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Association of Serum Carotenoids and Tocopherols with γ-Glutamyltransferase: The Cardiovascular Risk Development in Young Adults (CARDIA) Study

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Background: **Our previous studies suggest that serum γ-glutamyltransferase (GGT) activity may be related to oxidative stress, supporting findings of experimental studies. To further examine the role of GGT in relation to oxidative stress, we investigated the association between serum carotenoids and tocopherols, which have antioxidant properties, and serum GGT.**

Methods: **Study participants were 3128 black and white men and women 17–35 years of age in 1985–1986. Serum carotenoids and tocopherols were measured at years 0 and 7, and serum GGT was measured at years 0 and 10.**

Results: **Circulating concentrations of α-carotene, β-carotene, and β-cryptoxanthin inversely predicted the serum GGT concentration measured 10 years later in a dose–response manner (*P* for trend <0.01). Year 0 zeaxanthin/lutein was weakly inversely associated with year 10 GGT (*P* for trend = 0.08), and year 0 lycopene was unrelated to year 10 GGT. Adjusted geometric means of serum GGT at year 10 according to quintile of the sum of four carotenoids at year 0 (α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein) were 19.9, 19.4, 18.9, 17.8, and 17.3 U/L (*P* for trend <0.01). Year 0 α-tocopherol was also a significant inverse predictor of year 10 serum GGT concentration (*P* for trend = 0.03), whereas γ-tocopherol showed an inconsistent or possibly U-shaped association. However, year 0 serum GGT did not predict serum antioxidants measured 7 years later.**

Conclusion: **Our present findings support the contention that serum GGT concentration is a marker related with oxidative stress.**

Serum γ-glutamyltransferase (GGT)[5] is a well-known enzyme marker of alcohol consumption and liver disease *(1)*. However, serum GGT concentrations within the reference interval have also been associated with most cardiovascular risk factors and were a predictor of future heart disease, hypertension, stroke, and type 2 diabetes *(2–9)*. In particular, the serum GGT concentration has shown a strong graded relationship with incident diabetes, suggesting a role in the pathogenesis of diabetes *(4, 8, 9)*. Although the mechanism underlying the above associations remains largely unknown, serum GGT may be associated with these disease outcomes through a mechanism related to oxidative stress *(8, 9)*; recent experimental studies have shown that GGT plays an important role in antioxidant defense systems at the cellular level *(10–13)*.

Supporting the role of GGT in the oxidative stress mechanism, in our previous study *(9)*, serum GGT values within the reference interval at baseline predicted C-reactive protein, a marker of inflammation, and F2-isoprostanes, a marker of oxidative damage to arachidonic acid, measured after 15 years in a dose–response manner. Moreover, dietary heme iron was positively associated with serum GGT concentrations, whereas most dietary antioxidants, especially vitamin C and β-carotene, were inversely associated with serum GGT *(14)*.

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[5] Nonstandard abbreviations: GGT, γ-glutamyltransferase; YALTA, Young Adult Longitudinal Trends in Antioxidants; CARDIA, Cardiovascular Risk Development in Young Adults; and BMI, body mass index.

In the present study, to further examine the role of GGT in relation to oxidative stress, we investigated the association between serum carotenoids and tocopherols, which have known antioxidant properties, and serum GGT concentrations both cross-sectionally and longitudinally.

Materials and Methods

Young Adult Longitudinal Trends in Antioxidants (YALTA), an ancillary study to the Cardiovascular Risk Development in Young Adults (CARDIA) Study, measured serum antioxidant vitamin concentrations in frozen samples. CARDIA is a longitudinal, multicenter epidemiologic study of lifestyle and other factors on the evolution of coronary heart disease risk factors during young adulthood. The study design, recruitment of participants, and methods have been described elsewhere *(15)*. In 1985–1986, 5115 black and white men and women 17–35 years of age were recruited and examined at four clinical sites in the US: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants were reexamined at 2, 5, 7, 10, and 15 years post-baseline, with reexamination rates among surviving cohort members of 91%, 86%, 81%, 79%, and 74%, respectively.

