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ORIGINAL CONTRIBUTION

Association between leukocyte telomere length and serum carotenoid in US adults

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Abstract
Purpose Telomere length is a biomarker for aging. It is known that oxidative stress can accelerate telomere shortening, whereas antioxidants can delay their shortening. Carotenoids as antioxidants are favorably associated with health- and aging-related diseases caused by oxidative stress, but their association with telomere length is less certain. We investigated the association between blood carotenoid levels and leukocyte telomere length in a representative sample of US adults.
Methods We analyzed 3660 participants aged 20 years and older in the 1999–2002 National Health and Nutrition Examination Survey. The levels of carotenoids—alpha-carotene, beta-carotene (*trans* + *cis*), beta-cryptoxanthin, combined lutein/zeaxanthin, and *trans*-lycopene—were measured using high-performance liquid chromatography. The leukocyte telomere length (*T*/*S* ratio) was assayed using the quantitative polymerase chain reaction method.
Results A doubling of blood alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin was associated with approximately 2 % longer telomeres. Compared with the lowest carotenoid quartile of alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin, telomere length for adults with the highest quartiles was significantly increased by 5–8 %.
Conclusion We found that increasing levels of blood carotenoid were significantly associated with longer leukocyte telomeres in US adults. High intake of carotenoid-rich food may play a role in protecting telomeres and regulating telomere length.

Keywords Telomere · Oxidative stress · Carotenoid · Antioxidant

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Introduction

Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes, with a highly conserved repetitive DNA sequence (TTAGGG in human). Telomeres serve multiple functions: They maintain chromosome stability and integrity, regulate cellular proliferation, and prevent chromosome end fusions [1]. In the most somatic cells, telomeres are shortened with each cell division owing to incomplete DNA replication of chromosome ends [2]. In addition, endogenous (i.e., genetics, inflammation, and DNA damage) and environmental (i.e., smoking, alcohol, and life stress) factors are involved in telomere maintenance and regulation [3]. Notably, oxidative stress can accelerate telomere shortening, whereas antioxidants can delay their shortening in human cells [4–6]. Evidence from human studies suggests that dietary intake and blood levels of antioxidants (i.e., vegetable, vitamins C, and vitamin E) are positively associated with telomere length [7, 8].

Carotenoids are naturally occurring yellow, orange, or red pigments found in many fruits and vegetables. Epidemiologic studies have suggested that dietary carotenoid intake or circulating carotenoid serum levels are positively associated with health- and aging-related diseases caused by oxidative stress [9–11]. A recent study examined the

association between the plasma levels of antioxidative micronutrients and the leukocyte telomere length in 786 elderly adults, who had participated in the Austrian Stroke Prevention Study and reported a significantly protective effect of combined lutein/zeaxanthin on the telomere length [12]. Based on this finding, carotenoid may prevent telomere loss by protecting against oxidative stress-induced DNA damage. However, there is still insufficient evidence regarding the link between carotenoids and telomere length in subjects across a wider span of ages.

In this study, we investigated the association between blood carotenoid levels—provitamin A carotenoids (the carotenes, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin) and non-vitamin A carotenoids (combined lutein/zeaxanthin, and *trans*-lycopene)—and leukocyte telomere length in a representative sample of US adults who participated in the 2001–2002 National Health and Nutrition Examination Survey (NHANES).

Methods

Study population

The NHANES, conducted by the Centers for Disease Control and Prevention, is a nationally representative survey of the non-institutionalized US civilian population. The study protocols of the NHANES (1999–2002) were approved by the National Center for Health Statistics Institutional Review Board. Oral and written consent was obtained from all participants.

This study used data collected from 2001 to 2002, in simultaneous measurements of leukocyte telomere length and blood carotenoid levels. Of 5411 participants aged more than 20 years old (the age threshold was set to provide blood samples for DNA purification), we selected 4260 (79 % of those eligible) who agreed to provide DNA samples and specific consent for future genetic research. We then excluded 600 participants for whom there were no data regarding either carotenoids in the blood or at least one of the variables of interest. The result was a final sample size of 3660 participants.

Measurement of serum carotenoid level

Serum specimens for measurement of carotenoid in the blood were processed, stored, and shipped for the analysis to the Division of Laboratory Sciences at the Centers for Disease Control and Prevention's National Center for Environmental Health. The analytical laboratory followed extensive quality control procedures [13].

