Passive Smoke Exposure and Circulating Carotenoids in the CARDIA Study

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Key Words

Carotenoids • Micronutrients • Occupational health • Passive smoking • Smoke exposure • Tobacco smoke pollution

Abstract

Background/Aims: Our objective was to assess associations between passive smoke exposure in various venues and serum carotenoid concentrations. **Methods:** CARDIA is an ongoing longitudinal study of the risk factors for subclinical and clinical cardiovascular disease. At baseline in 1985/1986, serum carotenoids were assayed and passive smoke exposure inside and outside of the home and diet were assessed by self-report. Our analytic sample consisted of 2,633 black and white non-smoking adults aged 18–30 years. **Results:** Greater total passive smoke exposure was associated with lower levels of the sum of the three provitamin A carotenoids, α -carotene, β -carotene, and β -cryptoxanthin (–0.048 nmol/l per hour of passive smoke exposure, $p = 0.001$), unassociated with lutein/zeaxanthin, and associated with higher levels of lycopene (0.027 nmol/l per hour of passive smoke exposure, $p = 0.010$) after adjustment for demographics, diet, lipid profile, and supplement use. Exposure in both

home and non-home spaces was also associated with lower levels of the provitamin A carotenoid index. **Conclusion:** Cross-sectionally, in 1985/86, passive smoke exposure in various venues was associated with reduced levels of provitamin A serum carotenoids.

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Introduction

There is strong evidence that increased oxidative stress from the oxidant compounds contained in cigarette smoke is one mechanism through which active smoking harms health [1]. It is known that compared to non-smokers, individuals who smoke have reduced concentrations of certain serum carotenoids, which are antioxidant micronutrients [2–4]. There is evidence that non-smokers who are exposed to passive smoke also have lower levels of selected serum carotenoids [5–9], although a small study of workplace smoke exposure suggested the opposite [10]. However, individuals who are exposed to greater amounts of passive smoke consume generally poorer diets and have a lower mean dietary carotene intake compared to those individuals who are exposed to less passive

smoke [9, 11–15]. Despite this potential confounding by diet, two cross-sectional studies [5, 6] and one longitudinal study [6] that controlled for diet have reported an association between passive smoking and reduced carotenoid levels suggesting that passive smoke itself affects carotenoid levels.

There are a variety of types of indoor venues in which one could be exposed to passive smoke, including one's home, workplace, and/or other public indoor spaces, and the amount of exposure can vary greatly. However, several of the past studies on passive smoking and serum carotenoid connection have crudely defined passive smoke exposure as living with a smoker [6, 8, 9]. In the previous studies that took passive smoke outside of the home into account, dose was not quantified [5, 7, 10]. In the one study that measured passive smoke dose, only household exposure was assessed [8].

The CARDIA (Coronary Artery Risk Development in Young Adults) study is uniquely suited to further our understanding of the relationship between exposure to passive smoke in various venues and serum carotenoids. In CARDIA, the dose of passive smoke exposure both inside and out of the home was assessed through a self-reported questionnaire. We hypothesized that serum carotenoid levels would be lower in non-smokers who reported exposure to passive smoke in any venue, not just in the home.

Patients and Methods

Overview

CARDIA is an ongoing longitudinal study of the risk factors for subclinical and clinical cardiovascular disease [16, 17]. Briefly, 5,115 black and white men and women aged 18–30 years in four field centers (Birmingham, Ala.; Chicago, Ill.; Minneapolis, Minn., and Oakland, Calif., USA) were recruited in 1985/1986. YALTA (Young Adults Longitudinal Trends in Antioxidants) is an ancillary study to CARDIA, in which serum carotenoids were assayed. The CARDIA and YALTA studies have been approved by the Institutional Review Boards at all participating institutions; subjects gave informed consent for participation. All information in this report arises from the CARDIA baseline examination.

