Associations of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African-American and White Adults

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Abstract

High antioxidant intake has been shown to reduce cancer risk and may also mitigate the effects of oxidative DNA damage, which is hypothesized to be causally linked to carcinogenesis. This study examined potential racial differences in (*a*) dietary intakes and plasma concentrations of vitamin C, vitamin E, and carotenoids and oxidative DNA damage and (*b*) associations between plasma antioxidants and oxidative DNA damage. Data were from a cross-sectional study of 164 generally healthy nonsmoking African-Americans and Whites in North Carolina, ages 20 to 45 years, equally distributed by race and sex. Participants completed a demographic and health questionnaire, four 24-h dietary recalls, and a dietary supplement inventory; had height and weight measured; and provided a semifasting blood sample. African-Americans had statistically significantly lower plasma concentrations of vitamin E, α-carotene, β-carotene, and lutein + zeaxanthin than Whites, as well as lower self-reported intake of most antioxidants. Levels of oxidative DNA damage, measured using the alkaline comet assay, were lower in African-Americans than Whites. An inverse association between lycopene and oxidative DNA damage ($r = -0.20$; $P = 0.03$) was found in the combined study population after adjusting for sex, age, body mass index, passive smoke exposure, physical activity, education, income, and alcohol intake. There was also a positive association of vitamin E with oxidative DNA damage in the total population ($r = 0.21$; $P = 0.02$) and in African-American men ($r = 0.63$; $P = 0.01$) after adjusting for covariates. This study is among the first to examine these associations in a sample of healthy adults with an adequate representation of African-Americans. (Cancer Epidemiol Biomarkers Prev 2007;16(7):1428–36)

Introduction

Diet and nutrition-related factors play an important role in carcinogenesis (1). One mechanism by which it is hypothesized that diet reduces cancer risk is through consumption of antioxidants, which are substances found within many foods, such as fruits and vegetables, that decrease the adverse effects of reactive oxygen species on normal physiologic functions. High reactive oxygen species levels can lead to oxidative stress, in which the imbalance of radical-generating agent concentrations exceeds the defense mechanisms of the body (2). Humans have well-developed defense systems that generally maintain homeostasis by disposal of these oxidative products; however, under conditions of elevated oxidative stress (e.g., low antioxidant intakes), defenses may be overwhelmed. Oxidative stress is caused by exogenous factors (e.g., smoking) as well as endogenous processes during normal cell metabolism. Excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, altered gene expression, and ultimately mutagenesis (3, 4). Continuous oxidative damage to DNA is believed to be a significant contributor to the age-related development of many cancers, such as those of the breast, colon/rectum, and prostate (2, 5).

Numerous studies have examined associations of antioxidant intakes (from diet and/or supplements) with oxidative DNA damage and cancer risk. Most intervention trials that focused on intakes of fruits and/or vegetables have shown significant reductions in oxidative DNA damage levels (6-11); one study showed no effect (12). In a randomized crossover study of healthy nonsmoking males ages 27 to 40 years, Pool-Zobel et al. (6) found that supplementing the diet with tomato, carrot, or spinach products resulted in significantly decreased levels of endogenous strand breaks in lymphocyte DNA. However, studies that have examined relationships between individual antioxidant nutrients and oxidative DNA damage or cancer risk have been less consistent. Results from most observational studies provide support for a protective association of high dietary intakes and/or blood levels of antioxidant vitamins, especially β-carotene and vitamin C, on cancer risk (1, 13, 14) and oxidative DNA damage (15, 16). Several interventions with supplemental doses of antioxidants resulted in a significant decrease in endogenous DNA damage (17, 18). For example, in a randomized double-blind placebo-controlled intervention, Zhao et al. (18) showed significant decreases in endogenous DNA damage after 57 days of taking supplements of lutein, β-carotene, lycopene, and a combination of all three in a sample of postmenopausal women. Conversely, two notable randomized trials, ATBC and CARET, reported elevated risk of lung cancer with high-dose supplementation in high-risk populations, such as smokers and asbestos workers (19, 20). One possible explanation for these results is that the high doses used during the trial may have resulted in pro-oxidant activity in the radical-rich environment of smokers' lungs (21).

African-Americans are at disproportionately higher risk for many oxidative stress–related medical conditions and have the highest cancer burden of any racial or ethnic group in the United States (22). Moreover, survey data suggest that African-Americans consume fewer daily fruits and vegetables (i.e., antioxidant-rich foods; refs. 23, 24) and tend to have lower

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blood levels of most antioxidant nutrients (26-28) than do Whites. For example, according to data from the 2002 Behavioral Risk Factor Surveillance Survey, <19% of African-Americans in North Carolina consumed the recommended five fruit and vegetable servings per day, which is lower than the median for White adults in the United States (22.6%) and North Carolina (24.7%), and only 38% of African-Americans reported current use of multivitamins compared with 51% of Whites (25). Similarly, African-Americans had significantly lower serum levels of α-carotene and α-tocopherol among all racial/ethnic groups in the Third National Health and Nutrition Examination Survey (27) but had higher serum lutein + zeaxanthin concentrations than Whites (28). Nonetheless, it seems that African-Americans have overall dietary patterns that may put them at higher risk for oxidative stress and oxidative stress–related medical conditions, including cancer.

We collected and analyzed data from a convenience sample of African-Americans and Whites in North Carolina to (*a*) determine whether dietary intakes and blood levels of antioxidant nutrients (carotenoids, vitamin C, and vitamin E) and oxidative DNA damage differed between African-Americans and Whites, (*b*) examine associations between antioxidants and oxidative DNA damage, and (*c*) determine whether associations differed by race. This study is among the first to examine these relationships in a sample with adequate representation of both African-Americans and Whites and may provide mechanistic support for the higher cancer burden in African-Americans compared with Whites.