Carotenoid levels were measured in serum because serum carotenoid levels are useful biomarkers of the total dietary intake of vegetables and fruits. The most common types of carotenoids in humans—alpha-carotene, *trans*-beta-carotene, *cis*-beta-carotene, beta-cryptoxanthin, combined lutein/zeaxanthin, and *trans*-lycopene—were assayed in NHANES 2001–2002. Detailed procedures are described elsewhere [13]. High-performance liquid chromatography with photodiode array detection was used to quantify these assays. The median inter-assay coefficients of variation were 8.1 % for alpha-carotene, 5.2 % for *trans*-beta-carotene, 23.3 % for *cis*-beta-carotene, 6.4 % for beta-cryptoxanthin, 11.8 % for combined lutein/zeaxanthin, and 4.6 % for *trans*-lycopene [13].

Measurement of telomere length

DNA samples were extracted from whole blood using the Puregene (D-50K) kit protocol (Gentra Systems, Inc., Minneapolis, Minnesota) and stored at −80 °C. Telomere length assays were performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco. The quantitative polymerase chain reaction method described by Cawthon [14] and Lin et al. [15] was used to measure telomere length, which was determined by the ratio of the telomere repeat copy number (*T*) to the single-copy gene (*S*) copy number (*T*/*S* ratio) as a control to normalize DNA. The mean telomere length variable was derived by averaging two separate measurements of leukocyte telomere lengths with a maximum variability of 7 %. If the variation was >7 %, a third assay was performed and the average of the two closest values was used. Details on analytical methods for telomere length have been previously described [16].

Variables of interest

Questionnaire information included age (20–29, 30–39, 40–49, 50–59, 60–69, 70–79, or ≥80 years old), sex (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), annual household income (<$20,000 or ≥$20,000), smoking status (current, former, or never), and alcohol consumption (drinker or non-drinker). With regard to disease history, diabetes was defined as having a fasting plasma glucose level of at least 126 mg/dL, a non-fasting plasma glucose level of at least 200 mg/dL, and current insulin use or prior diagnosis of diabetes by a physician. Metabolic variables and other dietary variables, such as the body mass index (BMI), homocysteine, folate, and omega-3 fatty acids, were included as covariates. BMI, calculated by dividing the individual's weight by his or her height squared, was treated as a continuous variable. BMI was categorized into the following four groups: underweight (<18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), overweight (23–24.9 kg/m^2), and obese (≥25 kg/m^2). We grouped the serum homocysteine and folate levels into

Table 1 Mean leukocyte telomere length (*T*/*S* ratio) according to participants' characteristics ($N = 3660$)

	N (%)	Mean (95 % CI)	*p* value
Age (years)			
20–29	687 (18.8)	1.18 (1.16–1.20)	<0.0001
30–39	643 (17.6)	1.10 (1.08–1.12)	
40–49	677 (18.5)	1.06 (1.04–1.08)	
50–59	507 (13.9)	0.99 (0.97–1.01)	
60–69	536 (14.6)	0.93 (0.91–0.94)	
70–79	377 (10.3)	0.88 (0.86–0.90)	
≥80	233 (6.4)	0.84 (0.81–0.86)	
Gender			
Male	1781 (48.7)	1.00 (0.99–1.01)	<0.0001
Female	1879 (51.3)	1.05 (1.04–1.06)	
Ethnicity			
White	2008 (54.9)	1.00 (0.99–1.01)	<0.0001
Black	630 (17.2)	1.07 (1.05–1.09)	
Hispanic	913 (25.0)	1.04 (1.02–1.05)	
Others	109 (3.0)	1.05 (1.01–1.09)	
Monthly income ($)			
≤20,000	1078 (29.5)	1.01 (1.00–1.03)	<0.0001
>20,000	2582 (70.6)	1.03 (1.02–1.03)	
Smoking status			
Current smoker	828 (22.6)	1.05 (1.03–1.06)	0.3777
Ex-smoker	973 (26.6)	0.97 (0.96–0.98)	
Never smoked	1859 (50.8)	1.04 (1.03–1.05)	
Alcohol drinking			
Drinker	2513 (68.7)	1.03 (1.02–1.04)	0.0018
Non-drinker	1147 (31.3)	1.01 (0.99–1.02)	
History of diabetes			
Yes	399 (10.9)	0.94 (0.92–0.96)	<0.0001
No	3261 (89.1)	1.03 (1.02–1.04)	
BMI (kg/m^2)			
Underweight (<18.5)	63 (1.7)	1.09 (1.01–1.18)	0.0004
Normal weight (18.5–24.9)	1095 (29.9)	1.04 (1.03–1.06)	
Overweight (25–29.9)	1372 (37.5)	1.01 (1.00–1.02)	
Obese (≥30)	1130 (30.9)	1.01 (1.00–1.02)	
Homocysteine, serum (μmol/L)			
Q1 (<6.36)	788 (21.5)	1.10 (1.08–1.12)	<0.0001
Q2 (6.37–7.88)	942 (25.7)	1.05 (1.04–1.07)	
Q3 (7.89–10.00)	989 (27.0)	1.01 (0.99–1.02)	
Q4 (≥10.01)	941 (25.7)	0.95 (0.94–0.96)	
Folate, serum (ng/mL)			
Q1 (<9.1)	959 (26.2)	1.04 (1.03–1.06)	<0.0001
Q2 (9.1–12.8)	951 (26.0)	1.03 (1.01–1.04)	
Q3 (12.9–18.2)	954 (26.1)	1.03 (1.01–1.05)	
Q4 (≥18.3)	796 (21.8)	0.98 (0.96–1.00)	
Dietary eicosapentaenoic (g)			
Q1 (<0.001)	1056 (28.9)	1.02 (1.01–1.04)	0.3258
Q2 (0.001–0.003)	571 (15.6)	1.00 (0.98–1.02)	
Q3 (0.004–0.014)	1078 (29.5)	1.03 (1.02–1.05)	
Q4 (≥0.015)	955 (26.1)	1.03 (1.01–1.04)	