Measures

All interviewer-administered CARDIA questionnaires are available at the study website (www.cardia.dopm.uab.edu). Ethnicity, sex, and field center were ascertained at baseline. Education was categorized as the maximum reported at any examination – high school graduation or less, some college, and college degree or greater. Total passive smoke exposure was determined by the Tobacco Use Questionnaire by the following items: (1) On the average, how many hours per week are you exposed to ciga-

rette, cigar or pipe smoke in a small space other than your home? (2) On the average, how many hours per week are you exposed to cigarette, cigar or pipe smoke in your home because of smoking by others? (3) On average, how many hours per week are you exposed to cigarette, cigar or pipe smoke in a large indoor area (such as a restaurant, hotel lobby, or lecture hall) because of smoking by others? Total passive smoke exposure was the sum of these three exposures and has been shown to have reasonable validity with serum cotinine [18]. Fasting plasma total and HDL cholesterol and triglycerides were measured and LDL cholesterol was estimated as triglycerides/5. Serum cotinine was measured by radioimmunoassay [19, 20].

Using a modified high-performance liquid chromatography method [21], blood samples were used to assay the carotenoids α -carotene, β -carotene, β -cryptoxanthin, lutein/zeaxanthin, and lycopene. All participants were asked to avoid smoking and heavy physical activity for at least 2 h before each examination and to fast for at least 12 h before the examination. After plasma or serum separation, aliquots were stored at -70°C until shipped on dry ice to a central laboratory. Sera obtained at CARDIA baseline (and analyzed approximately 8 years later) were used in the YALTA study to assess the carotenoids (Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota, Minneapolis, Minn., USA) [22]. The HPLC-based assay of carotenoids was a modification of the method of Bieri et al. [21] to optimize detection of carotenoids with calibration as described by Craft et al. [23]. Calibration was performed with pure compounds (Hoffmann-La Roche; Sigma, St. Louis, Mo., USA). Quality control procedures included routine analyses of plasma and serum control pools containing high and low concentrations of each analyte. In addition, the laboratory routinely analyzed NIST (National Institute of Standards and Technology) reference sera and was a participant in the NIST Fat-Soluble Vitamin Quality Assurance Group. The coefficients of variation were $<10\%$ for all analytes and control pools. The intraclass correlation coefficients (ratio of between-person variance to between-person and within-person variance) were 0.93 for α -carotene, 0.98 for β -carotene, 0.73 for lutein and zeaxanthin, 0.97 for β -cryptoxanthin, and 0.73 for lycopene [24].

Since the goal of our study was to isolate the effect of passive smoking on carotenoids, we sought to adjust for dietary patterns to remove confounding that might be caused by the previously documented tendency for passive smokers to have less healthy diets and perhaps less healthy lifestyles [9, 11–15]. The CARDIA Diet History Interview was the source of the food group covariates; it asked over 100 general questions with open-ended responses about foods eaten and their frequencies, and yielded over 700 separate food codes as well as dietary supplement use in 1985/1986. Foods were grouped according to the Nutrient Data System (Nutrition Coordinating Center, University of Minnesota). First, we chose to adjust for food types that were plausibly associated with carotenoids due to their nutrient content. Thus, for the provitamin A carotenoids and lutein/zeaxanthin we chose carotenoid-rich fruits and vegetables (food groups: fruits, fruit juices, green vegetables, and yellow vegetables). For lycopene, we selected the CARDIA food group tomatoes, which included tomato-based sauces, salsas, and pastes (but does not include catsup, which was not separately available for analysis). Based on empirical regression findings using other food groups that plausibly affect oxidative stress, and could spare or reduce circulating carot-

enoids as a consequence, we added red and processed meat, beer, and sugar-sweetened soda groups to the carotenoid-rich fruits and vegetables in the prediction of the provitamin A carotenoids and lutein/zeaxanthin (r^2 for predicting the provitamin A index was 0.26; for lutein/zeaxanthin $r^2 = 0.12$). In prediction of lycopene, we added red and processed meat to tomatoes ($r^2 = 0.09$).