Materials and Methods

Study Population. Data are from the DIet, Supplements, and Health Study, which enrolled 168 generally healthy African-American and White adults (approximately equal by race and gender) from the Research Triangle Area of North Carolina between March and December 2005. Participants were recruited via flyers displayed in public venues throughout the Research Triangle area of North Carolina, including churches, gyms, campus-wide emails, and campus buildings. Eligible participants were 20 to 45 years of age, generally healthy, free of diseases related to oxidative stress (i.e., cancer, diabetes, heart disease, or Alzheimer's disease), and fluent in written and spoken English. Persons likely to have high levels of oxidative stress, such as current smokers and those with a self-reported body mass index (BMI) of 30 or greater, were ineligible. Other exclusion criteria included anorexia or bulimia nervosa, large weight change (>15 pounds) in the past year, inability to fast for 6 h, and pregnancy. Of the 191 respondents deemed eligible during the screening interview, 168 (88.0%) were enrolled and 164 (85.9%) completed all aspects of the study. Data for nine participants were excluded because of serum cotinine levels that were consistent with active smoking (≥15 ng/mL), leaving a total of 155 participants (76 African-American and 79 White).

Data Collection. Participants completed four unannounced telephone-administered 24-h dietary recall interviews and a self-administered demographic, health, and antioxidant questionnaire. During a one-time visit to the University of North Carolina's General Clinical Research Center, participants had height, weight, and waist circumference measured, provided urine and semifasting (≥6 h) blood samples, participated in a dietary supplement inventory, and answered questions about the use of nonsteroidal anti-inflammatory drugs and lipid-lowering drugs, current occupation, outdoor exposure, and last menstrual cycle (women only). Blood samples were analyzed for plasma levels of antioxidant nutrients, cholesterol, oxidative DNA damage, hemoglobin A1c (to confirm self-reported absence of diabetes), and serum cotinine (to validate self-reported smoking status). Each participant received $100 compensation for his/her time on completion of all study activities. This study was approved by the University of North Carolina's Institutional Review Board and written (signed) informed consent was obtained from all participants.

Dietary Recalls. Four unannounced telephone-administered 24-h dietary recalls were conducted by trained nutritionists from University of North Carolina's Clinical Nutrition Research Core using a computerized multiple pass approach (29) with the Nutrition Data System software (version 5.0.35; University of Minnesota, Minneapolis, MN) over a 1-month period. Two recalls each were conducted on weekdays and weekend days (i.e., Saturday or Sunday) to account for variability in eating patterns. The consumed foods, beverages, preparation methods, amounts, and recipes reported by the participant were entered by a trained nutritionist into the Nutrition Data System-R software package to obtain an estimate of intakes of various nutrients. The Nutrition Data System-R database combines United States Department of Agriculture's Nutrient Database for Standard Reference and information from scientific literature and food manufacturers, to maintain the most accurate and comprehensive nutrition calculation software available in the United States.

Demographic, Health, and Antioxidant Questionnaire. All participants completed a self-administered 12-page questionnaire, which included 37 questions pertaining to general health and a newly developed antioxidant food frequency questionnaire (FFQ). The questionnaire also included sections on physical activity, medical history, smoking and alcohol use, demographic characteristics, dietary supplement use, and attitudes and beliefs about diet. We conducted a small pilot study in a convenience sample that was equally divided by race and gender to test the questionnaire for feedback about the design, content, and ease of completion and made the necessary modifications.

Antioxidant FFQ. We developed a semiquantitative FFQ designed to capture usual dietary and supplemental intakes of carotenoids, vitamin C, and vitamin E. The 92-item questionnaire includes more than 80 foods that either are natural sources of carotenoids, vitamin C, and vitamin E (e.g., fruits and vegetables) or fortified sources (e.g., cold cereals). Participants were asked to report how often they ate each listed food in the past month, specifically, never or less than once per month, once per month, 2 to 3 per month, 1 to 2 per week, 3 to 4 per week, 1 per day, or 2+ per day. Participants also recorded whether they usually consumed a small, medium, or large amount (a medium serving size was shown as a reference). The Fred Hutchinson Cancer Research Center Nutrition Assessment Shared Resource analyzed all nutrient intake records using the Nutrition Data System.

Dietary Supplement Use. A closed-ended format was used to quantify self-reported use (frequency and dose) of various antioxidant nutrients in the past month. Specifically, for multivitamin use, participants selected from a list of common multivitamins or wrote in their brand if it was not listed and indicated the usual frequency of use (number of days per week). Next, they reported whether they took a single nutrient supplement of β-carotene, vitamin A, vitamin C, or vitamin E, and if yes, the frequency and usual dose (amount per day). Daily intake of each nutrient was calculated as follows: frequency (days per week) × dose per day / 7 (days) (30). For these analyses, participants are categorized as ''nonusers'' or ''users'' of dietary supplements from this instrument. Capturing supplemental intakes of antioxidants is crucial as supplements can contribute a large percentage of the total intake. This is especially true for vitamin E, as typical dietary intake (8-10 mg) is much smaller than typical doses in dietary supplements (e.g., 180 mg from single supplements; ref. 31).