Table 1 continued

	N (%)	Mean (95 % CI)	p value
Dietary docosahexaenoic (g)			
Q1 (<0.001)	732 (20.0)	1.03 (1.01–1.05)	0.7977
Q2 (0.001–0.021)	1011 (27.6)	1.01 (1.00–1.03)	
Q3 (0.022–0.059)	972 (26.6)	1.03 (1.01–1.05)	
Q4 (≥0.060)	945 (25.8)	1.02 (1.01–1.04)	

Table 2 Pearson's correlation structure of serum carotenoid levels

	Alpha-carotene	Beta-carotene (*trans* + *cis*)	Beta-cryptoxanthin	Combined lutein/zeaxanthin	*Trans*-lycopene
Alpha-carotene	1				
Beta-carotene (*trans* + *cis*)	0.65	1			
Beta-cryptoxanthin	0.34	0.41	1		
Combined lutein/zeaxanthin	0.39	0.42	0.47	1	
Trans-lycopene	0.13	0.14	0.16	0.20	1

quartiles based on their distribution. Omega-3 fatty acids were obtained from two diets containing eicosapentaenoic acid and docosahexaenoic acid, in which these two dietary intakes were also categorized into the quartiles.

Statistical analysis

We applied weighted estimates of the population parameters based on the NHANES Analytic and Reporting Guidelines. All the statistical analyses were performed using the PROC SURVEY procedures in SAS 9.2 (SAS Institute, Cary, NC, USA) to account for the complex sampling scheme. All the tests were two-sided, with the level of statistical significance set to $\alpha = 0.05$.

The mean telomere length in leukocytes and its 95 % confidence interval (CI) were computed according to the characteristics of the study participants. The adjusted mean and 95 % CI of the telomere length were also calculated by adjusting for age, gender, ethnicity, monthly income, smoking status, alcohol, BMI, and history of diabetes.

Each carotenoid level was log-transformed and treated as a continuous variable. Risk analysis was conducted using a linear regression model by providing the percent change $[(e^{(\ln 2 * \beta)} - 1) * 100\ \%]$ in the telomere length for a doubling of the blood carotenoid levels, with the estimated 95 % CI as $(e^{(\ln 2 * (\beta \pm 1.96 * \mathrm{SE}))} - 1) * 100\ \%$, where β is the estimated regression coefficient and SE is standard error. Additionally, the serum carotenoid level was categorized into quartiles, and logistic regression was used to assess the percent change in leukocyte telomere length for each quartile of the carotenoid distribution compared with the lowest quartile of blood carotenoid. For each analysis, age, gender, ethnicity, income, smoking, drinking, the presence of diabetes, BMI, serum homocysteine and folate levels, dietary eicosapentaenoic acid and docosahexaenoic intakes, and smoking–carotenes interaction were adjusted as potential confounders.