Statistical Analyses

The analyses used data from the CARDIA examinations at year 0, restricted to self-identified never and former smokers with serum cotinine ≤ 13 ng/ml at baseline for whom we have complete data on all predictor and outcome variables ($n = 2,633$ individuals at year 0). We used multivariable linear regression to estimate cross-sectional differences in carotenoid concentrations per hour of weekly passive smoke exposure adjusted for race, sex, field center, maximum attained education, diet, any supplement use, and lipid profile. We adjusted for lipid profile because blood lipid concentrations affect the distribution of fat-soluble antioxidants across fat tissue and blood [25] (models unadjusted for lipids were also run).

Results

The mean age was 25.0 (SD = 3.6) and for each additional year of age, there were 0.63 h per week less total passive smoke exposure, while the provitamin A index was 0.9 $\mu\text{g/dl}$ greater. Mean passive smoke exposure was 19.9 (SD = 24.7) h/week. As in the full sample including smokers [26], in the current sample of non-smokers (table 1), women, whites, individuals with greater educational attainment, and older individuals had higher levels of the provitamin A carotenoids (α -carotene, β -carotene, and β -cryptoxanthin). Food groups were significantly associated with both passive smoke exposure and carotenoid levels. As would be expected, mean serum cotinine levels were low in these non-smokers (1.0 ng/ml for women and 1.2 ng/ml for men).

Table 2 shows that greater total passive smoke exposure was significantly associated with lower levels of an index formed as the sum of the three provitamin A carotenoids after adjustment ($p = 0.001$). Greater total passive smoke exposure was also significantly associated with lower concentrations of each of the provitamin A carotenoids individually (α -carotene, β -carotene, and β -cryptoxanthin). In contrast, total passive smoke exposure was associated with higher levels of lycopene ($p = 0.009$). There was a significant association between the sum of the three carotenoids and exposure to passive smoke in small and large spaces outside and in the home. Associations of passive smoke exposure and circulating carotenoids without lipid adjustment but adjusting for all other variables as in table 2 were similar to those reported in

table 2, except that the association with lycopene was about 15% stronger, and for lutein/zeaxanthin the association was 8.3% weaker.

Table 3 displays mean carotenoid concentrations for each quartile of total passive smoke exposure. All of the individual provitamin A carotenoids as well as the provitamin A index displayed significant linear trends of reduced concentrations at the higher quartiles of passive smoke exposure. Those in the two highest quartiles of total passive smoke exposure (9–29 h/week and ≥ 30 h/week) had a mean provitamin A index that was nearly 5 $\mu\text{g/dl}$ lower than those in the lowest quartile of exposure (0 to <3 h/week of passive smoke exposure). Lycopene displayed a significant trend to higher concentrations as the quartile of passive smoke exposure increased. Lutein/zeaxanthin did not display any significant trend over the quartiles.

Discussion

This study confirms and extends the work of others: even after adjustment for dietary factors, passive smoke exposure was associated cross-sectionally with lower β -carotene [5, 6, 8] and other provitamin A serum carotenoids [6, 8]. Similar to past research on both the effects of active and passive smoking on circulating carotenoids, we saw no effect on the non-provitamin A carotenoid lutein/zeaxanthin [27], although lycopene actually increased with increasing passive smoke exposure. The reasons for the association of greater total hours exposed to passive smoke and higher concentrations of lycopene are not clear, though previous research in CARDIA has also shown a pattern where lycopene is associated with generally less healthy lifestyles [25, 26]. Despite our adjustment for relevant lifestyle factors, as in any observational study, residual confounding may be driving this association.