Table 1. Demographic, lifestyle, and other characteristics of study participants, by race and sex (n = 155)

Characteristic	African-Americans			Whites			P*
	Males, n = 36 (%)	Females, n = 40 (%)	Total, n = 76† (%)	Males, n = 38 (%)	Females, n = 41 (%)	Total, n = 79† (%)	
Age (y)							
20-28	21 (58)	13 (33)	34 (45)	13 (34)	13 (32)	26 (33)	
29-37	8 (22)	14 (35)	22 (29)	13 (34)	14 (34)	27 (34)	
38-45	7 (19)	13 (33)	20 (26)	12 (32)	14 (34)	26 (33)	0.32
BMI‡ (kg/m^2)							
Normal (18.5-24.9)	23 (58)	9 (25)	32 (42)	34 (83)	24 (63)	58 (73)	
Overweight (25-29.9)	16 (40)	22 (61)	38 (50)	7 (17)	12 (32)	19 (24)	
Obese (≥30)	1 (3)	5 (14)	6 (8)	0 (0)	2 (5)	2 (3)	<0.0001
Education							
Some college or less	12 (33)	18 (45)	30 (39)	10 (24)	10 (26)	20 (25)	
College graduate	19 (53)	14 (35)	33 (43)	13 (32)	19 (50)	32 (41)	
Advanced degree	5 (14)	8 (20)	13 (17)	18 (44)	9 (24)	27 (34)	0.03
Marital status							
Single/separated or divorced	20 (56)	25 (63)	45 (59)	19 (46)	20 (53)	39 (49)	
Married/living with partner	16 (44)	15 (38)	31 (41)	22 (54)	18 (47)	40 (51)	0.22
Income							
<$20,000	6 (19)	8 (22)	14 (21)	7 (19)	7 (18)	14 (19)	
$20,000-39,000	7 (23)	9 (25)	16 (24)	9 (24)	9 (24)	18 (24)	
$40,000-79,000	10 (32)	10 (28)	20 (29)	14 (38)	13 (34)	27 (36)	
≥$80,000	8 (26)	9 (25)	17 (25)	7 (19)	9 (24)	16 (22)	0.86
Dietary supplement use							
None	22 (61)	28 (70)	50 (66)	21 (55)	18 (44)	39 (49)	
Multivitamin only	9 (25)	6 (15)	15 (20)	10 (26)	6 (15)	16 (20)	
Single nutrient only	1 (3)	2 (5)	3 (4)	2 (5)	2 (5)	4 (5)	
≥2 Supplements	4 (11)	4 (10)	8 (11)	5 (13)	15 (13)	20 (25)	0.09
Passive smoke exposure							
Lives with a smoker	3 (8)	3 (8)	6 (8)	0 (0)	4 (10)	4 (5)	
No one at home smokes	33 (92)	37 (93)	70 (92)	38 (100)	36 (88)	74 (95)	0.49
Physical activity							
<1/wk	4 (11)	11 (28)	15 (20)	1 (3)	0 (0)	1 (1)	
1-2 Times/wk	12 (33)	14 (35)	26 (34)	8 (21)	12 (34)	20 (25)	
3-4 Times/wk	17 (47)	11 (28)	28 (37)	14 (37)	15 (37)	29 (38)	
5+ Times/wk	3 (8)	4 (10)	7 (9)	15 (39)	14 (34)	29 (35)	0.002
Alcohol consumption							
Never	12 (33)	22 (55)	34 (45)	5 (13)	10 (24)	15 (19)	
<1/wk	8 (22)	14 (35)	22 (29)	9 (24)	15 (37)	24 (30)	
1-6 Times/wk	13 (36)	4 (10)	17 (22)	18 (47)	15 (37)	33 (42)	
≥1/d	3 (8)	0 (0)	3 (4)	6 (16)	1 (2)	7 (9)	0.002
Self-rated health status							
Excellent	11 (31)	7 (18)	18 (24)	14 (37)	12 (29)	26 (33)	
Very good	16 (44)	21 (53)	37 (49)	16 (42)	22 (54)	38 (48)	
Good/fair	9 (25)	12 (30)	21 (27)	8 (21)	7 (17)	15 (19)	0.49
County of residence							
Urban	30 (83)	35 (88)	65 (86)	33 (87)	35 (85)	68 (86)	
Rural	6 (17)	3 (8)	9 (12)	3 (8)	3 (7)	6 (8)	
Not specified	0 (0)	2 (5)	2 (3)	2 (5)	3 (7)	5 (6)	0.39

*Overall P values were determined by χ^2 tests for differences between ''total African-Americans'' and ''total Whites.''
† Numbers may not add up to 76 for African-Americans and 79 for Whites and percentages may not add up to 100%, due to rounding and missing data.
‡ BMI calculated as kg/m^2, based on measured weight (kg) and height (m^2).

Dietary Supplement Inventory. Participants were instructed to bring the bottles for all vitamin, mineral, and herbal supplement(s) taken (even once) during the past month to the study interview. For each supplement, a trained nutritionist recorded the brand name, type of supplement (multivitamin, single nutrient, and multinutrient), usual frequency of use, total number of pills taken each time, amount of each ''nutrient'' per pill, when usually taken (morning, afternoon, and evening), and when the supplement was last taken. This open-ended approach is more valid than self-administered questionnaires (30). Average daily nutrient intake from the inventory was calculated as follows: frequency (days per week) × number of pills taken each time × dose per pill / 7 (30). We then summed intakes of each individual nutrient from all multivitamins and single supplements reported to determine a total average daily intake for each nutrient. β-Carotene, retinol, and vitamin E were converted into activity units as follows: 1 IU of vitamin A = 0.3 μg retinol and 3.6 μg β-carotene and 1 IU of vitamin E = 0.45 mg α-tocopherol (32).

Plasma Nutrients. Semifasting (≥6 h) blood samples that were protected from heat and light were analyzed for plasma concentrations of carotenoids, retinol, tocopherols, vitamin C, and cholesterol. The aliquot of plasma designated for ascorbic acid assessment was preserved with a 6% weight/volume metaphosphoric acid solution added in 1:4 plasma to metaphosphoric acid ratio to stabilize vitamin C. Plasma concentrations of retinol, tocopherols, vitamin C, and carotenoids (lutein + zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene) were measured using high-performance liquid chromatography with multiwavelength photodiode-array absorbance detection (33). Plasma cholesterol was measured by enzymatic/colorimetric analyses (''Trinder'' procedure) using adaptations of commercially available kits (kit no. 401-25P; Sigma-Aldrich), based on the method of Smith et al. (34). Multiple levels of quality control samples were included and 10% blinded duplicates were included in each batch. The assays were done by Craft Technologies, Inc.; the relative SD ranged from <3% to <7.5% in all samples. All samples were stored at −80°C and

analyzed within 1 year, well within guidelines for storage stability (35).

