# Socioeconomic Status, Antioxidant Micronutrients, and Correlates of Oxidative Damage: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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**Objective:** To examine whether socioeconomic status (SES) (education, occupation, income), is associated both cross sectionally and prospectively with circulating concentrations of a) two correlates of oxidative damage, $F_2$-isoprostanes ($F_2$-IsoPs) and gamma-glutamyltransferase (GGT); and b) antioxidant nutrients (ascorbic acid and carotenoids). We also examine whether the proposed associations are mediated by smoking, alcohol consumption, and depression. Risk for chronic disease increases with decreasing SES. One pathway by which low SES might influence disease risk is by promoting oxidative stress. **Methods:** Data from 1278 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study were used to examine the association of SES with oxidation correlates and antioxidant nutrients. Education, occupation, health behaviors, and body mass index (BMI) were assessed during Years 0, 10, and 15 of the study; income and depression were evaluated at Years 10 and 15. $F_2$-isoprostanes were measured at Year 15, gamma-glutamyltransferase (GGT) at Years 0 and 10, carotenoids at Years 0 and 15, and ascorbic acid at Years 10 and 15. **Results:** Cross sectionally, oxidation correlates decreased and antioxidant nutrients increased with increasing SES, estimated in several ways, independent of age, sex, race, and BMI. Prospectively, lower Year 0 education and occupation predicted greater increases in GGT and greater decreases in carotenoids over 10 to 15 years. Prospective associations of Year 0 SES with Year 15 carotenoids were independent of Year 15 SES. Smoking, drinking, and depression symptoms partially mediated these effects. **Conclusions:** Circulating oxidation correlates increase and antioxidant nutrients decrease with decreasing SES, both cross sectionally and prospectively. **Key words:** socioeconomic status, oxidative stress, antioxidants, prospective, CARDIA.

**BMI** = body mass index; **CARDIA** = Coronary Artery Risk Development in Young Adults; **GGT** = gamma-glutamyltransferase; $F_2$-IsoPs = $F_2$-isoprostanes; **SEI** = Stevens and Cho Socioeconomic Index; **SES** = socioeconomic status; **SE** = standard error.

## INTRODUCTION

Chronic disease risk increases steadily with decreasing socioeconomic status (SES) (1). Lower SES might increase disease risk through promotion of behaviors, environments, and psychological states that contribute to oxidative stress, a steady state imbalance favoring pro- to antioxidants. Prooxidants are molecules with a single unpaired electron (free radicals) that have the potential to damage healthy cells whereas antioxidants are chemical compounds that bind to free radicals, preventing cell damage. When the balance is shifted in the direction of prooxidants, an unfavorable and potentially damaging systemic environment is created that surpasses the body's detoxification capacity and, in turn, contributes to organic pathology (2).

Increasing prevalence of certain health risk behaviors with decreasing SES may contribute to higher levels of oxidative stress among persons of lower relative to higher status. Rates of smoking (3) and alcoholism (4) both have been found to increase with decreasing SES. In turn, smoking has been associated with higher levels of oxidative stress (5) and chronic alcohol consumption with increased free radical concentrations (6,7). Low SES also could influence oxidative stress through increased exposure to environmental toxins (hazardous wastes, pollution) at home and at work (8).

Finally, psychosocial factors associated with lower SES, such as depressive symptomatology (9), could also mediate the association of decreasing SES with increasing vulnerability to oxidative stress. Depression may influence oxidative stress directly via activation of the physiological stress axes. Metabolism of catecholamines, for example, results in the production of free-radical intermediates, which irreversibly bind to and damage cell constituents (10). It is possible that increases in catecholamines in response to stimulation of the sympatho-adrenal-medullary axis by depression may contribute to this source of prooxidant activity.

An important measure approximating oxidative stress in vivo is circulating $F_2$-isoprostane concentration ($F_2$-IsoP) (11). $F_2$-IsoPs are oxidatively damaged molecules that result from free radical peroxidation of arachidonic acid. One feature of $F_2$-IsoPs that has contributed to their frequent use marking oxidative stress is that their concentrations depend largely on production rather than metabolism and excretion, thus suggesting they accurately reflect in vivo levels of oxidative damage (12). Empirical support for using $F_2$-IsoPs as oxida-

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tive stress markers derives from comparative animal research wherein $F_2$-IsoPs have been found to increase post experimental oxidant injury (13) and decrease after administration of antioxidants (14).

