4·66†	70·50	2·06†	16·54	0·78†
> 25	5751	59·40	0·53†	1191·50	11·05†	903·04	15·57†	238·76	5·36†	60·89	1·02†	11·70	0·37†
Smoking status													
Never	4527	57·26	0·59†	1154·41	17·67†	902	15·32†	208·65	5·61†	68·66	1·39†	15·10	0·55†
Former	2325	63·10	0·67†	1278·56	13·74†	1050·52	18·81†	209·97	4·65†	66·39	1·76†	14·58	0·75†
Current	1890	58·14	0·69†	1035·42	14·32†	760·51	14·62†	228·51	4·28†	52·45	1·65	8·54	0·34†

NHANES, National Health and Nutrition Examination Survey.
Participant column total reflects total number of possible participants which varies by antioxidant nutrient level availability.
Wilcoxon rank test revealed significance at *$P < 0·05$ or †$P < 0·01$.

Table 2 All-cause and cause-specific mortality and serum antioxidant level, NHANES 1999–2002*

		All-cause mortality			CVD-specific mortality			Cancer-specific mortality		
Serum antioxidants (μg/dl)	Participants (*n*)	Deaths (*n*)	HR	95 % CI	Deaths (*n*)	HR	95 % CI	Deaths (*n*)	HR	95 % CI
Vitamin A										
Q_1 (< 48·43)	2648	470	1·14	0·92, 1·41	74	0·98	0·56, 1·72	92	1·29	0·88, 1·88
Q_2 (48·43–59·99)	2126	459	Reference	–	70	Reference	–	117	Reference	–
Q_3 (60·00–70·60)	2014	507	1·02	0·87, 1·21	92	1·22	0·70, 2·12	88	0·87	0·64, 1·18
Q_4 (> 70·60)	1970	647	1·06	0·91, 1·24	109	1·23	0·73, 2·07	132	0·96	0·72, 1·29
Vitamin E										
Q_1 (< 912·80)	2101	351	Reference	–	59	Reference	–	82	Reference	–
Q_2 (912·80–1156·35)	2189	475	1·22	1·00, 1·49	78	1·01	0·59, 1·73	101	0·97	0·70, 1·33
Q_3 (1156·36–1532·64)	2192	508	1·04	0·86, 1·25	91	1·09	0·64, 1·86	103	0·78	0·55, 1·10
Q_4 (> 1532·64)	2239	737	1·22	1·04, 1·43	116	1·23	0·72, 2·10	143	0·95	0·69, 1·30
α-Tocopherol										
Q_1 (< 649·11)	1933	374	Reference	–	61	Reference	–	88	Reference	–
Q_2 (649·12–890·80)	2072	424	1·17	0·92, 1·49	66	1·08	0·62, 1·87	88	0·99	0·70, 1·40
Q_3 (890·81–1290·08)	2063	431	0·99	0·80, 1·23	72	1·1	0·63, 1·93	98	0·86	0·60, 1·25
Q_4 (> 1290·08)	2088	692	1·18	0·97, 1·43	117	1·41	0·80, 2·48	123	0·91	0·62, 1·35
γ-Tocopherol										
Q_1 (< 137·90)	1965	556	Reference	–	92	Reference	–	103	Reference	–
Q_2 (137·90–215·59)	2107	422	0·90	0·75, 1·09	58	0·75	0·45, 1·27	103	1·11	0·74, 1·66
Q_3 (215·60–303·91)	2022	403	0·98	0·82, 1·14	75	1·12	0·70, 1·78	84	0·86	0·60, 1·22
Q_4 (> 303·91)	2101	551	1·05	0·90, 1·24	92	0·96	0·61, 1·51	107	0·97	0·71, 1·32
Total carotenoids										
Q_1 (< 46·58)	1090	309	Reference	–	45	Reference	–	61	Reference	–
Q_2 (46·58–63·62)	1132	237	0·86	0·74, 1·00	36	1·02	0·68, 1·55	54	1·10	0·75, 1·61
Q_3 (63·63–87·21)	1143	234	0·99	0·79, 1·25	27	0·89	0·36, 2·25	52	1·18	0·71, 1·97
Q_4 (> 87·21)	1268	255	0·80	0·63, 1·03	35	0·98	0·47, 2·05	50	0·92	0·54, 1·55
β-Carotene										
Q_1 (< 7·50)	1046	208	Reference	–	28	Reference	–	49	Reference	–
Q_2 (7·50–12·89)	1132	222	0·97	0·72, 1·33	31	1·05	0·42, 2·66	56	1·09	0·76, 1·58
Q_3 (12·90–23·27)	1224	278	1·03	0·78, 1·36	39	1·42	0·61, 3·31	48	0·86	0·50, 1·47
Q_4 (> 23·27)	1256	330	0·99	0·71, 1·41	46	1·09	0·48, 2·46	64	0·98	0·57, 1·69