Oxidative DNA Damage. Oxidative DNA damage was assessed using the single-cell gel electrophoresis or comet assay. The comet assay is a widely used method for measuring DNA strand breaks at the level of a single cell in which lymphocytes are digested with lesion-specific repair endonucleases (36). The comet assay used here was a slightly modified version in which formamidopyrimidine DNA glycosylase (provided by Dr. A.R. Collins, Oslo, Norway) was added to convert oxidized purines into strand breaks (36, 37). Peripheral whole blood lymphocytes were washed in PBS, counted using a hemacytometer, and cryopreserved in 1 mL RPMI 1640 + 15% bovine serum albumin + 10% DMSO. All samples were processed within 2 h of collection and stored at −80°C until assays were conducted, as recommended by the European Standards Committee on Oxidative DNA Damage (37). Lymphocytes were sandwiched between 0.5% agarose and 0.5% low-melting-point (37°C) agarose (Fisher). The resulting slides were placed into cold, freshly made lysis solution [10 mmol/L Tris (pH 10), 2.5 mol/L NaCl, 100 mmol/L EDTA, 1% sodium sarcosinate, 10% DMSO, and 1% Triton X-100] at 4 °C for 1 h, and then treated for 20 min in electrophoresis buffer [300 mmol/L NaOH, 1 mmol/L EDTA (pH 13); ref. 38]. After electrophoresis was done at 25 V and 300 mA for 20 min, slides were incubated thrice for 5 min in neutralization buffer [0.4 mol/L Tris (pH 7.5)] with formamidopyrimidine DNA glycosylase, washed with methanol, and stained with SYBR Green. Multiple levels of quality control samples (e.g., 10% blinded duplicate samples) were included in each batch and all assays were done by the University of North Carolina Clinical Nutrition Research Unit.

Comet tail length (the distance of DNA migration from the body of the nuclear core) was visualized by using a fluorescence microscope (typically, 100 cells per sample) and Scion Image software (38). The comet tail moment (defined as the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail) was calculated by using the NIH Image Analysis Macro language software.

Statistical Analyses. Data analyses were done using Stata (version SE 8.2, Stata Corp.). Descriptive statistics (means and percentages for continuous and categorical variables, respectively) were calculated for all variables. Missing data were excluded from analyses; on average, <1% of data were missing. For each demographic characteristic, χ^2 tests were used to test for comparability by race. Antioxidant nutrient intakes were assessed in four main ways: (*a*) biomarker (plasma)

Table 2. Unadjusted antioxidant intakes and plasma levels among study participants, stratified by race and sex (*n* = 155)

	African-Americans			Whites			*P**
	Males (*n* = 36)	Females (*n* = 40)	Total (*n* = 76)	Males (*n* = 48)	Females (*n* = 41)	Total (*n* = 79)	
Vitamin A (retinol)							
Biomarkers (μg/mL)	0.42	0.39	0.40	0.47	0.41	0.44	0.002
Dietary recalls (mg/d)	513.7	344.1	424.4	608.3	467.4	535.2	0.02
FFQ (mg/d)	1,435.2	481.4	933.2	1,893.9	880.3	1,367.8	0.48
Supplements only (mg retinol equivalents/d) †	117.4	217.5	170.0	140.5	119.2	129.4	0.66
Vitamin C (ascorbic acid)							
Biomarkers (μg/mL)	8.38	8.82	8.61	9.02	8.24	9.06	0.90
Dietary recalls (mg/d)	124.0	84.2	103.1	138.7	104.7	121.1	0.11
FFQ (mg/d)	191.0	124.4	156.0	210.7	139.5	173.7	0.13
Supplements only (mg/d)	105.7	61.9	82.7	86.1	132.0	109.9	0.57
Vitamin E (α-tocopherol)							
Biomarkers (μg/mL)	7.64	7.35	7.49	9.81	10.43	10.13	<0.001
Dietary recalls (mg/d)	9.0	7.0	8.0	12.4	9.9	11.1	<0.001
FFQ (mg/d)	12.1	8.0	9.9	16.8	12.8	14.7	0.004
Supplements only (mg α-tocopherol equivalents/d)	62.9	34.3	47.9	28.9	84.0	57.5	0.69
α-Carotene							
Biomarkers (μg/mL)	0.05	0.05	0.05	0.05	0.07	0.06	0.006
Dietary recalls (μg/d)	336.4	361.0	349.3	622.9	516.9	567.9	0.01
FFQ (μg/d)	600.5	517.5	556.8	1,037.5	712.8	869.0	0.04
β-Carotene							
Biomarkers (μg/mL)	0.18	0.18	0.18	0.19	0.27	0.23	0.007
Dietary recalls (μg/d)	3,044.4	2,249.1	2,625.8	4,134.1	3,096.1	3,595.4	0.02
FFQ (μg/d)	3,900.5	3,392.5	3,633.1	5,337.7	4,430.3	4,866.8	0.03
Supplements only (μg β-carotene equivalents/d)	704.2	1,305.0	1,020.4	843.0	715.2	776.7	0.66
β-Cryptoxanthin							
Biomarkers (μg/mL)	0.11	0.10	0.10	0.10	0.09	0.10	0.68
Dietary recalls (μg/d)	257.4	169.8	211.3	270.4	236.6	252.9	0.38
FFQ (μg/d)	339.0	171.6	250.9	372.8	177.4	271.4	0.76
Lutein + zeaxanthin							
Biomarkers (μg/mL)	0.12	0.12	0.12	0.12	0.14	0.13	0.05
Dietary recalls (μg/d)	3,064.1	2,075.6	2,543.8	3,637.3	2,774.5	3,189.5	0.21
FFQ (μg/d)	3,028.1	2,563.1	2,783.4	3,791.3	3,577.6	3,680.4	0.04
Supplements only (μg/d)	34.7	34.1	34.4	24.1	24.4	24.3	0.52
Lycopene							
Biomarkers (μg/mL)	0.48	0.40	0.44	0.44	0.38	0.41	0.73
Dietary recalls (μg/d)	4,819.7	4,152.3	4,468.4	10,690.8	5,861.9	8,184.7	0.005
FFQ (μg/d)	7,655.7	4,152.9	5,812.1	7,890.8	4,994.8	6,387.8	0.60
Supplements only (μg/d)	148.2	20.0	80.7	39.6	44.2	42.0	0.29

NOTE: No adjustments were made, except for total cholesterol levels for biomarker values for fat-soluble nutrients only.