Gamma-glutamyltransferase (GGT) is an ectoplasmic enzyme produced by many cell types. Traditionally, elevated serum GGT has been used as a clinical marker of excessive alcohol consumption. However, GGT concentrations also have been found to correlate with levels of biologic exposure to other known prooxidant-producing toxins (15,16). Thus, GGT may be an indirect marker of risk for future oxidative damage (17). An earlier report from the Coronary Artery Risk Development in Young Adults (CARDIA) study showed that serum GGT at recruitment and 10 years later correlated with circulating markers of inflammation and oxidative damage at 15 years post recruitment (18).

Antioxidants can be categorized as endogenous antioxidant enzymes and nonenzymatic antioxidant molecules. Nonenzymatic antioxidants include the innate compound glutathione as well as exogenous antioxidant vitamins obtained through the diet, such as $\alpha$-tocopherol, ascorbic acid, and $\beta$-carotene. Circulating antioxidant levels relate inversely to markers of oxidative stress and inflammation, both cross sectionally (19) and prospectively (20). Serum ascorbic acid and carotenoid levels also have been found to correlate inversely with serum GGT (21,22).

Several cross-sectional studies have found circulating antioxidant nutrient levels to vary directly with SES. Higher occupational class has been associated with higher ascorbic acid (23,24) and $\beta$-carotene (25) concentrations, and higher education with higher ascorbic acid (24) and lycopene (a carotenoid) (26) concentrations. Aside from the association of lower GGT with higher education at CARDIA study baseline (18), no published studies have examined whether SES is related to circulating $F_2$-IsoPs or GGT.

The present study has two primary aims. First, we examine whether SES is associated cross sectionally with concurrent concentrations of a) two correlates of oxidative damage ($F_2$-IsoPs and GGT) and b) two dietary nutrients with known antioxidant properties (carotenoids and ascorbic acid). We predict that GGT and $F_2$-IsoPs (hereafter referred to as "oxidation correlates") will increase and antioxidant nutrients will decrease with decreasing SES. Second, we examine whether SES during early adulthood predicts change in two of these biomarkers (GGT and carotenoids) over time. Specifically, we expect lower SES to predict greater increases in GGT and greater decreases in carotenoids over 10 to 15 years.

## METHODS

### Subjects

In 1985 to 1986, 5115 Black and White adults (46% male), aged 18 to 30 years, were recruited into the CARDIA study at four sites: Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. Recruitment was stratified on age (18–24 years, 25–30 years), race (Black, White), sex, and education (≤12 years, >12 years). Participants were recruited by telephone in three of the localities and by telephone and door-to-door contact in the fourth. Overall response rates ranged from 51% to 82% (27,28). Participants were examined at study entry (Year 0) and Years 2, 5, 7, 10, 15, and 20. The current analyses focus on Year 0, and the years that oxidative stress markers were assessed (Years 10 and 15). To obtain a single sample for all analyses, participant data were excluded from the present analyses if any of the following information was missing (see *Measures*): Year 0, 10, or 15 education or occupational class; Year 10 or 15 household income; Year 0 or 10 GGT; Year 15 $F_2$-IsoPs; Year 0 or 15 carotenoids; Year 10 or 15 ascorbic acid. We also excluded data from persons who reported being students at Year 0, as these individuals were in the process of achieving their "educational attainment" and likely were not yet established in the workforce. The resulting sample after exclusions were made was $n = 1278$. Relative to the original 5115 baseline CARDIA participants, the present sample was older (mean age at recruitment = 26.06 years versus 24.85 years), less likely to be female (51.2% versus 54.5%), and less likely to be Black (43.1% versus 51.6%). Alternative analyses that maximized sample sizes in individual analyses (different sample for each analysis) result in identical conclusions despite often substantially greater sample size. Site institutional review committee approval and informed consent were obtained. This manuscript has been approved by the CARDIA steering committee.

### Measures

The Young Adult Longitudinal Trends in Antioxidant (YALTA) ancillary study has assayed various circulating antioxidants and related biochemicals at several times throughout the CARDIA study.

