

High dietary antioxidant intakes are associated with decreased chromosome translocation frequency in airline pilots^{1–4}

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ABSTRACT

Background: Dietary antioxidants may protect against DNA damage induced by endogenous and exogenous sources, including ionizing radiation (IR), but data from IR-exposed human populations are limited.

Objective: The objective was to examine the association between the frequency of chromosome translocations, as a biomarker of cumulative DNA damage, and intakes of vitamins C and E and carotenoids in 82 male airline pilots.

Design: Dietary intakes were estimated by using a self-administered semiquantitative food-frequency questionnaire. Translocations were scored by using fluorescence in situ hybridization with whole chromosome paints. Negative binomial regression was used to estimate rate ratios and 95% CIs, adjusted for potential confounders.

Results: Significant and inverse associations were observed between translocation frequency and intakes of vitamin C, β -carotene, β -cryptoxanthin, and lutein-zeaxanthin from food ($P < 0.05$). Translocation frequency was not associated with the intake of vitamin E, α -carotene, or lycopene from food; total vitamin C or E from food and supplements; or vitamin C or E or multivitamin supplements. The adjusted rate ratios (95% CI) for $\geq$ median compared with $<$ median servings per week of high-vitamin C fruit and vegetables, citrus fruit, and green leafy vegetables were 0.61 (0.43, 0.86), 0.64 (0.46, 0.89), and 0.59 (0.43, 0.81), respectively. The strongest inverse association was observed for $\geq$ median compared with $<$ median combined intakes of vitamins C and E, β -carotene, β -cryptoxanthin, and lutein-zeaxanthin from food: 0.27 (0.14, 0.55).

Conclusion: High combined intakes of vitamins C and E, β -carotene, β -cryptoxanthin, and lutein-zeaxanthin from food, or a diet high in their food sources, may protect against cumulative DNA damage in IR-exposed persons. *Am J Clin Nutr* 2009;90:1402–10.

INTRODUCTION

The intake of antioxidants, which can neutralize reactive oxygen species (ROS) generated endogenously or exogenously, has been extensively investigated in relation to DNA damage and cancer risk (1–8). Ionizing radiation (IR) is an established human carcinogen (9) and an efficient inducer of chromosome aberrations (10), which have also been shown to be associated with increased cancer risk in prospective studies (11). In addition to causing direct damage to DNA, IR exposure is an exogenous source of a wide range of ROS, including the superoxide anion and hydroxyl radicals and other nonradical species such as hydrogen peroxide (7, 12, 13). These highly reactive species can cause various forms of DNA damage, such as DNA base mod-

ifications and DNA strand breaks, which can lead to the formation of chromosome aberrations if unrepaired (7, 8, 13, 14).

During past decades, numerous animal or in vitro studies have suggested that antioxidants may provide protection against several forms of DNA damage induced by IR (reviewed in 7, 14–17). To date, human data supporting these associations are limited. Of the dietary antioxidants, vitamins C and E and β -carotene have been the focus of most research (1, 2, 4, 6). However, there are other carotenoids with antioxidant properties, such as α -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin, which are found in relatively large amounts in the diet (3, 5). Studies of intakes of these dietary antioxidants in IR-exposed populations using validated biomarkers of cumulative DNA damage are therefore needed to clarify their possible protective role.

Airline pilots are exposed to elevated levels of cosmic IR and are considered an IR-exposed occupational group in many countries (18). Translocations, a stable form of chromosome aberrations that persist through cell divisions, are an established biomarker of cumulative exposure to chronic and low-dose IR (19). We previously reported that the translocation frequency in airline pilots increased significantly with an increase in the duration of their flight experience in years after adjustment for age and other potential confounders (20). In the present study, we examined whether the translocation frequency of these pilots, as a biomarker of cumulative DNA damage, was associated with their intakes of vitamins C and E and the specific carotenoids and

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² The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

³ Supported in part by an interagency agreement between the National Institute for Occupational Safety and Health and the National Cancer Institute (contract Y1CP802904) and by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute.

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Received June 10, 2009. Accepted for publication August 31, 2009.

First published online September 30, 2009; doi: 10.3945/ajcn.2009.28207.

fruit and vegetables, the major food sources, adjusted for potential confounders.

SUBJECTS AND METHODS

Study subjects

Between December 2001 and September 2002, 83 male airline pilots from a major US airline were enrolled for a biomarker study of cosmic radiation exposure and DNA damage. Details of the study design and methods are presented elsewhere (20). Briefly, based on a telephone screening interview, all subjects met the following study eligibility criteria: 1) age 35–56 y, 2) a never smoker (defined as a person who smoked a lifetime total of <100 cigarettes) or a light smoker (defined as a smoker who had not smoked in the past 10 y or who was currently smoking <10 cigarettes/d), 3) no personal history of cancer except for non-melanoma skin cancer, 4) no history of chemotherapy or radiotherapy (except routine diagnostic X-ray procedures), and 5) no family history of chromosomal instability disorders. Selection was also based on the duration of employment and years of flying international flights to ensure that there was a wide range of occupational cosmic radiation exposures.

At enrollment, all subjects provided a venipuncture blood sample and completed a self-administered study questionnaire. Data collected included health, medical and occupational history, height, weight, smoking and alcohol consumption history, recreational activity, and personal diagnostic X-ray procedures. In addition, dietary data were collected from a self-administered semiquantitative food-frequency questionnaire (FFQ). After the exclusion of a pilot with an implausible total energy intake of >4200 kcal/d, 82 pilots were available for the present analysis. The study was approved by the Human Subjects Review Boards of the National Institute for Occupational Safety and Health and the National Cancer Institute (NCI), and all subjects provided written informed consent.