*Tests for differences between total African-Americans and Whites were calculated by ANOVA using log-transformed variables.

† Data for ''supplements only'' were based on in-person dietary supplement inventory. Conversions into activity units were made as follows: 1 IU of vitamin A = 0.3 μg retinol and 3.6 μg β-carotene and 1 IU of vitamin E = 0.45 mg α-tocopherol. No values were presented for α-carotene and β-cryptoxanthin because dietary supplements contributed only negligible amounts to intake.

Table 3. Mean oxidative DNA damage levels (comet tail moment), by race and sex (n = 155)

Comet assay mean tail moment (SD)	Total population			Men			Women		
	African-American (n = 74)	White (n = 77)	P*	African-American (n = 35)	White (n = 37)	P	African-American (n = 39)	White (n = 40)	P
Crude model	1.404 (0.298)	1.559 (0.359)	0.005	1.410 (0.312)	1.534 (0.351)	0.12	1.399 (0.289)	1.582 (0.370)	0.02
Adjusted† model	1.398 (0.147)	1.563 (0.196)	0.01	1.399 (0.206)	1.535 (0.249)	0.24	1.396 (0.176)	1.587 (0.321)	0.03

NOTE: Comet assay results were not available for four participants due to missing samples.
*Overall P value was calculated by ANOVA using log-transformed oxidative DNA damage estimates.
† Mean values adjusted for age, BMI, cotinine levels, alcohol intake, physical activity level, income, education, and, for women, days since last menses.

concentrations; (*b*) average daily dietary intakes from the FFQ in the past month; (*c*) mean intakes across the four dietary recalls; and (*d*) the average daily intake from supplements as reported in the supplement inventory. The dietary recalls, FFQ, and supplement inventory captured intakes during the same month. Oxidative DNA damage was quantified as the comet tail moment. Log transformations were applied to the dietary and oxidative DNA damage distributions to help meet normality distribution assumptions, as they were right skewed. Crude mean levels of antioxidant nutrient and oxidative DNA damage were reported separately by sex and race and differences between African-Americans and Whites were evaluated using ANOVA. All analyses of fat-soluble plasma antioxidant levels were adjusted for plasma cholesterol, as it affects bioavailability (39). Multiple linear regression analyses (40) and partial Pearson correlations were computed separately by each race to assess associations between dietary estimates and blood levels of the antioxidant nutrients and oxidative DNA damage, controlling for relevant covariates. Age, sex, BMI [calculated as weight (in kilograms) / height (in meters squared)], income, physical activity, education, serum cotinine, and alcohol consumption were evaluated as potential confounders, as these factors have been found to be associated with both antioxidant intakes/blood levels and oxidative DNA damage (39, 41, 42). Tertiles of dietary intake estimates were computed and compared with oxidative DNA damage using multiple regression analyses and P for linear trend was calculated. To approximate total antioxidant concentration, Z-scores were calculated for each antioxidant biomarker value and averaged. Hypothesis tests and 95% confidence intervals were used to make inferences about the regression coefficients. Statistical tests were two sided and P values ≤ 0.05 were considered statistically significant.

Results

The distributions of demographic and lifestyle characteristics, stratified by race and sex (n = 155), are shown in Table 1. The mean age of African-Americans was 30.9 years (7.9 SD) and 53% were female; Whites were 32.5 years old on average (7.9 SD) and 52% were female. African-Americans had statistically significantly lower formal educational levels, physical activity, and alcohol consumption than Whites and were also more likely to be obese (BMI, ≥ 30 kg/m^2). African-American males were somewhat younger (20-28 years) than White males (58% versus 34%), and females of both races tended to have higher BMI and lower alcohol consumption than men.

Table 2 gives the mean antioxidant levels for vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (α-tocopherol), and carotenoids measured from plasma biomarkers, mean of four dietary recalls, average daily intakes from the FFQ, and the supplement inventory, by race and sex. We note that there was good agreement among the three dietary measures: adjusted Pearson correlations FFQ- and 24-h recall-derived intakes ranged from 0.42 (lycopene in African-Americans) to 0.58 (β-carotene in Whites) and correlations for the FFQ and biomarkers ranged from 0.32 to 0.54 (α-tocopherol and β-cryptoxanthin in African-Americans, respectively).[7] Compared with Whites, African-Americans had statistically significantly lower plasma concentrations and dietary intakes of most of the antioxidant nutrients. Specifically, they had lower plasma levels of α-carotene, β-carotene, lutein + zeaxanthin, α-tocopherol, and retinol and lower intakes of α-carotene, β-carotene, lutein + zeaxanthin (FFQ only), α-tocopherol, and retinol (recalls only). In addition, African-Americans had significantly lower dietary recall-based lycopene than did Whites. There were no statistically significant differences by race in supplemental intakes of any of the antioxidants examined. Intake of all antioxidants, except α-carotene for African-American men, was higher for men than women of both races. Mean antioxidant estimates were also evaluated adjusting for sex, age, BMI, cotinine, physical activity, education, income, and alcohol intake and adjusted estimates were comparable with the unadjusted estimates shown.