A limitation is that due to the CARDIA design we were not able to examine how changes in passive smoking relate to changes in serum carotenoid concentrations over time since there was no other CARDIA examination aside from year 0 that included all passive smoke items, diet, and serum carotenoid measurement. Furthermore, by later CARDIA examinations, the range of passive smoke exposure among non-smokers is greatly reduced [mean 4.5 (SD = 12.9) h/week by year 15], and the study had little power to assess associations with this limited amount of passive smoke exposure. A strength of this study is that it used measures of passive smoke exposure that include venues outside of the home and by quantified

Table 1. Unadjusted characteristics of the sample and associations with passive smoking and sum of three carotenoids (restricted to non-smokers who responded to the questions on passive smoke exposure and had a serum cotinine value ≤ 13 ng/ml)

Characteristics	n	Passive smoke exposure total h/week	p value	3 provitamin A carotenoids (sum) $\mu\text{g/dl}$	p value	Lutein/zeaxanthin	p value	Lycopene	p value
Sex									
Female	1,515	22.1	0.027	32.0	<0.001	19.4	0.179	28.3	<0.001
Male	1,118	18.9		26.8		19.0		31.6	
Ethnicity									
Blacks	1,210	23.1	<0.001	26.7	<0.001	18.9	0.058	30.2	0.071
Whites	1,423	17.7		32.4		19.5		29.2	
Center									
Birmingham	651	22.9	<0.001	24.7	<0.001	17.3	<0.001	31.0	<0.001
Chicago	578	21.0		29.9		21.3		32.2	
Minneapolis	633	20.9		30.0		19.9		32.2	
Oakland	771	16.6		33.8		18.8		24.7	
Education									
College or greater	1,479	16.6	<0.001	32.7	<0.001	20.3	<0.001	29.0	0.010
Some college	818	24.1		27.1		18.1		30.9	
High school or less	336	23.9		23.6		17.3		29.9	
Smoking status									
Former	458	20.3	0.918	32.1	0.009	20.3	0.008	29.5	0.703
Never	2,175	20.1		29.3		19.0		29.7	
Supplement use									
No	1,684	21.4	0.001	27.4	<0.001	18.9	0.021	30.4	0.001
Yes	949	17.9		33.9		19.8		28.4	
HDL (median = 53 mg/dl) ^a									
Low	1,316	19.8	0.515	26.7	<0.001	17.4	<0.001	28.8	0.003
High	1,317	20.5		32.9		21.1		30.5	
LDL (median = 107 mg/dl) ^a									
Low	1,267	19.5	0.187	29.0	0.070	18.0	<0.001	26.6	<0.001
High	1,366	20.8		30.5		20.4		32.6	
Triglycerides (median = 60 mg/dl) ^a									
Low	1,299	19.7	0.326	31.9	<0.001	19.6	0.058	28.5	<0.001
High	1,334	20.6		27.6		18.9		30.9	
Carotenoid-rich fruits and vegetables (median = 3.0 servings/day) ^a									
Low	1,292	21.0	0.099	24.4	<0.001	17.9	<0.001	31.0	<0.001
High	1,341	19.4		34.9		20.6		28.5	
Red, lean and processed meat (median = 3.12 servings/day) ^a									
Low	1,503	17.9	<0.001	33.6	<0.001	20.0	<0.001	27.7	<0.001
High	1,130	23.2		24.7		18.2		32.3	
Sugar-sweetened soda (median = 0.87 servings/day) ^a									
Low	1,486	16.9	<0.001	34.0	<0.001	20.3	<0.001	28.8	<0.001
High	1,147	24.4		24.3		17.9		30.9	
Beer (median = 0.07 servings/day) ^a									
Low	1,455	19.3	0.048	30.6	0.023	18.7	<0.001	28.5	<0.001
High	1,178	21.2		28.7		19.9		31.1	
Tomatoes (median = 0.34 servings/day) ^a									
Low	1,342	19.7	0.294	28.0	<0.001	18.5	<0.001	28.5	<0.001
High	1,296	20.7		31.6		20.0		30.9	

All p values from type III SS ANOVA tables.

^a Low/high is dichotomized at the median for the full baseline CARDIA sample (n = 5,111).