### F2-IsoPs

Plasma $F_2$-IsoPs were measured by gas chromatography-mass spectrometry of blood samples collected at Year 15. This method provides a measure of total free $F_2$-IsoPs, can detect plasma concentrations of 5 to 500 pg/ml, and has high specificity and sensitivity (2). The assay is reproducible with an intraindividual variation of <13% and an interassay coefficient of variation of <10%. Samples were stable for at least 8 years when stored at −80°C (mean ± standard deviation: 56.7 ± 5.7 pg/ml in 2000–2002 ($n = 135$) and 58.1 ± 7.0 pg/ml in 2008 ($n = 9$), with similar values throughout). $F_2$-IsoPs were measured at the Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota.

### GGT

Blood samples for GGT measurement were collected at Years 0 and 10, and frozen at −70°C within 90 minutes of blood drawing. Frozen samples were stored for a few days at the CARDIA clinic center until they were shipped to the laboratory for analysis or to the Solomon Park storage facility for long-term storage at −70°C. Year 0 serum GGT was measured using a continuous flow analyzer (SMAC 12, Technicon Instruments Corp., Tarrytown, New York) at American Bio-science Laboratories (now Smith-Kline Beecham), and Year 10 GGT was measured colorimetrically by a nitroanilide methodology at Linco Research, Inc. As GGT values were not compatible across the two measurement methods, 103 samples frozen for 16 years were reanalyzed. Year 0 values were recalibrated using the following formula (21): recalibrated Year 0 GGT = (1.9004 × original Year 0 GGT) + 2.7618. GGT concentrations from both years were natural log-transformed to reduce skew.

### Antioxidant Nutrients

#### *Carotenoids*

Blood samples for measuring serum carotenoids were collected at Years 0 and 15. Serum carotenoid concentrations ($\alpha$-carotene, $\beta$-carotene, $\beta$-cryptoxanthin, zeaxanthin-plus-lutein, and lycopene) were assayed by high-performance liquid chromatography (HPLC) and stored at −70°C. For analytic purposes, a sum of carotenoids score (hereafter referred to as "carotenoids") was computed by summing the z scores for concentrations of $\alpha$- and $\beta$-carotene, $\beta$-cryptoxanthin, and zeaxanthin-plus-lutein. Lycopene was excluded from the summed score because previous CARDIA reports showed that associations of lycopene with other variables differed substantially from those involving the other four carotenoids (20,29–31).

### *Ascorbic Acid*

Plasma ascorbic acid was measured using HPLC from blood samples collected at Years 10 and 15. Specimens were collected in vials containing metaphosphoric acid and frozen for up to 1 year at −70°C.

All antioxidants were measured at the Molecular Epidemiology and Biomarker Research Laboratory.

## SES

### *Education*

Education was measured by the highest grade of regular school completed. At each follow-up examination, participants were asked to select one code from 01 to 20, with 01 indicating the first year of elementary school and 20 indicating ≥4 years of graduate education.

### *Occupational Class*

Occupation data were collected at each follow-up examination. We used the Stevens and Cho Socioeconomic Index (SEI) (32) as our measure of occupational class. SEI scores are "predicted prestige ratings" assigned to census occupation codes. Scores are derived from a regression of the estimated proportion of people rating the prestige of an occupation as "good" or "excellent" based on a) the proportion of people in that occupation with at least some college education and b) the proportion whose previous year's personal income was ≥$10,000 in 1980 US dollars (32). For the present study, SEI scores were applied to census codes associated with participants' current occupations as per the sociodemographic interviews conducted at each examination.

### *Income*

Household income data were collected beginning at the third follow-up examination (Year 5), by which time it was assumed that all participants had moved out of their parents' homes. Income was divided into eight categories, ranging from <$5,000 to ≥$75,000. For analytic purposes, income was recoded to the midpoint of each of the eight categories ($87,500 for the highest category). Recoded income data were adjusted for household size by dividing by the square root of the number of persons living in the home (33), and for inflation by dividing by the consumer purchasing index relative to 1990 (Year 5). Resulting adjusted income scores were natural log-transformed to reduce skew.

## Smoking, Alcohol Consumption, and Body Mass Index (BMI)

Measurement of BMI (weight in kg/height in $m^2$) and self-reported health practices were obtained during each CARDIA study visit. Self-report measures included smoker status, drinker status, smoking rate (number of cigarettes usually smoked per day), and alcohol consumption (number of drinks usually consumed per week; one drink = one 12-oz. glass, bottle or can of beer, one 5-oz. glass of wine, one 1.5-oz. shot of liquor). Smoker and drinker statuses at each examination were represented by a three-level categorical variable (0 = never; 1 = former; 2 = current). For purposes of analysis, never and former smokers and drinkers were assigned scores of "0" for smoking rate and alcohol consumption, respectively.