Oxidative DNA damage levels, measured as the mean tail moment of 100 cells using the comet assay, are given in Table 3. Overall, African-Americans had significantly lower crude mean oxidative DNA damage than Whites (1.404 versus 1.559; P = 0.005). Both African-American men and African-American women had lower oxidative DNA damage than their White counterparts, although the difference was not statistically significant for men. Estimates of oxidative DNA damage changed only slightly when adjusted by age, BMI, cotinine levels, alcohol intake, physical activity level, income, education, and days since last menses for women.

Table 4 gives mean oxidative DNA damage levels by antioxidant plasma concentrations, dietary intakes, and supplemental intakes. Antioxidant intakes were categorized into tertiles and mean oxidative DNA damage values were calculated for African-Americans and Whites, adjusting for sex, age, BMI, cotinine levels, physical activity level, education, income, and alcohol intake. Although few associations were statistically significant, oxidative DNA damage was generally lower for the highest tertiles of plasma antioxidants compared with the lowest, with the exception of α-carotene, lutein + zeaxanthin, and ascorbic acid in Whites. This inverse relationship was evident for most of the self-reported estimates of antioxidant intakes (i.e., recalls, FFQ, and dietary supplement use). For almost all nutrients, mean levels of oxidative DNA damage were lower for nonusers than users of dietary supplements (based on the self-reported instrument); however, the only statistically significant association was with supplemental lycopene intake in Whites (P = 0.01). The proportion of the variance in each of the biomarker values explained by covariates examined ranged from 0.10 for α-carotene in African-Americans to 0.61 for β-carotene in Whites.

[7] J.A. Satia, et al. Validation of an antioxidant nutrient food frequency questionnaire in Whites and African Americans, in preparation.

Pearson partial correlations between antioxidant plasma concentrations and oxidative DNA damage, stratified by race and sex, are given in Table 5. For the total study population, only lycopene and α-tocopherol were statistically significantly associated with oxidative DNA damage; however, lycopene was inversely associated with oxidative DNA damage (Pearson $r = -0.20$; $P = 0.03$), whereas the association with α-tocopherol was positive ($r = 0.21$; $P = 0.02$). Although not significant when examined separately by race, associations with lycopene and α-tocopherol were in the same direction and of similar magnitude in both African-Americans and Whites. Other racial and gender differences were noted, although not all were statistically significant. For example, vitamin C was inversely associated with oxidative DNA damage in African-Americans; in contrast, associations tended to be positive in Whites. α-Tocopherol was positively associated with oxidative DNA damage in men ($r = 0.63$; $P = 0.01$ for African-American men) but was inversely associated among women of both races. Oxidative DNA damage was not statistically significantly associated with all antioxidants combined (based on Z-scores).

Discussion

In this cross-sectional study of generally healthy adults in North Carolina, African-Americans had significantly lower plasma levels of α-carotene, β-carotene, lutein + zeaxanthin, α-tocopherol, and retinol than Whites. African-Americans also had lower oxidative DNA damage, as assessed by the mean comet tail moment. The only statistically significant inverse association between plasma antioxidants and oxidative DNA damage was found for lycopene in the combined study population. Rather unexpectedly, there were also positive associations of α-tocopherol with oxidative DNA damage in the total population and in African-American men.

The lower self-reported intakes and plasma concentrations of antioxidants seen here among African-Americans compared with Whites agree with most national and North Carolina–specific data (24-27, 43). For example, mean intakes of β-carotene, lutein, α-tocopherol, and ascorbic acid reported here were similar to those of healthy controls in a recent population-based case-control study of African-Americans and Whites in North Carolina (26). African-American adults also had lower serum α-tocopherol and lycopene concentrations in the Third National Health and Nutrition Examination Survey, although serum lutein + zeaxanthin concentrations were higher than in Whites (27, 28). The fact that there were statistically significant differences by race in plasma antioxidant concentrations and at least one self-reported diet method (i.e., FFQ or recalls) for retinol, α-carotene, β-carotene, lutein + zeaxanthin, and α-tocopherol suggests that these differences are likely attributable to intake of antioxidant-rich foods rather than dietary supplement use. Although we saw no racial differences in supplement use, other studies have reported higher use in Whites than African-Americans (26, 43). Thus, our findings agree with other published data, suggesting that African-Americans may have dietary patterns that may lead to increased oxidative stress.

Oxidative DNA damage levels were lower in African-Americans than Whites for the total population and women alone; however, there was no racial difference in men. Other studies have reported higher oxidative DNA damage in men than in women, which was attributed to lower fruit intake in men in one study (44, 45). Men in our study had higher total self-reported intakes but similar plasma levels of antioxidants than women, which may explain (at least in part) why we did not observe significant differences in oxidative DNA damage levels by sex. Although we were unable to find other studies in which the potential race-sex interactions have been investigated, our findings are consistent with other published data (38, 46-48). For example, the overall mean oxidative DNA damage levels we observed are similar to baseline data in a recent study of choline depletion in African-American and White healthy adults; however, results were not stratified by race (38). In a randomized controlled study of vitamins C and E supplements by Huang et al. (46), oxidative DNA damage (assessed by urinary 7-hydroxy-8-oxo-2′-deoxyguanosine) was lower in African-American than White participants at baseline. The authors noted that these differences were not explained by diet or lifestyle factors and that all participants were nonsmokers (46). Similarly, Toraason et al. (47) found statistically significantly lower oxidative DNA damage levels in African-Americans than Whites in a study of female dry cleaners. Considering that oxidative DNA damage is a potential mechanism associated with cancer risk and that our study population was young and relatively healthy, the relationship between race and oxidative DNA damage needs to be explored further in other studies that also include those at elevated risk of oxidative DNA damage.