NHANES, National Health and Nutrition Examination Survey.
*All analyses were adjusted for age, sex, race/ethnicity, smoking status, alcohol use, physical activity level, BMI, history of CVD, cancer, liver problems and diabetes.

in the second quartile were also associated with an increased risk of all-cause mortality (HR = 1·22, 95 % CI 1·00, 1·49). Similarly, there was a suggestion of a positive association between high α-tocopherol level and all-cause mortality; however, results were not statistically significant. Compared with the first quartile, the second and highest quartile of serum total carotenoid levels tended to be associated with lower risk of all-cause mortality, but none of these associations were statistically significant. There were no associations of serum vitamin A, γ-tocopherol and β-carotene levels with all-cause mortality. Furthermore, no associations between the antioxidants analysed and risk of CVD or cancer mortality were observed for the overall study population. Additionally, no linear trends were observed in the overall study population.

We further conducted stratified analyses for associations of serum antioxidants with all-cause mortality by race/ethnicity (Table 3). For total vitamin E, the positive association with all-cause mortality remained significant among non-Hispanic Whites (HR = 1·37, 95 % CI 1·15, 1·63, highest *v.* lowest quartile, $P_{trend} < 0·01$), while non-Hispanic Blacks with total vitamin E concentrations in the third quartile showed suggestions of reduced risk (HR = 0·71, 95 % CI 0·48, 1·07). Similar patterns of association were observed for serum α-tocopherol levels (HR = 0·66, 95 % CI 0·42, 1·03, third *v.* lowest quartile) among non-Hispanic Blacks. Serum levels of antioxidants were not associated with risk of all-cause mortality among Hispanics. Figure 2 showed U-shaped associations between serum levels of vitamin E, α-tocopherol and all-cause mortality among non-Hispanic Blacks, with significant reduction in risk in the third quartile. Additionally, although suggestions of linear associations with all-cause mortality were observed among non-Hispanic Blacks, tests for trend revealed no significance for serum total carotenoids ($P_{trend} = 0·08$) and γ-tocopherol levels ($P_{trend} = 0·08$). No linear associations were observed for other antioxidants and all-cause mortality in race/ethnicity stratified analysis.

Results for cancer and CVD mortality, stratified by race/ethnicity, are presented in online supplementary material, Supplemental Tables 1 and 2, respectively. Among non-Hispanic Blacks, the highest quartile of serum total vitamin E and third quartile of serum α-tocopherol levels were inversely associated with cancer mortality, when compared with the lowest quartiles (total vitamin E: HR = 0·46, 95 % CI 0·21, 1·00, $P_{trend} < 0·01$, and α-tocopherol: HR = 0·30, 95 % CI 0·12, 0·75, $P_{trend} < 0·01$). There was suggestion of an inverse association between high total carotenoid levels and cancer mortality among non-Hispanic Blacks; meanwhile; for CVD mortality, the pattern of association was similar, with the second quartile of total carotenoids showing an inverse association with CVD mortality (HR = 0·26; 95 % CI 0·07, 0·97). The second and highest quartiles of serum β-carotene were associated with lower risk of CVD mortality (HR = 0·38; 95 % CI 0·16, 0·88; HR = 0·05; 95 % CI 0·01, 0·38, respectively) among Hispanics when compared with the lowest quartile, and the test for trend was statistically significant ($P_{trend} < 0·01$). There were no significant differences in the risk of cancer or CVD mortality for different levels of serum antioxidants among non-Hispanic Whites. There were no significant interactions between serum antioxidant levels and race/ethnicity in relation to all-cause mortality, CVD mortality or cancer mortality.