## Depression Symptoms

Symptoms of depression were measured at Year 15 using the Center for Epidemiologic Studies Depression Scale (34). Scores were natural-log transformed to approximate a normal distribution.

## Statistical Analyses

Partial correlations were used to examine cross-sectional associations of education, occupational class, and household income with concurrent levels of oxidation correlates and antioxidant nutrients. All analyses controlled for a set of standard covariates: age, sex, race, and concurrent BMI.

Multiple regression was used to conduct lag analyses examining prospective associations of Year 0 SES with Year 10 GGT and Year 15 carotenoids. All lag models controlled for the standard covariates as well as for GGT or carotenoids measured concurrently with the predictor (Year 0), thus examining the change in the dependent variable from the time of its baseline. To determine whether prospective associations between SES and the outcome variables were independent of cross-sectional SES effects, a second set of prospective analyses was conducted that included control for SES measured concurrently with the outcomes.

**TABLE 1. Sample Characteristics[a]**

| | Year 0 | Year 10 | Year 15 |
|---|---|---|---|
| % Smokers | | | |
| Current | 27.78 (355) | 24.80 (317) | 22.07 (282) |
| Former | 14.24 (182) | 19.72 (252) | 22.85 (292) |
| Never | 57.98 (741) | 55.48 (709) | 55.09 (704) |
| Median daily cigarette consumption | 12.0 (5.0–20.0) | 10.0 (5.0–20.0) | 10.0 (5.0–20.0) |
| % Consumers of alcohol | | | |
| Current | 87.95 (1124) | 80.36 (1027) | 79.97 (1022) |
| Former | 5.48 (70) | 15.88 (203) | 16.35 (209) |
| Never | 6.57 (84) | 3.76 (48) | 3.68 (47) |
| Median weekly alcohol consumption | 3.0 (0–8.0) | 3.0 (0–8.0) | 3.0 (0–8.0) |
| Median BMI ($kg/m^2$) | 23.54 (21.48–26.30) | 26.11 (23.32–30.00) | 27.33 (24.16–31.78) |
| Median depression symptoms | NA | 8.0 (0–56) | 7.0 (0–54) |
| Median education (years) | 14.0 (12.0–16.0) | 15.0 (13.0–16.0) | 15.0 (13.0–16.0) |
| Median occupational class | 31.05 (22.58–54.42) | 36.84 (23.88–52.99) | 46.27 (25.23–54.48) |
| Median income (thousands $US)[b] | NA | 21.81 (12.61–36.32) | 27.34 (15.80–46.87) |
| Median GGT ($\mu$g/ml) | 8.00 (5.00–12.00) | 18.00 (12.00–28.00) | NA |
| Median $F_2$-IsoPs (pg/ml) | NA | NA | 50.60 (38.34–69.52) |
| Median carotenoids ($\mu$g/dl) | 40.95 (29.31–56.47) | NA | 55.74 (38.65–78.60) |
| Median ascorbic acid (mg/L) | NA | 8.43 (5.86–10.68) | 8.55 (5.90–11.12) |

BMI = body mass index; GGT = gamma-glutamyltransferase; $F_2$-IsoPs = $F_2$-isoprostanes; CPI = consumer purchasing index.

[a] $n$ = 1278; values displayed as percent ($n$) or median (interquartile range).

[b] Adjusted for CPI relative to 1990 and number of persons living in the household.

## RESULTS

### Sample Characteristics

Sample characteristics are displayed in Table 1. As expected, all three SES indicators increased from Year 0 to Year 15. GGT concentrations increased from Year 0 to Year 10, as did carotenoid concentrations from Year 0 to Year 15. Ascorbic acid levels increased slightly between Years 10 and 15.

### Intercorrelations of Independent and Dependent Variables

*Independent Variables*

Correlations among SES indicators measured during a given examination year ranged from $r = .39$ to .64. Between years, education was most stable ($r > .85$), followed by income ($r = .73$) and occupation ($r = .50–.68$). All correlations were significant at $p < .001$.