Standard questionnaires were used to maintain consistency in the assessment of demographic and behavioral information across CARDIA examination visits. Sex, race, date of birth, weekly alcohol consumption, and cigarette smoking were determined by structured interview or by self-administered questionnaire. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire *(16)*. Alcohol intake (mL/day) was computed from the self-reported frequency of beer, wine, and liquor consumed per week. Body weight with light clothing was measured to the nearest 0.09 kg (0.2 pounds), and height without shoes was measured to the nearest 0.5 cm. Body mass index (BMI) was computed as weight divided by height squared (kg/m^2). The CARDIA diet history, an interviewer-administered quantitative food frequency questionnaire including ~700 foods, was obtained at years 0 and 7.

All participants were asked to fast at least 12 h and to avoid smoking and heavy physical activity for at least 2 h before each examination. Blood was collected with minimal stasis for GGT. After plasma or serum separation, aliquots were stored at −70 °C until shipped on dry ice to a central laboratory. Serum GGT was measured at year 0 and at year 10. At year 0, GGT was measured with a SMAC II continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically with nitroanilide methodology on a Roche Cobas Mira Plus chemistry instrument at Linco Research Inc. The methodologies for measuring GGT were not comparable between year 0 and year 10. To identify an appropriate recalibration formula, GGT in 103 baseline samples with original GGT values of 3–228 U/L that had been stored at −70 °C for 17 years (since 1985–1986) was remeasured at Linco Research Inc. with the year 10 methodology. The correlation between measurements made in year 0 and those measured with year 10 methodology was 0.995; accordingly, the year 0 GGT values reported here are 2.7618 + (1.9004 × original year 0 values). Year 0 lipids were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Total triglycerides and total HDL-cholesterol were measured by enzymatic procedures. HDL-cholesterol was measured after dextran sulfate–magnesium precipitation. LDL-cholesterol was calculated using the Friedewald equation.

Serum obtained at CARDIA years 0 and 7 was used to assay α- and γ-tocopherol and the carotenoids α- and β-carotene, lycopene, zeaxanthin plus lutein, and β-cryptoxanthin (Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota). The tocopherols and carotenoids were measured by an HPLC-based assay. The assay was a modification of the method of Bieri et al. *(17)* with calibration as described by Craft et al. *(18)* and sample handling as described by Gross et al. *(19)*. The primary modification was simultaneous detection of tocopherols and carotenoids. α-Tocopherol and γ-tocopherol were detected by the use of a second absorbance channel set at 292 nm. The retention times of α- and γ-tocopherol were 5.0 and 4.5 min, respectively. Other modifications were the addition of diisopropylethylamine (0.15 g/L) in the HPLC solvent as an aid to analyte recovery and the use of tocol as an internal standard for tocopherols, rather than tocopherol acetate. Calibration was performed with pure compounds (Hoffmann-La Roche; Sigma Chemical Co.). Quality-control procedures included routine analysis of plasma and serum control pools containing high and low concentrations of each analyte. In addition, the laboratory routinely analyzed NIST reference sera and was a participant in the NIST Fat-Soluble Vitamin Quality Assurance Group. The CV were <10% for all analytes and control pools. Previously, we have shown that the intraclass correlation coefficients (ratio of between-person variance to between- plus within-person variance) were 0.93 for α-carotene, 0.98 for β-carotene, 0.73 for lutein plus zeaxanthin, 0.97 for β-cryptoxanthin, 0.73 for lycopene and 0.93 for α-tocopherol *(20)*.

For this analysis, we excluded those who were missing either year 0 or year 10 GGT (n = 1289), were missing either year 0 or year 7 serum carotenoids and tocopherols (n = 1401), or self-reported diabetes at baseline or during follow-up (n = 194). Some individuals satisfied more than one exclusion criterion, leaving 3128 study participants for analysis.

statistical analysis

We examined associations between serum antioxidant vitamin and serum GGT concentrations in three ways: (*a*)

Table 1. Geometric means of year 0 serum GGT and year 0 serum carotenoids and tocopherols by their quintiles.

	Geometric means of row variables by quintiles of the row variables				
	Q1	Q2	Q3	Q4	Q5
GGT, U/L	7.9	12.2	16.1	21.4	44.4
α-Carotene, μg/L	5	11	17	27	64
β-Carotene, μg/L	45	82	118	169	315
β-Cryptoxanthin, μg/L	31	52	70	96	164
Zeaxanthin, μg/L	86	131	167	214	315
Sum of carotenoids,[a] μg/L	204	308	396	508	792
Lycopene, μg/L	111	208	284	367	511
α-Tocopherol, mg/L	6	8	9	10	13
γ-Tocopherol, mg/L	1	2	2	2	3

[a] Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.

the cross-sectional association between year 0 serum antioxidants (independent variables) and year 0 serum GGT (dependent variable); (*b*) the longitudinal association between year 0 serum antioxidants (independent variables) and year 10 serum GGT (dependent variable); (*c*) the longitudinal association between year 0 serum GGT (independent variable) and year 7 serum antioxidants (dependent variables). Longitudinal associations between year 7 serum antioxidants and year 10 serum GGT were very similar to (*b*); we therefore do not show these findings.