Results

Table 1 shows the mean leukocyte telomere length according to the study characteristics. The mean (95 % CI) telomere length had a 1.05 (95 % CI 1.03–1.07) *T/S* ratio for the study population. The telomere length differed significantly by age, gender, ethnicity, income, alcohol drinking, BMI, and history of diabetes, and the serum homocysteine and folate levels were more likely to be low in subjects who were older, male, non-drinkers, white, and overweight (BMI of 25–29.9 kg/m^2) and had a low income, diabetes, and high serum homocysteine or folate levels. However, the data did not show a significant difference in smoking status, and dietary eicosapentaenoic and docosahexaenoic intake.

Table 2 shows the correlation structure of serum carotenoid levels. The carotenoid levels were significantly correlated with each other, and the coefficients were ranged from 0.13 to 0.65.

Table 3 shows the estimated beta coefficients (SE) and percent changes in leukocyte telomere length (*T/S* ratio) by blood carotenoid levels. There was a significant association between provitamin A carotenoids (i.e., alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin) and telomere length, whereas there was no association between non-vitamin A carotenoids (i.e., combined lutein/zeaxanthin and *trans*-lycopene) and telomere length. For continuous carotenoid levels, a doubling of blood alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin was associated with 1.76 % ($\beta = 0.017$; SE = 0.007), 2.22 % ($\beta = 0.022$; SE = 0.008), and 2.02 % ($\beta = 0.020$;

Table 3 Beta coefficient (SE) and percent change (%) in leukocyte telomere length (*T/S* ratio) by blood carotenoid levels

	N	Model 1				Model 2				Model 3			
		β	(SE)	% change	*p* value	β	(SE)	% change	*p* value	β	(SE)	% change	*p* value
Alpha-carotene (µg/dL)													
Per doubling of alpha-carotene		0.017	(0.006)	1.75	0.0108	0.018	(0.007)	1.84	0.0148	0.017	(0.007)	1.76	0.0211
Quartile of alpha-carotene													
Q1 (≤1.4)	946	Reference		Reference		Reference		Reference		Reference		Reference	
Q2 (1.5–2.7)	876	0.019	(0.012)	1.87	0.1331	0.022	(0.015)	2.23	0.1604	0.019	(0.015)	1.91	0.2264
Q3 (2.8–5.1)	922	0.043	(0.026)	4.37	0.1162	0.029	(0.028)	2.91	0.3264	0.025	(0.027)	2.54	0.3710
Q4 (≥5.2)	914	0.059	(0.024)	6.04	0.0287	0.065	(0.025)	6.73	0.0217	0.060	(0.024)	6.14	0.0253
Beta-carotene (*trans* + *cis*) (µg/dL)													
Per doubling of beta-carotene		0.018	(0.007)	1.79	0.0307	0.025	(0.008)	2.51	0.0071	0.022	(0.008)	2.22	0.0124
Quartile of beta-carotene													
Q1 (≤7.9)	902	Reference		Reference		Reference		Reference		Reference		Reference	
Q2 (8.0–13.8)	916	0.025	(0.012)	2.57	0.0495	0.030	(0.015)	3.04	0.056	0.024	(0.025)	2.46	0.3503
Q3 (13.9–24.4)	928	0.037	(0.018)	3.73	0.0578	0.029	(0.020)	2.86	0.1517	0.010	(0.018)	1.01	0.5932
Q4 (≥24.5)	912	0.071	(0.023)	7.34	0.0085	0.086	(0.023)	8.90	0.0014	0.076	(0.029)	7.94	0.0183
Beta-cryptoxanthin (µg/dL)													
Per doubling of beta-cryptoxanthin		0.022	(0.009)	2.23	0.0212	0.025	(0.007)	2.54	0.0022	0.020	(0.006)	2.02	0.0062
Quartile of beta-cryptoxanthin													
Q1 (≤4.9)	901	Reference		Reference		Reference		Reference		Reference		Reference	
Q2 (5–7.9)	900	0.020	(0.013)	1.99	0.1545	−0.001	(0.013)	−0.15	0.9127	−0.003	(0.015)	−0.29	0.8436
Q3 (8–13.2)	921	0.044	(0.023)	4.52	0.0731	0.047	(0.022)	4.85	0.0475	0.045	(0.022)	4.56	0.0639
Q4 (≥13.3)	921	0.053	(0.022)	5.42	0.0291	0.052	(0.018)	5.36	0.0127	0.049	(0.017)	5.06	0.0120
Combined lutein/zeaxanthin (µg/dL)													
Per doubling of lutein/zeaxanthin		0.020	(0.010)	2.05	0.0557	0.021	(0.011)	2.13	0.0801	0.014	(0.007)	1.37	0.0856
Quartile of lutein/zeaxanthin													
Q1 (≤10.2)	893	Reference		Reference		Reference		Reference		Reference		Reference	
Q2 (10.3–14.4)	944	0.021	(0.015)	2.10	0.1794	−0.001	(0.017)	−0.08	0.9605	−0.004	(0.016)	−0.42	0.8014
Q3 (14.5–20.4)	907	0.019	(0.020)	1.95	0.3581	0.016	(0.024)	1.64	0.5156	0.011	(0.024)	1.09	0.6521
Q4 (≥20.5)	912	0.027	(0.019)	2.79	0.1679	0.026	(0.017)	2.61	0.1575	0.019	(0.014)	1.88	0.2176
Trans-lycopene (µg/dL)													
Per doubling of *trans*-lycopene		0.016	(0.008)	1.59	0.0593	0.016	(0.011)	1.60	0.1826	0.008	(0.007)	0.82	0.2888