*Dependent Variables*

At Years 0 and 10, respectively, higher GGT concentrations were associated with lower carotenoids ($r = -.14$) and ascorbic acid ($r = -.18$). At Year 15, higher $F_2$-IsoPs also were associated with lower carotenoids ($r = -.36$) and ascorbic acid ($r = -.18$). GGT concentrations were correlated across Years 0 and 10 ($r = .65$), carotenoids across Years 0 and 15 ($r = .60$), and ascorbic acid across Years 10 and 15 ($r = .34$). All correlations were significant at $p < .001$.

### Cross-Sectional Analyses

*SES and Oxidation Correlates*

Cross-sectional analyses were conducted for each year that oxidation correlates were collected. Education and income showed the strongest associations with GGT, with higher levels of each being associated with lower concurrent GGT (partial $r = -.08$ to $-.11$, $p < .003$). Occupation also demonstrated associations in the predicted direction, but achieved statistical significance only at Year 10 (Year 0: partial $r = -.05$, $p < .10$; Year 10: partial $r = -.07$, $p < .02$).

Findings for Year 15 $F_2$-IsoPs were similar to those reported for Years 0 and 10 GGT in that education and especially income showed stronger cross-sectional associations with $F_2$-IsoPs than did occupation (education: $r = -.08$, $p < .004$; income: $r = -.21$, $p < .001$; occupation: $r = -.04$, $p = .12$).

*SES and Antioxidant Nutrients*

As expected, higher education, occupation, and income each were associated with higher concurrent carotenoid concentrations at Years 0 and 15 (partial $r = .13–.28$, $p < .001$) and higher ascorbic acid concentrations at Years 10 and 15 (partial $r = .09–.20$, $p < .002$).

### Prospective Analyses

With lag analyses, we examined the prospective associations of Year 0 education and occupation with concentrations of a) the oxidation correlate GGT at Year 10 and b) carotenoids at Year 15. Models included control for age, sex, race, Year 0 GGT or carotenoids, and BMI concurrent with the outcome. Because income was not measured at Year 0, we could not examine 10- and 15-year prospective associations with income as the predictor.

*GGT*

Higher Year 0 education was associated with lower GGT levels at Year 10 ($b = -0.07$, standard error (SE) = 0.007, $p < .01$, partial $R^2 = .004$). When Year 10 education was included as a covariate, the prospective effect of Year 0 education was reduced to nonsignificance ($p = .14$). Higher Year 0 occupation also was associated with lower GGT at Year 10 ($b = -0.06$, SE = 0.001, $p < .05$, partial $R^2 = .003$). Inclusion of Year 10 occupation in the model also resulted in a loss of statistical significance for Year 0 occupation, but a marginal trend remained apparent ($p < .08$). Neither Year 10 education nor Year 10 occupation emerged as independent predictors when entered simultaneously with the analogous Year 0 SES indicator ($p > .60$).

*Carotenoids*

As expected, higher Year 0 education predicted higher carotenoid concentrations at Year 15 ($\beta = 0.12$, SE = 0.006, $p < .001$, partial $R^2 = .011$). When Year 15 education was included as a covariate, the association of Year 0 education with Year 15 carotenoids was unaffected. Higher Year 0 occupation also predicted higher Year 15 carotenoids ($\beta = 0.13$, SE = 0.001, $p < .001$, partial $R^2 = .013$); there was no change in effect size when additional control was added for Year 15 occupation. Neither Year 15 education nor Year 15 occupational class emerged as an independent predictor when entered simultaneously with the analogous Year 0 SES indicator ($p > .13$).

*Mediation of Lagged Association of Year 0 SES with Year 15 Carotenoids*

Smoking and alcohol consumption each have been associated with lower circulating antioxidant concentrations (35,36). Antioxidant concentrations also have been found to decrease with increasing symptoms of depression (36). Thus, we examined whether smoking, alcohol consumption, and depression symptoms may have mediated the associations of Year 0 education and occupation with Year 15 carotenoids.

With univariate analyses, we examined whether each of the potential mediators was associated with Year 15 carotenoid concentrations. Results of univariate analyses indicated that smoker status and daily cigarette consumption at Years 0 and 15 each were related to Year 15 carotenoids ($r = -.27$ to $-.28$, $p < .001$). Drinker status at Year 15 was positively related to concurrent carotenoid levels ($r = .09$, $p < .01$); however, Year 0 drinker status was unrelated to Year 15 carotenoids ($p > .66$). Weekly alcohol consumption at Years 0 and 15 were negatively related to Year 15 carotenoids ($r = -.11$, $p < .001$), as were Year 15 depression symptoms ($r = -.19$, $p < .001$).