All results are presented as geometric means of dependent variables across quintiles of independent variables because serum GGT concentration was highly right skewed and carotenoids were also right skewed. Similar associations were observed between each of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein and GGT; we therefore also present associations for the sum of these four carotenoids.

Adjustment for potential confounding was done by linear regression. Adjusting variables were baseline values of study center, race, sex, age (years), alcohol consumption (mL/day), BMI (kg/m^2), cigarette smoking (current smoker, ex-smoker, and nonsmoker), and physical activity (continuous). LDL-cholesterol (continuous), HDL-cholesterol (continuous), and triglycerides (continuous) were adjusted in both minimal and fully adjusted models because the distribution of the lipophilic antioxidant vitamin is influenced by circulating lipoprotein concentrations *(21)*. Further adjustment for year 0 intake of meat, vegetables, fruit, and whole grains did not change results; therefore, results concerning food groups are not presented. We repeated the same analyses after stratifying by race (black vs white), sex (men vs women), alcohol consumption status at year 0 (nondrinkers vs drinkers), smoking status at year 0 (never smokers vs current smokers), BMI at year 0 (<25 vs ≥ 25 kg/m^2), and supplement use at year 0 (nonuser vs user of vitamin A, C, or E).

Results

The mean (SD) age of the sample at baseline was 25.0 (3.6) years (range, 17–35 years) with similar percentages of black (45.7%) and white (54.3%) participants. There were more females (55.7%) than males (44.3%) in the sample, and 28% of participants reported being current smokers. Table 1 lists year 0 quintile categories of serum GGT concentration and serum antioxidants at year 0 as geometric means within quintile categories.

Shown in Table 2 are the correlation coefficients between serum carotenoids and tocopherols, serum GGT, and the demographic/health behavior variables at baseline. α-Carotene, β-carotene, cryptoxanthin, zeaxanthin/lutein, and α-tocopherol showed similar associations; individuals with higher serum concentrations tended to be white, female, older, nonsmokers, nondrinkers, lean, and physically active. In contrast, the direction of association between serum GGT and demographic/health behavior variables, except age, were the opposite from those of α-carotene, β-carotene, cryptoxanthin, zeaxanthin/lutein, and α-tocopherol. On the other hand, individuals with higher serum lycopene tend to be male and drink

Table 2. Simple or partial correlation coefficients between serum carotenoids and tocopherols, serum GGT, and demographic or health behavior variables at baseline.

	Race[a]	Sex[a]	Age	Alcohol consumption	BMI	Physical activity	Current smoker
α-Carotene	0.36[b]	0.15[b]	0.21[b]	−0.13[b]	−0.20[b]	0.09[b]	−0.20[b]
β-Carotene	0.14[b]	0.16[b]	0.15[b]	−0.20[b]	−0.15[b]	0.03	−0.21[b]
β-Cryptoxanthin	0.01	0.03	0.004	−0.14[b]	−0.14[b]	0.09[b]	−0.22[b]
Zeaxanthin/Lutein	0.03	0.006	0.13[b]	−0.03	−0.12[b]	0.08[b]	−0.11[b]
Sum of carotenoids[d]	0.11[b]	0.10[b]	0.15[b]	−0.15[b]	−0.18[b]	0.08[b]	−0.22[b]
Lycopene	−0.004	−0.11[b]	−0.02	0.07[b]	−0.001	0.02	0.02
α-Tocopherol[e]	0.28[b]	0.008	0.15[b]	−0.06[b]	−0.14[b]	0.11[b]	−0.12[b]
γ-Tocopherol[e]	−0.10[b]	−0.04[c]	−0.01	−0.03	0.17[b]	−0.02	−0.01
GGT	−0.27[b]	−0.26[b]	0.08[b]	0.22[b]	0.18[b]	0.01	0.14[b]

[a] Race (0 = black; 1 = white); sex (0 = male; 1 = female).
[b] $P < 0.01$; [c] $P < 0.05$.
[d] Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.
[e] Adjusted for LDL-cholesterol, HDL-cholesterol, and triglycerides.