Assessment of dietary intake

Usual dietary intake was assessed with a 138-item semiquantitative FFQ developed by Willett et al (21). Subjects were asked about the average frequency of consumption of a given unit or portion size for each food using the past year as a reference period. There were 9 possible responses: never or <1/mo, 1–3/mo, 1/wk, 2–4/wk, 5–6/wk, 1/d, 2–3/d, 4–5/d, or ≥6/d. The questionnaire also collected information on the use of supplements of vitamins C and E, β -carotene (dose and duration), and multivitamins (brand, type, frequency, and duration).

Intakes of vitamins C and E and of the specific carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin) from foods were computed by multiplying the frequency of consumption of each food by the nutrient content of the portion specified and then summing over all food items. The food composition values were primarily derived from the nutrient database of the US Department of Agriculture (USDA) (22) and supplemented with manufacturer information. The carotenoid values were based on the USDA-NCI carotenoid database (23, 24). Values for lutein and zeaxanthin were combined because of the difficulty in separating these 2 carotenoids in laboratory analyses (23). For vitamins C and E, total intakes

were also computed by adding the contributions from food and vitamin supplements. However, the specific carotenoid intakes reported here were from dietary sources only. This is because the current use of β -carotene supplements was reported by only 2 subjects, and information on use of other carotenoid supplements was not collected because they were not routinely available in the United States during the time of the study.

The intakes of fruit and vegetables were calculated by summing the intakes (in servings/wk) across the foods belonging to each group. Fruit and vegetables were also categorized a priori into groups according to their type or nutrient content as defined by Steinmetz et al (25), but with modifications to correspond to our study FFQ. The specific groups include 1) high-vitamin C fruit and vegetables (>40 mg vitamin C per serving) consisting of cantaloupes, oranges, orange juice, grapefruit, grapefruit juice, other fruit juices, strawberries, broccoli, and green peppers; 2) citrus fruit consisting of oranges, orange juice, grapefruit, and grapefruit juice; 3) high-carotenoid vegetables consisting of tomatoes, tomato juice, tomato sauce, yellow squash, and carrots (raw or cooked); 4) high- β -carotene fruit and vegetables consisting of carrots (raw or cooked), yams or sweet potatoes, yellow squash, spinach (raw or cooked), cantaloupe, and peaches (item includes apricots and plums); 5) high-lycopene vegetables consisting of tomatoes, tomato juice, and tomato sauce; 6) cruciferous vegetables consisting of broccoli, cabbage or coleslaw, cauliflower, Brussels sprouts, and kale (item also includes mustard and chard greens that do not belong to the cruciferous family); and 7) green leafy vegetables consisting of spinach (raw or cooked), kale, and romaine or leaf lettuce (excluding iceberg and head lettuce).

Assay for chromosome translocations

The analysis of translocations using the established cytogenetic method of fluorescence in situ hybridization with whole chromosome paints has been described in detail previously (20). Cell cultures and slides were prepared by using standardized methods (26, 27). Chromosomes 1, 2, and 4 were painted red, and chromosomes 3, 5, and 6 were simultaneously painted green. The slides were then counterstained with 4',6-diamidino-2-phenylindole. Approximately 1800 cells in metaphase were evaluated for translocations for each subject, which yields information equivalent to 1000 cells in metaphase, as if the full genome had been scored (defined as cell equivalents). The translocations in all cells of each subject were counted and totaled as the translocation frequency. To permit comparisons between subjects, the translocation frequency was converted to the full genome level, ie, expressed per 100 cell equivalents per subject.

Statistical analysis

Descriptive statistics were computed for the dietary antioxidant intakes and translocation frequency across categories of age by using analysis of variance and categories of lifestyle factors and duration of flight experience in years ("flight years"), adjusted for age by using analysis of covariance. Because the distributions of the antioxidant intakes were skewed, they were log_e-transformed, and geometric means and their 95% CIs are reported. In this group of subjects, total energy intake was correlated with the intakes of vitamin C, vitamin E, α -carotene, β -carotene, β -cryptoxanthin,

lycopene, and lutein-zeaxanthin from food ($r = 0.52, 0.78, 0.32, 0.52, 0.33, 0.34$, and 0.53 , respectively) and the total intakes of vitamins C and E ($r = 0.22$ and 0.20 , respectively). Therefore, before all analyses, antioxidant intakes were energy-adjusted by using the residual method to obtain measures of intakes that are not correlated with total energy intake (28).

Separate negative binomial regression models were used to assess the relation between the frequency of translocations (as the dependent variable) and 1) individual antioxidant intake, 2) combinations of antioxidant intakes, 3) vitamin supplement use, and 4) intakes of fruit and vegetables. Negative binomial regression was selected because it provides an efficient approach for the control of overdispersion of the count data of translocation frequency, which can result in increased unexplained variance and biased SEs for the parameter estimates (29). Before the analyses, the energy-adjusted antioxidant intakes were categorized into tertiles based on the distribution of all subjects. This was to avoid assumptions about the shape of the antioxidant intake-translocation frequency relation and to provide sufficient power to compare subjects in the extreme categories of intake. Rate ratios with Wald 95% CIs were estimated for the categories of antioxidants or fruit and vegetables relative to a reference category. The P values for the likelihood ratio chi-square statistic were also computed because it is preferable for small sample sizes.