To determine whether levels of each of the potential mediators increased with decreasing SES, we examined univariate associ-

**TABLE 2. Change in Association of Year 0 Education With Year 15 Carotenoids When Potential Mediators Are Included in the Model**

| | Association With Year 15 Carotenoids | | | | |
|---|---|---|---|---|---|
| | *B* | SEB | $\beta$ | $\Delta\beta$ | Partial $R^2$ |
| Year 0 education<br>Standard covariates | 0.027 | 0.010 | 0.116** | — | .003 |
| Year 0 education<br>Standard covariates<br>Year 0 and 15 drinker status and alcohol consumption | 0.028 | 0.010 | 0.117** | <0.10% | .004 |
| Year 0 education<br>Standard covariates<br>Year 0 and 15 smoker status and cigarette consumption | 0.020 | 0.010 | 0.085* | −27% | .002 |
| Year 0 education<br>Standard covariates<br>Year 15 depression symptoms | 0.024 | 0.010 | 0.102* | −12% | .003 |
| Year 0 education<br>Standard covariates<br>Year 0 and 15 drinker status and alcohol consumption<br>Year 0 and 15 smoker status and cigarette consumption<br>Year 15 depression symptoms | 0.019 | 0.010 | 0.079† | −32% | .002 |

$\Delta\beta$ = change in $\beta$ in comparison to the base model containing Year 0 education and the standard covariates.
Standard covariates: age, sex, race, Year 15 BMI, Year 0 carotenoids, Year 15 education.
† $p < .06$; * $p < .05$; ** $p < .01$.

ations of SES indicators with each of the mediators. Lower Year 0 education and occupational class each were associated with greater likelihood of being a smoker and greater cigarette consumption at Years 0 and 15 ($p < .001$). Likewise, both Year 0 SES variables were associated with drinker status at Years 0 and 15 ($p < .001$), such that lower SES was associated with a greater likelihood of being a consumer of alcohol.

In separate models that included the standard covariates and Year 15 SES, we examined the reduction in association of Year 0 SES with Year 15 carotenoids when each of the potential mediators was included in the model. When examining smoking and drinking as mediators, we included simultaneously measures from Year 0 and Year 15.

As indicated by Table 2, smoking explained roughly a quarter of the association of Year 0 education with Year 15 carotenoids, and depression symptoms explained about 12% of the association. Explanatory effects of drinking were virtually nonexistent. To determine the degree of overlap between potential mediators in accounting for the association of Year 0 education with Year 15 carotenoids, we entered smoking, drinking, and depression symptoms simultaneously into the model. As shown in the last row of Table 2, the combination of all three mediators accounted for nearly one third of the association between Year 0 education and Year 15 carotenoids. Because this proportion of the association was less than the roughly 40% that would have been accounted for had each of the three mediators made independent contributions to the association, we can assume some overlap in effects.

As indicated by Table 3, smoking explained approximately 20% of the association of Year 0 occupation with Year 15 carotenoids; the proportion explained by drinking was much smaller (2%). Year 15 depression symptoms did not contribute to the association of Year 0 occupation with Year 15 carotenoids. When examined in combination, the mediating variables explained almost one fourth of the association of Year 0 occupation with Year 15 carotenoids, suggesting that smoking and, to a lesser extent, drinking each accounted for a unique proportion of the association.

### *SES by Oxidative Stress Vulnerability*

In exploratory analyses, we examined whether SES might have a greater influence on future GGT levels among persons who already were vulnerable to oxidative stress. Specifically, we asked the question of whether SES effects on future risk for oxidative damage might be stronger among individuals with reduced antioxidant defense. Thus, we examined the interaction of Year 0 education and occupation, respectively, with Year 0 carotenoid levels in the prediction of Year 10 GGT. These models included the standard covariates, Year 0 GGT, Year 0 carotenoids, Year 0 SES, and the interaction of Year 0 carotenoids and Year 0 SES. Results showed no moderating effect of Year 0 carotenoids on the association of either education or occupation with Year 10 GGT ($p > .80$).

## DISCUSSION

Lower SES, estimated in several ways, was associated cross sectionally with higher levels of oxidation correlates and lower levels of antioxidant nutrients. Lower Year 0 education and occupation also predicted greater increases in GGT and decreases in carotenoids over 10 to 15 years. All associations were independent of age, sex, race, and BMI; prospective associations were independent of baseline measures of the outcomes.