Discussion

Utilising a representative sample of US adults, we found that serum antioxidant levels related to mortality, but the associations differed by race/ethnicity, and were non-linear for all-cause and cause-specific mortality. To our knowledge, few studies have examined the associations of serum antioxidant levels with mortality by race/ethnicity, and limited research has examined non-linear associations with mortality[26].

Vitamin E is a chain-breaking antioxidant with the function of preventing propagation of free radical reactions; it is primarily composed of α- and γ-tocopherol, with higher concentrations of α-tocopherol than any other tocopherol in serum[31]. Furthermore, γ-tocopherol inhibits cancer cell progression and proliferation[31] and may play a role in CVD prevention through the decrease of lipid peroxidation, platelet accumulation and monocyte proatherogenic effects, to name a few[32]. The mechanisms through which vitamin E acts to prevent disease remain to be fully elucidated. The Institute of Medicine has identified a concentration of 5·16 μg/ml (516 μg/dl) of α-tocopherol as sufficient to prevent acute vitamin E deficiency, while pointing out the need for more research to determine the levels and types of tocopherols important for optimal nutritional status and their mechanisms in chronic disease prevention[33]. In our population, which consisted of over 50 % non-Hispanic Whites, we found that high total vitamin E or α-tocopherol concentration was associated with increased risk of all-cause mortality, although no dose–response relationships were observed. These patterns of association were similar only among non-Hispanic Whites. A recent Mendelian randomisation of subject of predominantly European ancestry found non-linear associations of circulating vitamin E levels with risk of coronary artery disease and myocardial infarction and reported significant increase in the risk of coronary artery disease and myocardial infarction for those with higher circulating levels of vitamin E[34], which may support our findings among non-Hispanic Whites. Consistent with previous studies[26,30], we observed U-shaped curves in the association of total vitamin E or α-tocopherol levels with all-cause mortality among non-Hispanic Blacks, with greatest reductions in mortality risk with serum vitamin E

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Table 3 All-cause mortality and serum antioxidant level by race/ethnicity, NHANES 1999–2002*