The energy-adjusted intakes of vitamins C and E and carotenoids were highly correlated, especially for vitamin C and β -cryptoxanthin ($r = 0.81$), β -carotene and α -carotene ($r = 0.80$), β -carotene and lutein-zeaxanthin ($r = 0.70$), vitamin C and lutein-zeaxanthin ($r = 0.47$), vitamin C and β -carotene ($r = 0.36$), and vitamin E and lutein-zeaxanthin ($r = 0.34$), but to a lesser extent for vitamins C and E ($r = 0.20$). Because of multicollinearity, it would be statistically difficult to adjust the intake of each antioxidant for that of the others by introducing them simultaneously in the same model. Therefore, we examined their combined intakes on translocation frequency by categorizing subjects into groups of 1) low intake with <median intake of each antioxidant studied, 2) intermediate intake with a combination of < and $\geq$ median intakes of the antioxidants studied, and 3) high intake with $\geq$ median intake of each antioxidant studied. Because fruit and vegetables are major sources of vitamin C and carotenoids, and green vegetables are a source of vitamin E (30), we also examined their intakes in relation to translocation frequency. Subjects were first categorized by their median intake (number of servings/wk) and the nearest whole number was used as the cutoff to create categories of low and high intakes to permit easy interpretation.

In the multivariate regression models, we adjusted for flight years (quartiles: <13.2, 13.2–17.4, 17.5–23.2, or ≥ 23.3) as well as the known confounders (20): age at blood draw (≤ 40 , 41–45, 46–50, or > 50 y), cumulative red bone marrow X-ray dose score (< 0.5 , 0.5 – 1.9 , or ≥ 2.0), and military flying (yes or no). Additional adjustments were made for the following lifestyle factors that were found to be associated with the intakes of the antioxidants or the frequency of translocations: pack-years of cigarette smoking, alcohol intake, months of vigorous recreational activity, and BMI (weight in kg divided by the square of height in m) as continuous variables. Total energy intake was also included in all models to account for confounding and to reduce measurement errors due to general over- or under-reporting of food intake in the FFQ (28). Because the associa-

tions were slightly strengthened by additional adjustment for the lifestyle factors, all results are presented for the full model. All analyses were performed by using the SAS software version 9.2 (SAS Institute Inc, Cary, NC), and a P value < 0.05 (2-sided) was considered statistically significant.

RESULTS

The mean dietary intakes of vitamins C and E and β -carotene as well as the mean frequency of translocations across categories of age and selected lifestyle characteristics are presented in **Table 1**. None of the antioxidant intakes were significantly associated with age. However, because of a general tendency for the antioxidant intake to increase with increasing age, the relations with other covariates were adjusted for age. The only significant relation was for the age-adjusted vitamin E intake, which was observed to be higher among former smokers with > 1 pack-year of smoking than among never smokers ($P = 0.01$). The other carotenoids were not significantly associated with the above lifestyle characteristics, except for β -cryptoxanthin, for which intake was found to increase significantly with age ($P = 0.04$) (data not shown). For translocation frequency, a significant increase was observed with increasing age ($P = 0.01$) as well as flight years ($P = 0.02$) and the cumulative red bone marrow X-ray dose score ($P = 0.02$) after the adjustment for age (Table 1). The age-adjusted translocation frequency did not vary significantly with BMI, months of vigorous recreational activity, intakes of alcohol and total energy, military flying, and use of vitamin supplements.

The results of separate negative binomial regression models relating translocation frequency with the individual intakes of vitamins C and E and the specific carotenoids for the full model are shown in **Table 2**. Translocation frequency was significantly and inversely associated with the intakes of vitamin C, β -carotene, β -cryptoxanthin, and lutein-zeaxanthin ($P < 0.05$) but not with vitamin E, α -carotene, or lycopene from food. The adjusted rate ratios (95% CIs) for subjects in the highest compared with the lowest tertile were 0.56 (0.38, 0.82) for vitamin C, 0.66 (0.44, 0.97) for β -cryptoxanthin, and 0.60 (0.41, 0.86) for lutein-zeaxanthin. For β -carotene, the adjusted rate ratio (95% CI) was significant for subjects in the middle, but was of borderline significance in the highest tertile compared with those in the lowest tertile: 0.61 (0.41, 0.91) and 0.70 (0.47, 1.03), respectively. These results for intakes from food remained unchanged after the further adjustment for the use of vitamin C, vitamin E, or multivitamin supplements (data not shown). Translocation frequency was not associated with the total intakes of vitamin C or E from food and supplements. In addition, the use of multivitamins (past or current) was not associated with the frequency of translocations, even among current users with a long duration of use (≥ 5 y) or a high frequency (> 5 times/wk) of use (**Table 3**). Similar results were observed for vitamin C or E supplements, including among the current users with respect to duration or dose.