We found significant associations with oxidative DNA damage for two antioxidant nutrients, although one relationship was not in the hypothesized direction. In the combined sample, there was a significant positive association for α-tocopherol and an inverse association for lycopene with oxidative DNA damage. Although not significant when analyzed separately by race, the directions of these associations were consistent for both African-Americans and Whites. There seem to be differences by sex in the association between α-tocopherol and oxidative DNA damage among African-Americans, as there is a strong positive association in men and a nonstatistically significant inverse association in women. Other studies comparing α-tocopherol and oxidative DNA damage have not reported a positive association in men (45, 49) and α-tocopherol supplementation has been associated with lower oxidative stress levels in healthy young adults (49). There is some evidence that in the presence of copper (50) or in smokers consuming a high fat diet (49), α-tocopherol can act as a strong pro-oxidant, but it is somewhat surprising to see a positive association of α-tocopherol in this sample of healthy, nonsmoking young adults. Conversely, the inverse association with lycopene is not surprising as intervention trials with lycopene or tomatoes (the richest food source of lycopene) have consistently shown lower levels of oxidative DNA damage (6, 8, 9, 11). Decreased levels of oxidative DNA damage were shown after consuming only a single serving of tomatoes in a trial of five participants (11) and after 3 weeks of consuming tomato sauce–based pasta dishes in a study of 32 men with prostate cancer (8).

Although not shown here, other investigations have reported inverse associations of vitamin C and several carotenoids with oxidative DNA damage. For example, two intervention studies that showed a reduction in endogenous DNA damage with supplemental doses of antioxidants, including vitamin C, vitamin E, and carotenoids, were conducted in persons over age 50 years (17, 18); in contrast, two studies of younger, healthy adults (25-45 and 35-64 years) found no association (45, 51). There are several possible reasons why we did not observe more significant associations. First, our study population consisted of healthy, nonsmoking, nonobese young adults that were likely to have low levels of oxidative stress relative to other populations. Second, although we did not find a significant association for total antioxidant plasma concentration as measured by Z-scores, it is possible that a synergistic effect exists among all antioxidants not seen for each individual nutrient. In addition, we acknowledge that there are more sophisticated methods to measure total antioxidants, such as an antioxidant index, which combined various antioxidants using principle components analysis described by Wright et al. (52). Third, perhaps, antioxidants

Table 4. Adjusted mean oxidative DNA damage level (comet tail moment) by antioxidant intakes and plasma levels, by race

	Vitamin A (retinol)		Vitamin C (ascorbic acid)		Vitamin E (α-tocopherol)	
	African-American	White	African-American	White	African-American	White
Biomarkers						
Highest tertile	1.348	1.472	1.309	1.602	1.337	1.526
Middle tertile	1.373	1.571	1.327	1.545	1.408	1.543
Lowest tertile	1.394	1.584	1.496	1.439	1.367	1.560
P for linear trend	0.98	0.84	0.08	0.03	0.52	0.84
Dietary recalls						
Highest tertile	1.406	1.409	1.307	1.515	1.485	1.515
Middle tertile	1.335	1.667	1.424	1.577	1.571	1.577
Lowest tertile	1.389	1.597	1.374	1.511	1.593	1.511
P for linear trend	0.33	0.39	0.81	0.84	0.59	0.32
FFQ						
Highest tertile	1.369	1.461	1.346	1.574	1.378	1.499
Middle tertile	1.431	1.544	1.426	1.518	1.373	1.590
Lowest tertile	1.322	1.664	1.341	1.501	1.377	1.529
P for linear trend	0.17	0.54	0.38	0.33	0.27	0.63
Supplements only*						
Users	1.386	1.545	1.392	1.534	1.386	1.532
Nonuser	1.380	1.539	1.378	1.553	1.380	1.553
Overall *P* value	0.72	0.85	0.76	0.69	0.91	0.33

NOTE: Associations were adjusted for sex, age, BMI, cotinine, physical activity, education, income, alcohol intake, and plasma cholesterol for the fat-soluble nutrients.
Abbreviation: NA, not available.
*Dietary supplement estimates were from open-ended in-person dietary supplement inventory.
†Estimate is not available due to limited number of observations (cell size, <5).

that we did not measure are more strongly related to oxidative DNA damage. For example, a recent study modeled the ''total antioxidant capacity'' and found that uric acid was the greatest independent predictor of total antioxidant capacity (53). Fourth, it is plausible that associations between some of the antioxidants we examined and oxidative DNA damage may be better captured using other measures of oxidative DNA damage. Fifth, although we used recommended sample collection and processing techniques, the possibility of artifactual oxidation is a concern. Finally, it is possible that the distributions of antioxidant concentrations and/or oxidative DNA damage in this study sample were not variable enough to detect associations or that associations do not exist.

We note that comparing results of oxidative DNA damage across studies can be problematic, as oxidative DNA damage can be assessed in several ways: qualitatively by visual scoring, a subjective method whereby comets are classified into categories of damage by eye, or expressed as the tail length, relative tail intensity (percentage of DNA in tail), or the comet tail moment using computer-based image analysis (54). All these approaches are used in research studies, although objective measures are considered optimal (54). In particular, the comet tail moment (used in the present study) is the most sensitive approach for low levels of damage, such as those seen here in healthy participants (55). Second, it is important to note that we only assessed oxidative DNA damage using the comet assay with formamidopyrimidine DNA glycosylase, a measure of direct oxidation of purines; however, there are other sources of oxidative DNA damage including oxidation of the sugar backbone and lipid peroxidation that form additional types of DNA damage, such as malondialdehyde-derived adducts and etheno adducts (56). These DNA lesions are repaired by different pathways, which could affect the results. Oxidative stress alters many other biomolecules, including glutathione and isoprostanes, which have not been evaluated. Future studies would benefit from including several measures of oxidative stress that reflect these divergent pathways.