	Non-Hispanic Whites			Non-Hispanic Blacks			Hispanics		
Serum antioxidants (μg/dl)	Deaths/Cohort	HR	95 % CI	Deaths/Cohort	HR	95 % CI	Deaths/Cohort	HR	95 % CI
Vitamin A									
Q_1 (< 48·43)	173/857	1·16	0·88, 1·51	113/677	0·97	0·65, 1·44	176/1012	1·22	0·88, 1·68
Q_2 (48·43–59·99)	249/996	Reference	–	82/387	Reference	–	121/686	Reference	–
Q_3 (60·00–70·60)	325/1141	0·99	0·81, 1·22	70/285	0·85	0·65, 1·10	98/514	1·00	0·74, 1·36
Q_4 (> 70·60)	445/1312	1·04	0·87, 1·24	102/254	1·15	0·75, 1·76	94/357	1·12	0·82, 1·52
Vitamin E									
Q_1 (< 912·80)	139/800	Reference	–	121/607	Reference	–	84/626	Reference	–
Q_2 (912·80–1156·35)	239/938	1·32	1·05, 1·67	102/486	0·95	0·61, 1·47	130/717	1·22	0·79, 1·89
Q_3 (1156·36–1532·64)	285/1113	1·12	0·89, 1·41	77/312	0·71	0·48, 1·07	136/686	1·22	0·71, 2·10
Q_4 (> 1532·64)	519/1420	1·37	1·15, 1·63	65/194	0·98	0·64, 1·49	139/542	1·26	0·64, 2·48
α-Tocopherol									
Q_1 (< 649·11)	161/800	Reference	–	138/614	Reference	–	70/461	Reference	–
Q_2 (649·12–890·80)	224/883	1·30	0·93, 1·80	94/468	0·83	0·53, 1·29	99/670	0·93	0·60, 1·45
Q_3 (890·81–1290·08)	246/1039	1·06	0·82, 1·39	63/283	0·66	0·42, 1·03	114/662	1·03	0·56, 1·89
Q_4 (> 1290·08)	500/1364	1·29	1·01, 1·65	63/194	0·87	0·57, 1·33	116/456	1·04	0·54, 2·00
γ-Tocopherol									
Q_1 (< 137·90)	389/1187	Reference	–	51/227	Reference	–	104/489	Reference	–
Q_2 (137·90–215·59)	234/972	0·89	0·71, 1·10	77/392	0·88	0·53, 1·45	102/668	0·96	0·64, 1·43
Q_3 (215·60–303·91)	221/943	1·01	0·82, 1·24	87/451	0·88	0·55, 1·40	90/565	0·94	0·61, 1·44
Q_4 (> 303·91)	296/1017	1·02	0·85, 1·23	145/494	1·29	0·82, 2·02	103/528	0·91	0·63, 1·32
Total carotenoids									
Q_1 (< 46·58)	196/624	Reference	–	63/229	Reference	–	46/219	Reference	–
Q_2 (46·58–63·62)	137/588	0·85	0·68, 1·06	49/221	0·88	0·47, 1·65	48/288	1·02	0·59, 1·76
Q_3 (63·63–87·21)	150/592	1·10	0·81, 1·49	33/202	0·56	0·31, 0·99	47/295	1·23	0·96, 1·58
Q_4 (> 87·21)	171/637	0·90	0·70, 1·16	30/196	0·44	0·15, 1·29	51/387	0·83	0·40, 1·73
β-Carotene									
Q_1 (< 7·50)	121/533	Reference	–	45/237	Reference	–	40/249	Reference	–
Q_2 (7·50–12·89)	129/590	0·94	0·61, 1·44	41/222	1·13	0·49, 2·62	48/282	1·03	0·65, 1·62
Q_3 (12·90–23·27)	175/619	1·05	0·73, 1·51	46/212	1·09	0·40, 2·92	52/348	0·95	0·63, 1·42
Q_4 (> 23·27)	231/717	1·07	0·72, 1·59	43/179	0·80	0·27, 2·39	53/315	0·85	0·52, 1·39

NHANES, National Health and Nutrition Examination Survey.
*All analyses were adjusted for age, sex, smoking status, alcohol use, physical activity level, BMI, history of CVD, cancer, liver problems and diabetes.