As shown in **Table 4**, the translocation frequency was significantly and inversely associated with the combined intakes of 1) vitamins C and E and 2) β -carotene, β -cryptoxanthin, or lutein-zeaxanthin that included vitamins C and E. However, the strongest inverse association was observed for the combined intakes of β -carotene, β -cryptoxanthin, and lutein-zeaxanthin

TABLE 1

Energy-adjusted dietary antioxidant intake and translocation frequency/100 cell equivalents across categories of selected characteristics among airline pilots ($n = 82$)

Covariates	Subjects	Dietary antioxidant intake ¹			Translocation frequency/100 cell equivalents ²
		Vitamin C	Vitamin E	β -carotene	
	<i>n</i> (%)	<i>mg/d</i>	<i>mg/d</i>	<i>μg/d</i>	
Age					
≤40 y	13 (15.9)	96.50 (76.24, 122.15)	7.86 (6.61, 9.35)	2123.82 (1638.03, 2753.68)	0.27 (0.09, 0.44) ³
41–45 y	24 (29.3)	107.68 (90.53, 128.07)	7.82 (6.88, 8.89)	2569.09 (2122.09, 3110.25)	0.26 (0.13, 0.39)
46–50 y	16 (19.5)	136.70 (110.53, 169.05)	8.00 (6.84, 9.36)	2665.82 (2109.40, 3369.02)	0.48 (0.32, 0.64)
>50 y	29 (35.4)	117.24 (100.13, 137.28)	9.07 (8.07, 10.18)	2981.38 (2505.51, 3547.62)	0.49 (0.38, 0.61)
Age-adjusted					
BMI					
≤25.22 kg/m ²	27 (32.9)	124.62 (105.77, 146.83)	8.30 (7.34, 9.39)	2857.28 (2385.23, 3422.76)	0.39 (0.27, 0.51)
25.23–27.74 kg/m ²	27 (32.9)	114.14 (96.10, 135.57)	8.17 (7.18, 9.30)	2283.42 (1889.35, 2759.69)	0.40 (0.27, 0.53)
≥27.75 kg/m ²	28 (34.2)	102.88 (87.44, 121.04)	8.04 (7.12, 9.08)	2547.93 (2130.46, 3047.21)	0.34 (0.22, 0.46)
Cigarette smoking					
Never	67 (81.7)	111.65 (100.39, 124.17)	7.94 (7.37, 8.54) ³	2563.45 (2282.14, 2879.44)	0.36 (0.29, 0.44)
Former					
≤1 pack-years	5 (6.1)	124.39 (83.96, 184.29)	7.66 (5.83, 10.06)	2041.92 (1328.49, 3138.48)	0.56 (0.27, 0.85)
>1 pack-years	10 (12.2)	126.32 (94.18, 169.42)	11.18 (9.12, 13.71)	2976.59 (2159.19, 4103.43)	0.36 (0.15, 0.58)
Vigorous recreational activity					
≤6/mo	18 (22.0)	108.56 (88.53, 133.13)	7.78 (6.70, 9.04)	2887.89 (2310.47, 3609.61)	0.35 (0.20, 0.50)
>6/mo	64 (78.0)	115.05 (102.97, 128.56)	8.29 (7.64, 8.99)	2482.68 (2198.90, 2803.07)	0.38 (0.30, 0.46)
Alcohol intake					
≤7.1 g/d	27 (32.9)	115.63 (97.79, 136.72)	8.40 (7.42, 9.52)	2578.96 (2142.95, 3103.67)	0.35 (0.22, 0.47)
7.20–15.47 g/d	28 (34.2)	105.01 (88.91, 124.01)	7.90 (6.99, 8.94)	2735.61 (2276.18, 3287.77)	0.40 (0.28, 0.53)
≥15.48 g/d	27 (32.9)	120.65 (102.19, 142.46)	8.22 (7.27, 9.30)	2398.12 (1995.88, 2881.43)	0.37 (0.25, 0.50)
Total energy intake					
≤1772.27 kcal/d	28 (34.2)	109.54 (91.82, 130.70)	8.27 (7.26, 9.42)	2612.24 (2150.11, 3173.70)	0.38 (0.25, 0.51)
1772.28–2291.49 kcal/d	26 (31.7)	119.70 (100.83, 142.10)	7.89 (6.95, 8.95)	2666.13 (2206.63, 3221.30)	0.44 (0.31, 0.57)
≥2291.50 kcal/d	28 (34.2)	111.82 (95.02, 131.59)	8.35 (7.40, 9.41)	2444.73 (2042.90, 2925.58)	0.32 (0.20, 0.44)
Supplement intake					
Multivitamin					
Never	15 (18.3)	134.83 (107.82, 168.61)	8.18 (6.91, 9.68)	2639.59 (2049.05, 3400.32)	0.31 (0.14, 0.48)
Past	18 (22.0)	121.86 (99.84, 148.74)	7.57 (6.52, 8.80)	2657.54 (2120.53, 3330.53)	0.37 (0.22, 0.52)
Current	49 (59.8)	103.68 (90.98, 118.15)	8.42 (7.63, 9.29)	2504.31 (2159.82, 2903.75)	0.40 (0.30, 0.50)
Vitamin C					
Never	27 (32.9)	110.35 (93.46, 130.28)	8.32 (7.36, 9.40)	2639.09 (2194.09, 3174.35)	0.31 (0.19, 0.43)
Past	34 (41.5)	121.68 (104.74, 141.37)	8.47 (7.58, 9.46)	2487.72 (2105.65, 2939.12)	0.41 (0.30, 0.52)
Current	21 (25.6)	104.65 (85.60, 127.94)	7.47 (6.44, 8.66)	2607.37 (2085.38, 3260.02)	0.40 (0.25, 0.55)
Vitamin E					
Never	45 (54.9)	114.10 (99.71, 130.56)	8.16 (7.39, 9.00)	2689.18 (2319.36, 3117.97)	0.37 (0.27, 0.47)
Past	15 (18.3)	106.88 (85.44, 133.70)	8.93 (7.58, 10.52)	2336.91 (1827.78, 2987.87)	0.33 (0.16, 0.49)
Current	22 (26.8)	117.73 (96.19, 144.08)	7.68 (6.62, 8.90)	2478.46 (1985.53, 3093.76)	0.42 (0.27, 0.57)
Flight years					
<13.17	21 (25.6)	126.94 (103.63, 155.49)	8.66 (7.43, 10.10)	2699.73 (2151.77, 3387.24)	0.32 (0.18, 0.47) ⁴
13.17–17.49	19 (23.2)	109.90 (90.32, 133.72)	7.64 (6.59, 8.87)	2812.48 (2258.42, 3502.47)	0.40 (0.26, 0.54)
17.50–23.24	21 (25.6)	95.67 (78.89, 116.03)	8.31 (7.18, 9.62)	2160.54 (1741.32, 2680.68)	0.25 (0.11, 0.39)
≥23.25	21 (25.6)	124.58 (96.98, 160.05)	8.04 (6.65, 9.72)	2606.60 (1969.73, 3449.37)	0.57 (0.39, 0.75)
Cumulative red bone marrow X-ray dose score					
<0.5	33 (40.2)	105.95 (91.05, 123.29)	7.93 (7.09, 8.86)	2478.80 (2102.40, 2922.60)	0.27 (0.16, 0.38) ⁴
0.5–1.9	38 (46.3)	118.15 (102.56, 136.12)	8.15 (7.34, 9.04)	2486.81 (2132.24, 2900.33)	0.45 (0.35, 0.55)
≥2.0	11 (13.4)	126.18 (95.46, 166.78)	9.38 (7.64, 11.51)	3393.90 (2506.38, 4595.71)	0.47 (0.27, 0.67)
Military flying					
No	25 (30.5)	103.96 (87.66, 123.29)	7.51 (6.62, 8.50)	2615.39 (2163.24, 3162.05)	0.32 (0.20, 0.45)
Yes	57 (69.5)	118.97 (105.29, 134.44)	8.54 (7.81, 9.34)	2540.90 (2217.79, 2911.08)	0.40 (0.31, 0.49)