Our study has several strengths. To our knowledge, it is among the first to examine associations of antioxidant nutrient levels and oxidative DNA damage in a sample of generally healthy African-American and White adults. We collected dietary intake data using two self-report methods (diet recalls and FFQ) and biological markers, which has been suggested

Table 5. Pearson partial correlations of antioxidant nutrient plasma levels and oxidative DNA damage, by race

	Total population			Men		Women	
	Total (*n* = 136)	African-American (*n* = 66)	White (*n* = 70)	African-American (*n* = 31)	White (*n* = 35)	African-American (*n* = 35)	White (*n* = 35)
Vitamin A (retinol)	−0.01	−0.13	0.13	0.45	−0.14	−0.41	0.05
Vitamin C (ascorbic acid)	−0.02	−0.19	0.15	−0.27	0.01	−0.36	0.27
Vitamin E (α-tocopherol)	0.21*	0.13	0.17	0.63*	0.20	−0.05	−0.17
α-Carotene	0.07	0.01	0.06	0.35	0.08	−0.20	−0.06
β-Carotene	0.01	0.05	−0.08	0.15	0.02	−0.24	−0.33
β-Cryptoxanthin	−0.11	−0.13	−0.05	−0.13	−0.23	−0.12	−0.10
Lutein + zeaxanthin	0.12	0.02	0.08	0.01	−0.23	−0.10	0.08
Lycopene	−0.20*	−0.16	−0.12	−0.34	0.10	−0.26	−0.05
All antioxidants combined †	−0.05	−0.15	0.02	0.18	−0.13	−0.36	−0.05

NOTE: Associations were adjusted for age, BMI, cotinine levels, physical activity level, education, income, alcohol intake, plasma cholesterol for the fat-soluble nutrients, and sex for the total population. Oxidative DNA damage was measured as mean comet tail moment of 100 cells via the comet assay.
*P value <0.05.
† Z-scores for the distribution of each antioxidant were calculated and averaged to provide a relative estimate of total antioxidant concentrations.

Table 4. Adjusted mean oxidative DNA damage level (comet tail moment) by antioxidant intakes and plasma levels, by race (Cont'd)

α-Carotene		β-Carotene		β-Cryptoxanthin		Lutein + zeaxanthin		Lycopene	
African-American	White	African-American	White	African-American	White	African-American	White	African-American	White
1.465	1.584	1.444	1.456	1.287	1.521	1.276	1.555	1.296	1.523
1.247	1.420	1.217	1.641	1.399	1.542	1.427	1.619	1.434	1.457
1.439	1.566	1.491	1.542	1.515	1.550	1.373	1.422	1.430	1.673
0.75	0.77	0.70	0.54	0.03	0.35	0.96	0.34	0.73	0.65
1.381	1.590	1.433	1.587	1.273	1.587	1.455	1.502	1.363	1.468
1.354	1.533	1.345	1.517	1.538	1.563	1.365	1.559	1.315	1.556
1.385	1.433	1.356	1.468	1.345	1.430	1.335	1.554	1.429	1.629
0.81	0.81	0.61	0.65	0.54	0.10	0.29	0.18	0.92	0.61
1.421	1.561	1.403	1.550	1.385	1.536	1.405	1.555	1.372	1.528
1.300	1.533	1.392	1.567	1.360	1.575	1.432	1.538	1.410	1.510
1.387	1.469	1.333	1.466	1.374	1.470	1.305	1.497	1.349	1.578
0.34	0.34	0.31	0.68	0.88	0.64	0.40	0.76	0.24	0.99
NA [†]	NA	1.413	1.566	NA	NA	NA	NA	1.385	1.730
NA	NA	1.383	1.548	NA	NA	NA	NA	1.377	1.511
		0.16	0.50					0.64	0.01

as the optimal approach for capturing dietary intake (57). In addition to self-administered queries in the FFQ, information about dietary supplement intake was collected during an open-ended interview and recorded directly from the supplement bottles, a method shown to be superior to self-administered queries (30). Finally, oxidative DNA damage was measured using a modified comet assay with formamidopyrimidine DNA glycosylase, which is considered to be an optimal measure for oxidative stress (36).

This study also has some limitations. First, self-reported dietary data are subject to both random and systematic bias (39) and because blood was collected at only one time point, seasonal variability in antioxidant intakes could not be assessed. Nonetheless, the results using self-reported and biological measures of diet were comparable, and the coefficients of variation for the laboratory assays were within acceptable standards. Second, the capacity for DNA repair activity was not measured; thus these estimates represent the oxidative DNA damage level only at the time of collection. It is also worth pointing out that oxidative DNA damage may not be an optimal intermediate marker of cancer risk, as it is possible that oxidative DNA damage is induced by carcinogenesis. As noted by Loft and Moller (58), ascertaining whether oxidative DNA is a risk factor for, or a result of, carcinogenesis (or both) would be best examined in a prospective cohort investigation. Third, although we controlled for several covariates, residual confounding is still a concern. Fourth, the fact that our study population consisted of generally healthy volunteers may limit generalizability. Finally, due to the cross-sectional nature of this study, we were unable to examine changes in oxidative DNA damage over time and no inferences about causality can be drawn.

In summary, this is among the first studies to examine the relationship between antioxidants (from self-report and biomarkers) and oxidative DNA damage in African-Americans and Whites. It has been suggested that oxidative DNA damage is associated with elevated cancer risk and that antioxidants may mitigate the effects of oxidative DNA damage. In addition, diets high in fruits and vegetables, and which are also rich in antioxidants, have consistently been linked to lower risk of many cancers, including those of the breast, colon/rectum, and prostate, all of which disproportionately affect African-Americans (22). Our findings agree with other studies, suggesting that African-Americans may have dietary patterns that put them at higher risk for cancer and oxidative DNA damage. However, we found that oxidative DNA damage levels were actually lower among African-Americans than Whites in this study population, which has also been reported in several other studies. Participants were healthy and young (20 to 45 years), and it is possible that the DNA repair activity can compensate for diets low in antioxidants in healthy, nonsmoking young adults. Continued research, optimally involving prospective cohort investigations, is needed to assess the relationship among antioxidant nutrients, oxidative damage, and cancer risk, especially in minority populations who suffer a disproportionately high cancer burden.

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