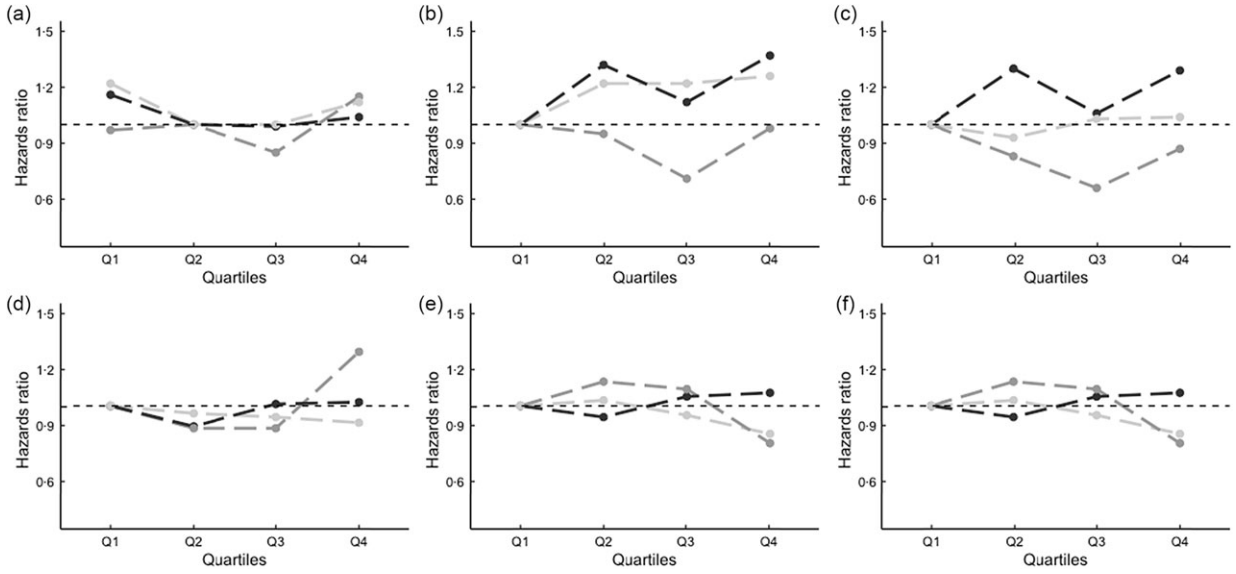

Fig. 2 Lipid-soluble antioxidants serum levels and all-cause mortality by race/ethnicity*.*Interaction tests were performed evaluating a multiplicative term using the Wald test. (a) Vitamin A, (b) vitamin E, (c) α-Tocopherol, (d) γ-Tocopherol, (e) total carotenoids, (f) β-carotene. NH, non-Hispanic; ●, NH White; ●, NH Black; ●, Hispanic

at 1156·35–1532·64 μg/dl or α-tocopherol at 890·81–1290·08 μg/dl, comparable to previously reported optimal ranges[30]. Additionally, total vitamin E concentrations were inversely associated with cancer mortality in the linear dose–response analysis among non-Hispanic Blacks.

Variations in associations of total vitamin E and α-tocopherol with risk of mortality across different racial/ethnic groups may be explained, partially, by differences in serum concentrations of these micronutrients. In our study, serum total vitamin E and α-tocopherol levels were considerably higher among non-Hispanic Whites than non-Hispanic Blacks and Hispanics, and the prevalence of vitamin E deficiency was higher in the two minority groups. Differences in dietary intake of vitamin E might contribute to the observed variations in serum vitamin E levels between non-Hispanic Whites, non-Hispanic Blacks and Hispanics[15]. Supplement use also mirrored serum α-tocopherol deficiency, with highest use among non-Hispanic Whites (62 %) followed by Hispanics (38 %) and non-Hispanic Blacks (37 %), suggesting that use of dietary supplements may be necessary in inadequate populations to prevent mortality. It is possible that over-supplementation may have contributed, at least partially, to the observed positive association between vitamin E or α-tocopherol and all-cause mortality among non-Hispanic Whites in our study, suggesting the potential harms of over-supplementation[35]. Future studies are warranted to confirm these findings and to elucidate the underlying mechanisms for racial/ethnic differences in the relationships between total vitamin E levels, α-tocopherol and mortality.

With respect to γ-tocopherol, a recent report found positive associations for serum γ-tocopherol with all-cause, cancer and CVD-specific mortality in the overall population and among all-cause mortality in Whites, with suggestions of a positive association among non-Hispanic Blacks[36]. Similar patterns of association among non-Hispanic Whites and non-Hispanic Blacks were observed in our study, although associations were not statistically significant. This inconsistency could be the result of overall higher γ-tocopherol levels in our study population, possibly due to significant age differences between the two populations considering that serum γ-tocopherol levels may decrease with older age[37]. Potential differences in physiological properties and functions of α- and γ-tocopherol could further complicate the interpretation of our observed results.