¹ Values are geometric means; 95% CIs in parentheses. *P* values (unadjusted from ANOVA and age-adjusted from ANCOVA on the basis of log_e antioxidant intake) were not statistically significant unless noted otherwise.

² Values are arithmetic means; 95% CIs in parentheses. All covariates were treated as categorical variables in separate negative binomial regression models. *P* values (likelihood ratio chi-square statistic) were not statistically significant unless noted otherwise.

³ *P* = 0.01.

⁴ *P* = 0.02.

TABLE 2
Association between energy-adjusted dietary antioxidant intakes and translocation frequency/100 cell equivalents among airline pilots (*n* = 82)

Dietary antioxidant	Tertile of intake			<i>P</i> value ²
	1 ¹	2	3	
Vitamin C from food only				
Median intake (mg/d)	71.53	120.90	170.95	
No. of subjects	28	27	27	
Rate ratio (Wald 95% CI) ³	1.00	0.59 (0.40, 0.88)	0.56 (0.38, 0.82)	0.01
Total vitamin C				
Median intake (mg/d)	110.41	191.40	663.14	
No. of subjects	27	28	27	
Rate ratio (Wald 95% CI)	1.00	0.92 (0.59, 1.42)	0.81 (0.53, 1.26)	0.65
Vitamin E from food only				
Median intake (mg/d)	6.38	7.89	10.83	
No. of subjects	28	27	27	
Rate ratio (Wald 95% CI)	1.00	1.02 (0.68, 1.54)	0.68 (0.44, 1.04)	0.09
Total vitamin E				
Median intake (mg/d)	7.20	18.80	192.98	
No. of subjects	27	27	28	
Rate ratio (Wald 95% CI)	1.00	1.05 (0.69, 1.60)	0.84 (0.56, 1.27)	0.51
β-Carotene from food only				
Median intake (μg/d)	1736.76	2639.33	3961.57	
No. of subjects	28	27	27	
Rate ratio (Wald 95% CI)	1.00	0.61 (0.41, 0.91)	0.70 (0.47, 1.03)	0.04
α-Carotene from food only				
Median intake (μg/d)	284.21	593.17	1070.93	
No. of subjects	27	27	28	
Rate ratio (Wald 95% CI)	1.00	1.03 (0.69, 1.54)	0.73 (0.49, 1.07)	0.17
β-Cryptoxanthin from food only				
Median intake (μg/d)	45.76	99.84	200.26	
No. of subjects	28	26	28	
Rate ratio (Wald 95% CI)	1.00	0.59 (0.39, 0.88)	0.66 (0.44, 0.97)	0.03
Lycopene from food only				
Median intake (μg/d)	3377.42	4982.25	8272.73	
No. of subjects	28	27	27	
Rate ratio (Wald 95% CI)	1.00	0.85 (0.57, 1.25)	0.81 (0.54, 1.21)	0.55
Lutein-zeaxanthin from food only				
Median intake (μg/d)	1317.83	1995.47	3020.46	
No. of subjects	27	28	27	
Rate ratio (Wald 95% CI)	1.00	0.61 (0.40, 0.92)	0.60 (0.41, 0.86)	0.01