Table 2. Differences in mean serum carotenoid levels (nmol/l per hour per week of passive smoke exposure), adjusted for diet, dietary supplement intake, sex, ethnicity, center, lipid profile, and maximum attained education (restricted to non-smokers who responded to the questions on passive smoke exposure and had a serum cotinine value ≤ 13 ng/ml; CARDIA, 1985/1986, n = 2,633)

Home			Small space			Large space			Total		
Est.	SE	p value	Est.	SE	p value	Est.	SE	p value	Est.	SE	p value
Lutein/zeaxanthin											
-0.001	0.012	0.930	-0.011	0.013	0.417	-0.015	0.015	0.330	-0.005	0.006	0.392
Lycopene											
0.038	0.019	0.048	0.036	0.022	0.101	0.043	0.025	0.080	0.027	0.010	0.009
β -Cryptoxanthin											
-0.011	0.008	0.166	-0.018	0.009	0.057	-0.023	0.011	0.032	-0.012	0.004	0.009
α -Carotene											
-0.011	0.005	0.030	-0.006	0.006	0.266	-0.011	0.006	0.084	-0.007	0.003	0.013
β -Carotene											
-0.038	0.019	0.043	-0.031	0.021	0.142	-0.058	0.024	0.017	-0.029	0.010	0.004
Provitamin A carotenoid index (β -cryptoxanthin + α -carotene + β -carotene)											
-0.060	0.026	0.023	-0.055	0.030	0.067	-0.092	0.034	0.007	-0.048	0.015	0.001

Table 3. Estimated mean serum carotenoid concentrations at quartiles of total passive smoke exposure adjusted for diet, dietary supplement intake, sex, ethnicity, center, lipid profile, and maximum attained education (restricted to non-smokers who responded to the questions on passive smoke exposure and had a serum cotinine value ≤ 13 ng/ml; CARDIA, 1985/1986, n = 2,633)

Q1 (n = 557): 0 to <3 h/week		Q2 (n = 703): 3 to <9 h/week		Q3 (n = 687): 9 to <30 h/week		Q4 (n = 686): ≥30 h/week		p for trend
mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	
Lutein/zeaxanthin								
19.54	18.86–20.21	19.44	18.85–20.03	19.30	18.70–19.90	18.76	18.16–19.37	0.087
Lycopene								
28.35	27.25–29.45	28.83	27.86–29.79	30.54	29.56–31.51	30.81	29.82–31.80	<0.001
β-Cryptoxanthin								
10.14	9.67–10.61	9.47	9.07–9.88	9.02	8.61–9.44	8.84	8.42–9.26	<0.001
α-Carotene								
3.73	3.45–4.01	3.30	3.05–3.55	2.98	2.73–3.23	3.06	2.81–3.32	<0.001
β-Carotene								
19.04	17.96–20.12	17.44	16.50–18.39	16.39	15.43–17.34	16.24	15.27–17.21	<0.001
Provitamin A carotenoid index (β-cryptoxanthin + α-carotene + β-carotene)								
32.91	31.39–34.43	30.22	28.89–31.55	28.39	27.04–29.74	28.14	26.78–29.51	<0.001

All units are nmol/l. CI = Confidence interval.

dose. We suspect that just using exposure to smoke in the home, which is only modestly correlated with the exposure to passive smoke in public ($r = 0.19$ – 0.23 in the baseline CARDIA sample of non-smokers), is not ideal since much time is spent outside of the home.

Cigarette smoke contains reactive free radicals and other pro-oxidants that are known to cause oxidative

damage [1]. This study provides additional evidence of oxidative stress and potential damage from tobacco smoke even for non-smokers who are exposed to the smoke of others either inside or outside of the home. These findings highlight one biological mechanism which may be operative in the associations of passive smoking with cancer and cardiovascular disease.

Acknowledgment

CARDIA and YALTA were supported by contracts N01-HC-95095, N01-HC-48047, N01-HC-48048, N01-HC-48049, and N01-HC-48050 and a grant R01-HL-53560 from the National Heart, Lung, and Blood Institute, National Institutes of Health. R.W. was in part supported by the National Cancer Institute

(NCI) Centers for Transdisciplinary Research on Energetics and Cancer (TREC) (U54CA1164849). This material is a result of work supported with resources and use of the facilities at the Minneapolis, MN Veterans Affairs Medical Center. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

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