Table 3 continued

	N	Model 1 β	(SE)	% change	p value	Model 2 β	(SE)	% change	p value	Model 3 β	(SE)	% change	p value
Quartile of *trans*-lycopene													
Q1 (≤14.1)	880	Reference		Reference		Reference		Reference		Reference		Reference	
Q2 (14.2–20.7)	917	0.010	(0.014)	0.96	0.5048	−0.006	(0.016)	−0.56	0.7338	−0.006	(0.016)	−0.64	0.6954
Q3 (20.8–28.6)	910	0.002	(0.011)	0.24	0.8203	−0.028	(0.015)	−2.72	0.0953	−0.027	(0.016)	−2.70	0.1004
Q4 (≥28.7)	949	0.040	(0.014)	4.13	0.0119	0.024	(0.016)	2.45	0.1514	0.024	(0.015)	2.47	0.1271

Model 1 was adjusted for age, gender, and ethnicity

Model 2 was further adjusted for family income, smoking status, alcohol drinking, BMI, the presence of diabetes, and interactions with smoking * serum carotene levels

Model 3 was further adjusted for dietary omega 3 (eicosapentaenoic and docosahexaenoic) and serum levels of folate and homocysteine

SE = 0.006) longer telomeres, respectively. Compared with the lowest carotenoid quartiles (Q1), the fully adjusted percent changes for telomere length in the highest carotenoid quartiles (Q4) increased by 6.14 % (β = 0.060; SE = 0.024) for alpha-carotene, 7.94 % (β = 0.076; SE = 0.029) for beta-carotene (*trans* + *cis*), and 5.06 % (β = 0.049; SE = 0.017) for beta-cryptoxanthin.

Discussion

We found that increased levels of blood carotenoid were significantly associated with longer leukocyte telomeres in US adults. Specifically, a doubling of blood alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin levels was associated with approximately 2 % longer telomeres. Compared with the lowest carotenoid quartile of alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin, the telomere length for adults with the highest quartiles was significantly increased, by 5–8 %. Our results support a link between antioxidant status and telomere length and suggest that a high intake of carotenoid-rich foods may play a role in protecting telomeres and regulating telomere length.

Telomeres, the specialized terminal regions of chromosomes, do not encode any proteins; however, they protect the chromosome from damage during cell division [1]. Telomeres are essential for regulating the life span of human cells, and their progressive shortening has been implicated in cellular senescence and apoptosis [2]. Although telomere length is highly heterogeneous and inherited [17], it has been proposed as a biomarker for aging, health, and survival [18]. Accumulating evidence has indicated that shorter telomeres are associated with biological age, life span, and increased risk of aging-related diseases and stressful life circumferences [16, 19–22]. Increased oxidative stress has been considered a key player in the mechanism of telomere shortening associated with these conditions [4, 23]. In telomeres, guanine is highly sensitive to damage by oxidative stress. Furthermore, the production of reactive oxygen species from oxidative stress induces modified bases (mainly 8-oxoG) and single-strand breaks that interfere with the replication fork at telomeric repeats, thus leading to an increase in the rate of telomere shortening by oxidative stress.