¹ Reference category.
² For the likelihood ratio chi-square statistic (overall test) from separate negative binomial regression models.
³ Adjusted for age (≤40, 41–45, 46–50, or >50 y), flight years (<13.2, 13.2–17.4, 17.5–23.2, or ≥23.3), cumulative red bone marrow X-ray dose score (<0.5, 0.5–1.9, or ≥2.0), and military flying (yes or no) as categorical variables and lifestyle factors (total energy/kcal, pack-years of smoking, months of vigorous recreational activity, alcohol intake, and BMI) as continuous variables.

that included vitamins C and E (adjusted rate ratio: 0.27; 95% CI: 0.14, 0.55 for ≥median compared with <median intake). In addition, the adjusted rate ratios (95% CIs) were significant for subjects with ≥median compared with <median intake of high-vitamin C fruit and vegetables, citrus fruit, and green leafy vegetables: 0.61 (0.43, 0.86), 0.64 (0.46, 0.89), and 0.59 (0.43, 0.81), respectively (Table 5). Although not significant, there was a tendency for rate ratios to be reduced for high intakes of all fruit and vegetables or those that are high in β-carotene, all fruit, all vegetables or those that are high in carotenoids or lycopene, and the cruciferous vegetables.

DISCUSSION

In this group of subjects with IR exposure, we observed significant and inverse associations between the frequency of translocations and the intake of vitamin C, β-carotene, β-cryptoxanthin,

and lutein-zeaxanthin from food, after adjustment for potential confounders. Translocation frequency was not associated with the intake of vitamin E, α-carotene, or lycopene from food; total intake of vitamin C or E from food and supplements; or the use of vitamin C or E or multivitamin supplements. To our knowledge, no previous study has examined the intakes of antioxidants in an IR-exposed population in relation to translocation frequency as a biomarker of cumulative DNA damage with which we can directly compare our findings.

Of the many biological mechanisms, the extensively studied antioxidant function of vitamin C (1, 2, 4) and vitamin E (6) may provide a plausible explanation for some of our findings. In addition, the different effects of the specific carotenoids against DNA damage may reflect their distinct antioxidant properties (3, 5). Another possible explanation is the varying quality of the specific carotenoid values used for the estimation of their intakes. Although the USDA-NCI carotenoid database is the best

TABLE 3
Association between vitamin supplement use and translocation frequency/100 cell equivalents among airline pilots ($n = 82$)¹

Supplement use	Never users ²	Past users	Current users										P value ³	
			Duration				Frequency		Dose					
			<5 y	≥5 y	<10 y	≥10 y	≤5 times/wk	>5 times/wk	≤700 mg/d	≥750 mg/d	≤250 IU/d	≥300 IU/d		
Multivitamins	15 (18.5)	18 (22.2)	20 (24.7)	28 (34.6)	—	—	—	—	—	—	—	—	—	0.49
Rate ratios	1.00	1.37	1.50	1.29	—	—	—	—	—	—	—	—	—	—
(Wald 95% CI)		(0.80, 2.36)	(0.89, 2.52)	(0.77, 2.15)										
Vitamin C	27 (32.9)	34 (41.5)	—	—	11 (13.4)	10 (12.2)	—	—	—	—	—	—	—	0.61
Rate ratios	1.00	1.14	—	—	0.87	1.20	—	—	—	—	—	—	—	—
(Wald 95% CI)		(0.75, 1.71)			(0.49, 1.52)	(0.71, 2.02)								
Vitamin E	45 (55.6)	15 (18.5)	9 (11.1)	12 (14.8)	—	—	—	—	—	—	—	—	—	0.71
Rate ratios	1.00	0.76	0.90	0.89	—	—	—	—	—	—	—	—	—	—
(Wald 95% CI)		(0.48, 1.20)	(0.55, 1.47)	(0.57, 1.41)										
Multivitamins	15 (18.3)	18 (22.0)	—	—	—	—	18 (22.0)	31 (37.8)	—	—	—	—	—	0.65
Rate ratios	1.00	1.38	—	—	—	—	1.41	1.29	—	—	—	—	—	—
(Wald 95% CI)		(0.79, 2.40)					(0.79, 2.48)	(0.78, 2.16)						
Vitamin C	27 (33.3)	34 (42.0)	—	—	—	—	—	—	15 (18.5)	5 (6.2)	—	—	—	0.91
Rate ratios	1.00	1.16	—	—	—	—	—	—	1.03	1.10	—	—	—	—
(Wald 95% CI)		(0.76, 1.75)							(0.62, 1.70)	(0.54, 2.24)				
Vitamin E	45 (54.9)	15 (18.3)	—	—	—	—	—	—	—	—	5 (6.1)	17 (20.7)	—	0.58
Rate ratios	1.00	0.78	—	—	—	—	—	—	—	—	1.07	0.79	—	—
(Wald 95% CI)		(0.49, 1.25)									(0.57, 2.01)	(0.52, 1.21)		

¹ Adjusted for age (≤40, 41–45, 46–50, or >50 y), flight years (<13.2, 13.2–17.4, 17.5–23.2, or ≥23.3), cumulative red bone marrow X-ray dose score (<0.5, 0.5–1.9, or ≥2.0), and military flying (yes or no) as categorical variables and lifestyle factors (total energy/kcal, pack-years of smoking, months of vigorous recreational activity, alcohol intake, and BMI) as continuous variables.

² Reference category.

³ For the likelihood ratio chi-square statistic (overall test) from separate negative binomial regression models.

⁴ One missing for current user.