Although there is some variability in the size of the associations of education, occupation, and income with each of the outcomes, all three indicators seem to play a role. That cor-

**TABLE 3. Change in Association of Year 0 Occupation With Year 15 Carotenoids When Potential Mediators Are Included in the Model**

| | Association With Year 15 Carotenoids | | | | |
|---|---|---|---|---|---|
| | *B* | SEB | $\beta$ | $\Delta\beta$ | Partial $R^2$ |
| Year 0 occupation<br>Standard covariates | 0.003 | 0.001 | 0.108** | — | .008 |
| Year 0 occupation<br>Standard covariates<br>Year 0 and 15 drinker status and alcohol consumption | 0.003 | 0.001 | 0.106** | −2% | .007 |
| Year 0 occupation<br>Standard covariates<br>Year 0 and 15 smoker status and cigarette consumption | 0.002 | 0.001 | 0.085* | −21% | .005 |
| Year 0 occupation<br>Standard covariates<br>Year 15 depression symptoms | 0.003 | 0.001 | 0.107** | <0.10% | .008 |
| Year 0 occupation<br>Standard covariates<br>Year 0 and 15 drinker status and alcohol consumption<br>Year 0 and 15 smoker status and cigarette consumption<br>Year 15 depression symptoms | 0.002 | 0.001 | 0.082* | −24% | .004 |

Standard covariates: age, sex, race, Year 15 body mass index, Year 0 carotenoids, Year 15 occupational class.
* $p < .01$; ** $p < .001$.

relations of SES with concurrent carotenoids concentrations were larger than correlations of SES with ascorbic acid, GGT or $F_2$-IsoPs might be explained by differences in how each outcome was measured. Carotenoid concentrations were estimated by aggregating across four separate carotenoid measures ($\alpha$-carotene, $\beta$-carotene, $\beta$-cryptoxanthin, zeaxanthin-plus-lutein) whereas each of the other outcome variables was estimated using only a single measurement. Therefore, the carotenoid measure may be a more reliable and more sensitive indicator of the underlying construct it is intended to represent (e.g., total antioxidant capacity) than are the ascorbic acid, GGT, or $F_2$-IsoP measures. An alternative explanation may be that the differing contributions of SES to the variance in each of the outcomes are indicative of a real difference in the association of SES with antioxidant capacity and risk for oxidative damage.

In addition to the cross-sectional analyses, we conducted lag analyses examining the prospective association of Year 0 SES with Year 10 GGT and Year 15 carotenoids, respectively. Results suggest that Year 0 education and occupation each predict changes in GGT and carotenoids across 10 to 15 years, independent of Year 0 biomarker levels. Year 0 education and occupation, respectively, remained independent predictors of Year 15 carotenoids when controlling for Year 15 SES, whereas associations with Year 10 GGT lost significance when controlling for Year 10 SES.

We propose three possible models to explain these findings. a) Year 0 SES influences GGT and carotenoid concentrations measured 10 to 15 years later, regardless of subsequent SES. b) The stability of the association of SES with GGT and carotenoids over time is the factor driving the association of Year 0 SES with Year 10 and 15 biomarker levels. c) Year 10 and 15 SES may be the most important determinant of concurrent GGT and carotenoids, respectively; thus, the primary reason Year 0 SES correlates with later outcomes is that Year 0 SES is a strong predictor of future SES. That control for Year 15 SES did not eliminate the association of Year 0 SES with Year 15 carotenoids reduces the plausibility of the third model and thus suggests that earlier exposure was key. By comparison, associations of Year 0 SES with Year 10 GGT lost significance when Year 10 SES was controlled. These findings, however, also fail to support the hypothesis that this association is primarily attributable to concurrent SES because neither Year 10 SES indicator emerged as an independent correlate of Year 10 GGT.

We examined the possibility that smoking, alcohol consumption, and depression symptoms might account for some of the prospective association of Year 0 education and occupation with Year 15 carotenoid concentrations. Neither singly nor in combination did the three proposed mediators explain the entire association (although the association of Year 0 education with carotenoids lost statistical significance). However, simultaneous inclusion of the three proposed mediators substantially reduced the variance in Year 15 carotenoids accounted for by Year 0 SES, with much of that reduction being attributable to the effects of smoking, with smaller reductions attributable to drinking and depression symptoms.