Based on this knowledge, antioxidants may play an important role in protecting against cellular damage, slowing the aging process, and preventing diseases driven by oxidative stress. A series of studies demonstrated the possible role of dietary antioxidants on telomere length [7, 8]. A recent study of García-Calzón et al. [24] suggested that dietary total antioxidant capacity was associated with leukocyte telomere length. Notably, dietary patterns with antioxidant properties, such as Mediterranean dietary intake and

a high consumption of vegetables and fruits, and omega-3 fatty acids, have been linked with telomere length, emphasizing its protective role on telomere length [7, 25–27].

Only one recent study has shown a beneficial effect of carotenoids, as a potential antioxidant, on maintaining telomere length [12]. Sen et al. [12] investigated the association between plasma antioxidant levels and leukocyte telomere length in 786 elderly adults. A strong and significant effect of combined lutein/zeaxanthin on longer telomeres ($\beta = 0.120$, p value <0.01) was observed, whereas no other carotenoids were associated with telomere length. However, we observed that the serum levels of provitamin A carotenoids, including alpha-carotene, beta-carotene (*trans* + *cis*), and beta-cryptoxanthin, were significantly associated with longer telomeres, whereas non-vitamin A carotenoids, including combined lutein/zeaxanthin and *trans*-lycopene, did not acquire statistical significance. Such a discrepancy may arise from the use of different methodologies, such as the size of the study population, age distribution, and confounding variables. Additional, carefully designed studies are needed to clarify the effect of carotenoid on telomeres.

Despite the potential controversy, the role of carotenoid in the protection of telomere loss is biologically plausible. Oxygen is essential to life, but too much oxygen is potentially harmful. Although the human body produces oxygen free radicals and other reactive oxygen species as by-products of various metabolic processes, overproduction induces oxidative damage to relevant molecules (DNA, proteins, and lipids), eventually leading to an increased risk of many chronic diseases and aging [28, 29]. Antioxidants have attracted attention as an efficient tool in counteracting oxidative stress [30, 31]. Carotenoids are particularly promising antioxidants, because they are capable of exerting antioxidant protection by scavenging singlet molecular oxygen and peroxyl radicals [10, 32]. Several human studies have supported carotenoid protection against oxidation and oxidative stress-induced, age-related conditions [33, 34]. However, although carotenoids are well-known antioxidants due to their health benefits, their effect on DNA damage appears to differ for the pro- and non-vitamin A carotenoids. Results from cell culture, animal experiments, and human trials show that non-vitamin A carotenoids are almost invariably reported to protect against DNA damage [35]. In contrast, provitamin A carotenoids have complicated effects, sometimes protecting and sometimes increasing DNA damage [35, 36]. At high concentrations or at a high partial pressure of oxygen, the carotenoids, mostly beta-carotene, may have pro-oxidant properties rather than playing a protective antioxidant role. Thus, subsequent studies are required to identify whether there are different effects on protecting the telomere length by specific types of carotenoids.

To our knowledge, this is the first study to report longer telomeres in leukocyte associated with blood carotenoid levels using a large-scale, high-power, and strict quality control procedures survey of the NHANES data. However, several limitations should be considered. First, the cross-sectional design of this study does not permit a causal inference for the observed association. Second, although blood carotenoid level is commonly used in nutrition epidemiologic studies, it is not an accurate reflection of life time exposure. Finally, because higher serum carotenoid levels are considered to be a marker of a healthy lifestyle [37], their association with the leukocyte telomere length may solely reflect the protective role of a healthy lifestyle on biological aging. Despite making adjustments for lifestyle-related variables (i.e., smoking, BMI, and alcohol use), we cannot exclude the possibility of residual confounding effects by unmeasured confounders.

In conclusion, we found a significant, positive association between leukocyte telomere shortening and blood carotenoid levels, especially for alpha-carotene, beta-carotene (*trans* + *cis*), beta-cryptoxanthin, and *trans*-lycopene, in US adults. Although the findings need to be confirmed, they suggest that carotenoids have a protective effect on telomere loss and that a high intake of these carotenoids may potentially prevent or delay the aging process and age-related diseases.

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Compliance with ethical standards

Conflict of interest None declared.

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