TABLE 4
Association between combinations of energy-adjusted dietary antioxidant intakes and translocation frequency/100 cell equivalents among airline pilots (*n* = 82)

Dietary antioxidants	Combined intakes ¹			<i>P</i> value ³
	Low ²	Intermediate	High	
Vitamins C and E				
No. of subjects	25	32	25	
Rate ratio (Wald 95% CI) ⁴	1.00	0.62 (0.42, 0.90)	0.42 (0.26, 0.68)	0.001
Vitamins C and E and β-carotene				
No. of subjects	19	47	16	
Rate ratio (Wald 95% CI)	1.00	0.67 (0.45, 0.99)	0.37 (0.21, 0.65)	0.003
Vitamins C and E and β-cryptoxanthin				
No. of subjects	21	41	20	
Rate ratio (Wald 95% CI)	1.00	0.53 (0.37, 0.75)	0.33 (0.20, 0.56)	<0.0001
Vitamins C and E and lutein-zeaxanthin				
No. of subjects	20	45	17	
Rate ratio (Wald 95% CI)	1.00	0.68 (0.46, 1.01)	0.37 (0.22, 0.63)	0.002
Vitamins C and E, β-carotene, β-cryptoxanthin, and lutein-zeaxanthin				
No. of subjects	15	58	9	
Rate ratio (Wald 95% CI)	1.00	0.53 (0.36, 0.79)	0.27 (0.14, 0.55)	0.001

¹ Low intake: <median intake of each antioxidant studied; intermediate intake: a combination of < and ≥median intakes of the antioxidants studied; and high intake: ≥median intake of each antioxidant studied.
² Reference category.
³ For the likelihood ratio chi-square statistic (overall test) from separate negative binomial regression models.
⁴ Adjusted for age (<40, 41–45, 46–50, or >50 y), flight years (<13.2, 13.2–17.4, 17.5–23.2 or ≥23.3), cumulative red bone marrow X-ray dose score (<0.5, 0.5–1.9, or ≥2.0), and military flying (yes or no) as categorical variables and lifestyle factors (total energy/kcal, pack-years of smoking, months of vigorous recreational activity, alcohol intake, and BMI) as continuous variables.

available at this time, data for some carotenoids are more limited than others, and the values for the different carotenoids in foods are influenced by many factors such as varietal differences, growth and harvesting conditions, and food-preparation methods (31).

Despite a high percentage of current users of vitamin C or E (26%) or multivitamin (59%) supplements, we found no evidence for their protective effects on translocation frequency. Additionally, translocation frequency was significantly and inversely associated with the intake of vitamin C from food but not from the higher combined intake from food and supplements. These data may suggest that there is no additional protective effect from supplements beyond that provided from food for those with adequate intake. This is further supported by our findings on intake of β-carotene or β-cryptoxanthin from food in which a larger decrease in translocation frequency was observed for those in the middle than the highest tertile as compared with the lowest tertile. This pattern of a lesser protective effect for intake beyond the middle tertile despite a 1.5–1.8-fold difference in intake between the middle and highest tertile was also observed for the other carotenoids. Therefore, consistent with findings from recent epidemiologic (32) and intervention (33) studies based on cancer incidence and mortality as endpoints, supplements may only benefit those with low intakes from food.

Our data indicate a greater decrease in translocation frequency for the combined intakes of β-carotene, β-cryptoxanthin, or lutein-zeaxanthin from food that included vitamins C and E than for their individual intake. For example, the decrease in translocation frequency for those with high compared with low intakes of vitamins C and E was 58%, but larger decreases were found when this was further combined with β-carotene,

β-cryptoxanthin, or lutein-zeaxanthin (63–67%) and for the combined intakes of all 5 antioxidants (73%). These findings may be explained by the interactive or synergistic effects of antioxidants against DNA damage. In particular, there is in vitro (2, 4, 34) and in vivo (35) evidence that vitamins C and E may exert their antioxidant effects synergistically at their respective lipid- and water-soluble sites and that vitamin C may have the ability to regenerate vitamin E from its oxidized state. Likewise, it is also possible that the specific carotenoids interact with each other, or with vitamins C and E, to prevent DNA damage (2, 5, 36). Thus, it is not the intake of individual but a combination of various antioxidants found in food that may provide the most protection against DNA damage.

The intakes of vitamin C and carotenoids and to a lesser extent, vitamin E, are correlated; therefore, their estimated intakes may be indicators of consumption of fruit and vegetables—the major food sources. The foods that are high in vitamin C include citrus fruit and vegetables such as broccoli and green peppers, whereas lutein-zeaxanthin is predominantly found in green leafy vegetables such as spinach, kale, and romaine lettuce (30). In contrast with β-carotene, which is distributed in more fruit and vegetables, α-carotene is concentrated in carrots, lycopene in tomatoes, and β-cryptoxanthin in fruit, particularly citrus fruit (24). Therefore, it is difficult to distinguish the independent effects of the individual antioxidants.