The results of these analyses suggest that smoking, drinking, and depression symptoms may comprise three potential pathways by which SES influences future antioxidant nutrient concentrations. Smoking may relate directly to reductions in antioxidants as exposure of human plasma in vitro to gaseous phase tobacco smoke has been found to result in an overall degradation in lipophilic antioxidant nutrients including all four of the carotenoids examined here (37). Both smoking and excessive alcohol consumption might indirectly influence carotenoid concentrations by promoting the generation of free radicals (5,7). Because circulating carotenoids are known

scavengers of free radicals (38), it is possible that excess generation of these prooxidant species by damaging properties of tobacco smoke and metabolism of ethanol may deplete available carotenoid stores. Depression has been associated to increased production of proinflammatory cytokines (39), which in turn lead to production of prooxidant species (40). Thus, depression symptoms, as well, may indirectly influence carotenoid concentrations via promotion of oxidative stress.

Because SES remained an independent predictor of carotenoid concentrations after controlling for previous carotenoid levels, and concurrent smoking, drinking, and depression symptoms, one might infer that additional factors not examined here may further explain the association. For example, exposure to environmental tobacco smoke has been associated with lower circulating antioxidant concentrations (41,42). Thus, insofar as lower SES persons, regardless of their own smoking status, are more likely to share living or workspace with smokers, they may be at increased risk of oxidative stress. Poor dietary habits, especially low fruit and vegetable intake, can directly influence circulating antioxidant nutrient concentrations. The Year 0 cross-sectional association of higher fruit and vegetable intake with increased carotenoid levels has previously been demonstrated in the CARDIA study (30). However, when included in the multivariate lag analyses reported here, Year 0 fruits and vegetables were unrelated to Year 15 carotenoids, and thus, did not reduce the prospective association of Year 0 SES with Year 15 carotenoids (data not shown). It is likely that dietary habits at Year 15 would have demonstrated a stronger association with Year 15 carotenoids. These data, however, are not available.

Although independent in association, SES explained a rather small amount of the variance in biomarker concentrations, both cross sectionally and prospectively. In regard to cross-sectional analyses, partial $R^2$ ranged from .002 to .044 for oxidative correlates and from .008 to .078 for antioxidant nutrients. By comparison, the more conservative prospective analyses—that controlled for the cross-sectional associations with Year 15 carotenoids—yielded smaller effects (partial $R^2$ = .003–.008). These latter values are much smaller than those associated with the contributions of smoking at Years 0 and 15 to Year 15 carotenoids (univariate analyses: $r^2$ = .07–.08; analyses including standard controls and SES: partial $R^2$ = .01–.02), which are comparable to those which have been reported previously (35). Despite the relative smallness of effect when compared with that associated with smoking, the effects of SES on carotenoid levels may still be of substantial importance when considered in terms of public health. Even modest differences in levels of a given risk factor can make a large difference in population attributable risk, as opposed to relative risk. Accordingly, were the entire population to shift downward in SES by even a small amount, the impact on antioxidant capacity and ultimately oxidative damage could be considerable.

There are limitations to the present study. First, identical circulating antioxidants were not measured at all years. Likewise, $F_2$-IsoPs were measured only at Year 15. Thus, we were unable to examine prospective associations of Year 0 SES with ascorbic acid or $F_2$-IsoPs. Second, levels for each oxidative correlate and antioxidant marker were based on single measures at the relevant follow-up examinations. Because of variability in actual levels and in the assays, this measurement strategy could result in underestimates of the real effect sizes. Third, because Year 0 income data were not available, we could not examine 10- and 15-year prospective associations of income with oxidation correlates and antioxidant nutrients.

In conclusion, lower SES was associated both cross sectionally and prospectively with higher oxidation correlate concentrations and lower antioxidant nutrient levels. Prospective analyses controlling for concurrent SES suggest that education and occupational class during early adulthood may affect antioxidant levels measured 15 years later independently of current status. Impressively, the effects reported in this article were consistent across cross-sectional (at different examination years) and prospective analyses. It is not clear whether the obtained effect sizes are clinically significant. However, the very existence of the consistent associations reported here, regardless of their size, invite further examination of a possible link between SES and oxidative stress. Refinements in the conceptualization and measurement of both SES and oxidative stress may enable the detection of stronger associations between socioeconomic factors and risk for oxidative damage.

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