Blood carotenoids are a reliable biomarker of usual intake of fruits and vegetables[33,38]. Carotenoids can reduce damage from reactive oxidative species, inhibit lipid peroxidation and are involved in cellular communications that regulate proliferation and apoptosis[39,40]. Inverse associations between dietary or circulating levels of carotenoids and mortality have been previously observed[23,41], suggesting the potential benefits of carotenoids for optimal health and prevention of disease. In our study, we did not observe a strong protective association for serum carotenoid levels with all-cause mortality. However, there appeared to be threshold effects in the non-Hispanic Black population, in which reduced risk of CVD mortality was observed for total carotenoid levels between 46·58 and 63·62 μg/dl. This observation is in concordance with a study[23] that reported incremental benefits of serum total carotenoid levels in relation to mortality that plateaued at 1·0 mmol/l (~53·73 μg/dl). Furthermore, our results were similar to another study that found non-linear relationships for serum carotenoid levels with risk of mortality among colorectal cancer patients[3]. In our study, among Hispanics, an inverse association between the second and highest quartiles of serum β-carotene level with CVD

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mortality was observed. However, carotenoid levels were only assessed in one cycle; thus, cause-specific mortality was low in stratified analysis and results should be interpreted cautiously. Future studies with larger representation of minority populations are necessary to validate our findings. Finally, other factors likely influence the absorption, transportation, storage and utilisation of carotenoids. Lower levels of serum carotenoids have been observed among smokers[42] and obese individuals[43], both of which increase chronic inflammation and oxidative stress[44,45], and may lead to increased mobilisation of these antioxidants. Physiological relationships between inflammation, oxidative stress, carotenoids and mortality risk, as well as differences between racial/ethnic populations must be investigated in future studies, particularly minority populations.

The strengths of the current study include a nationally representative sample of the USA, large sample size and long follow-up time, which enabled us the power to detect relatively weak associations and further assess associations by race/ethnicity. Rigorous methods utilised by the NHANES for obtaining mortality status reduce the chances of information bias influencing results. Several limitations must be mentioned. The use of one serum sample limited our ability to assess the impact of long-term antioxidant levels on mortality. Furthermore, the use of a single measurement for serum antioxidant level may have misclassified exposures for some participants, attenuating risk estimates; however, serum levels of tocopherol, total carotenoids and β-carotene measured 2–4 weeks apart showed good stability (correlation ranging from 0·79 to 0·84)[46]. Another limitation secondary data analysis is that cause-specific events were defined broadly in the study population. These cause-specific outcomes (e.g., CVD, cancer) possess heterogeneous mechanisms which we were unable to assess in the current study. In addition, the distribution of all-cause and cause-specific mortality likely differs across racial/ethnic groups for reasons other than inflammation, and race/ethnicity specific analysis may have masked relationships linking antioxidant serum status to inflammation-related cause of death. Furthermore, our population was younger than most cohorts assessing these associations; thus, average follow-up of 14 years may have been insufficient to detect effects if the primary benefit of antioxidants is to extend life expectancy or slow progression of disease in its earliest stages. Additionally, we have baseline information for potential confounders but no information on those factors over the follow-up period. We cannot rule out that we did not have enough power to detect weak associations in some strata. Further, the imbalanced and relatively small number of participants in each racial/ethnic group likely influenced the subgroup specific findings.

In summary, we found that over a certain level, there was no additional benefit of higher serum concentrations of certain lipid-soluble antioxidants on overall survival. We found that the associations of serum antioxidant levels and mortality may differ between racial/ethnic groups, suggesting race/ethnicity-specific thresholds. In the current study, particularly, for total vitamin E, α-tocopherol and total carotenoids, there were different thresholds for the associations with all-cause or cause-specific mortality across racial/ethnic groups. Studies with greater representation of minority populations and longer follow-up are warranted to confirm these findings.

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Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980020004619

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