On the other hand, a significant decrease in translocation frequency was observed for those with high compared with low intakes of the following food groups but not for all or other types of fruit and vegetables: 39% for high-vitamin C fruit and vegetables, 36% for citrus fruit, and 41% for green leafy vegetables. These findings may be supported by the study of Sauvaget et al

TABLE 5Association between intakes of fruit and vegetables and translocation frequency/100 cell equivalents among airline pilots (*n* = 82)

Food group	Servings/wk ¹	<i>n</i>	Rate ratio (Wald 95% CI) ²	<i>P</i> value ³
All fruit and vegetables				
Low intake ⁴	<27.0	40	1.00	0.57
High intake	≥27.0	42	0.90 (0.61, 1.31)	
All fruit				
Low intake	<10.0	43	1.00	0.70
High intake	≥10.0	39	0.94 (0.68, 1.30)	
All vegetables				
Low intake	<16.0	41	1.00	0.44
High intake	≥16.0	41	0.87 (0.60, 1.25)	
High-β-carotene fruit and vegetables				
Low intake	<2.5	39	1.00	0.34
High intake	≥2.5	43	0.85 (0.61, 1.19)	
High-carotenoid vegetables				
Low intake	<4.5	44	1.00	0.60
High intake	≥4.5	38	0.90 (0.62, 1.32)	
High-lycopene vegetables				
Low intake	<3.0	41	1.00	0.20
High intake	≥3.0	41	0.79 (0.56, 1.13)	
Cruciferous vegetables				
Low intake	<1.0	37	1.00	0.09
High intake	≥1.0	45	0.72 (0.50, 1.04)	
High-vitamin C fruit and vegetables				
Low intake	<7.5	39	1.00	0.005
High intake	≥7.5	43	0.61 (0.43, 0.86)	
Citrus fruit				
Low intake	<3.5	42	1.00	0.008
High intake	≥3.5	40	0.64 (0.46, 0.89)	
Green leafy vegetables				
Low intake	<2.0	43	1.00	0.001
High intake	≥2.0	39	0.59 (0.43, 0.81)	

¹ On the basis of the median cutoff, rounded to the nearest whole number.² Adjusted for age (≤40, 41–45, 46–50, or >50 y), flight years (<13.2, 13.2–17.4, 17.5–23.2, or ≥23.3), cumulative red bone marrow X-ray dose score (<0.5, 0.5–1.9, or ≥2.0), and military flying (yes or no) as categorical variables and lifestyle factors (total energy/kcal, pack-years of smoking, months of vigorous recreational activity, alcohol intake, and BMI) as continuous variables.³ For the likelihood ratio chi-square statistic (overall test) from separate negative binomial regression models.⁴ Reference category.

(37), which found that a daily intake compared with an intake of ≤1 time/wk of green-yellow fruit and vegetables was significantly associated with a 13% reduction in cancer mortality in an IR-exposed cohort of >36,000 atomic bomb survivors of Hiroshima and Nagasaki. Fruit and vegetables, however, are a source of other phytochemicals with potential antioxidant properties besides vitamins C and E and carotenoids (30). Thus, the possibility remains that other protective factors found in similar fruit and vegetables may account for the apparent benefits we observed.

Our study had several strengths. To our knowledge, this is the first report of intakes of antioxidants in a group of airline pilots. Because of their job requirements and the frequent medical surveillance to maintain fitness and health throughout their career (20), pilots represent a highly selected occupational group with lifestyle characteristics that differ from those of the general population (eg, they exercise more, smoke less, tend to maintain weight more, and have a better diet). Consequently, pilots are a unique healthy and homogenous group with IR exposure for the examination of the protective effects of antioxidants in relation to DNA damage. In addition to IR and cigarette smoke, ROS are

also generated exogenously through lifestyle activities and endogenously through normal metabolic activity (1–3, 8). Although residual confounding cannot be excluded, it seems unlikely because we have adjusted for potential confounders that included age, flight experience, cumulative X-ray dose score, and military flying (20). Additional adjustments for several major lifestyle factors did not alter the results, except for a slight strengthening of the translocation frequency-antioxidant association.

A limitation of the study was that diet was assessed by using a self-administered FFQ, which could have resulted in some misclassification of the antioxidant intake. However, previous validation studies among subsets of men of comparable socioeconomic status in the Health Professionals Follow-up Study have indicated that estimates of fruit and vegetables and nutrients reflect their long-term intakes or plasma concentrations reasonably well (38–40). It was also possible that some of our observed associations may reflect chance findings due to the number of tests and antioxidants examined. This may have been less likely because the associations were consistent across the antioxidants and their major food sources.

In summary, our data indicate that high intakes of vitamin C, β -carotene, β -cryptoxanthin, and lutein-zeaxanthin from food, particularly their combined intakes that include vitamin E, are significantly associated with a decrease in the translocation frequency of airline pilots with IR exposure. These findings suggest that a diet consisting of a variety of fruit and vegetables that provide a natural source of these antioxidants as well as other potential protective factors may offer the best protection against cumulative DNA damage associated with IR exposure. Our results may be applicable to flight crews worldwide, astronauts in space flight, and frequent flyers in the general population. Further studies of larger IR-exposed populations are needed to examine effect modification of the translocation frequency-IR dose association as well as cancer risk by intake of these antioxidants.

We thank the airline pilots, whose participation made this study possible; the staff of Battelle Centers for Public Health Research and Evaluation (particularly Louise Glezen, Katrina Spencer, and James Kerrigan) for data collection; and Lian Luo of SRA International for assistance with data programming.

The authors' responsibilities were as follows—LCY: developed the concept, designed the study and statistical analysis plan, performed the statistical analysis, wrote the first draft of the manuscript, and obtained funding; MRP: provided statistical expertise and contributed to the design of the statistical analysis plan; LAS: assisted with the assessment of dietary intakes; and AJS and EMW: contributed to the development of the concept and design of the study and obtained funding. All authors played a role in data interpretation and writing of the manuscript and approved the final version of this manuscript. None of the authors had a conflict of interest to declare.

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