Table 3. Adjusted[a] geometric means (SE) of year 0 serum GGT by quintiles of year 0 serum carotenoids and tocopherols (cross-sectional association).

Serum carotenoids and tocopherols at year 0	Geometric mean (SE) serum GGT at year 0,[b] U/L					*P* for trend
	Q1	Q2	Q3	Q4	Q5	
α-Carotene	18.8 (0.48)	16.8 (0.38)	16.9 (0.36)	16.7 (0.34)	16.1 (0.36)	<0.01
β-Carotene	18.6 (0.43)	17.2 (0.37)	16.8 (0.35)	16.3 (0.34)	16.3 (0.35)	<0.01
β-Cryptoxanthin	18.2 (0.41)	16.8 (0.36)	17.1 (0.36)	17.1 (0.35)	15.9 (0.34)	<0.01
Zeaxanthin/Lutein	17.9 (0.40)	16.4 (0.35)	17.0 (0.36)	16.9 (0.36)	16.7 (0.36)	0.15
Sum of carotenoids[c]	18.1 (0.42)	17.4 (0.38)	16.9 (0.36)	16.3 (0.34)	16.2 (0.35)	<0.01
Lycopene	17.5 (0.38)	17.2 (0.37)	16.6 (0.35)	16.8 (0.35)	16.8 (0.37)	0.13
α-Tocopherol	18.1 (0.45)	16.9 (0.37)	16.5 (0.34)	16.7 (0.36)	16.9 (0.39)	0.09
γ-Tocopherol	17.9 (0.37)	17.2 (0.36)	16.4 (0.34)	16.8 (0.36)	16.4 (0.39)	<0.01

[a] Adjusted for study center, race, sex, age, BMI, alcohol consumption, smoking, physical activity, LDL-cholesterol, HDL-cholesterol, and triglycerides; dependent variable was lnGGT.
[b] Q1–Q5 are quintiles of the row variables.
[c] Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.

more. γ-Tocopherol was higher among black, male, and obese individuals.

In cross-sectional analyses restricted to year 0 data, serum GGT showed inverse associations with most serum carotenoids and tocopherols (Table 3). The strongest associations were with α-carotene, β-carotene, and β-cryptoxanthin.

Longitudinal associations were clearer than cross-sectional associations. Year 0 α-carotene, β-carotene, and cryptoxanthin strongly and inversely predicted year 10 GGT (Table 4). Year 0 serum zeaxanthin/lutein and α-tocopherol were also inversely associated with year 10 serum GGT. However, serum lycopene did not predict serum GGT concentration, and γ-tocopherol showed an inconsistent or U-shaped association. Findings were attenuated by additional adjustment for year 0 GGT, suggesting that a substantial part of the longitudinal association of serum GGT with year 0 serum antioxidants was already present at year 0, but that there was some expansion of the inverse associations over the course of 10 years. On the other hand, year 0 GGT did not predict any of year 7 serum antioxidants after full adjustment (Table 5).

In analyses stratified on baseline variables, the associations between year 0 sum of carotenoids and year 10 serum GGT were consistently present among most subgroups, including race, sex, and vitamin supplement usage; however, the relationships tended to be more strongly inverse among year 0 drinkers and smokers (Table 6). In the case of BMI, although the *P* value for interaction was significant, the difference in shape between the two BMI categories was based solely on the GGT concentration in the highest carotenoid quintile among individuals with BMI ≥25 kg/m^2.

Discussion

Antioxidants such as carotenoids or tocopherols have been known to contribute to the body's defense against reactive oxygen species *(22, 23)*. High blood concentrations of these nutrients were prospectively shown to be associated with low incidence of cardiovascular disease and several cancers *(24, 25)*, although a causal link is

Table 4. Adjusted[a] geometric means (SE) of year 10 serum GGT by quintiles of year 0 serum carotenoids and tocopherols (longitudinal association).

Serum carotenoids and tocopherols at year 0	Geometric mean (SE) serum GGT at year 10,[b] U/L					*P* for trend
	Q1	Q2	Q3	Q4	Q5	
α-Carotene	21.1 (0.61)	18.7 (0.48)	18.7 (0.45)	17.9 (0.43)	17.5 (0.44)	<0.01
β-Carotene	20.6 (0.55)	19.8 (0.49)	18.4 (0.44)	18.0 (0.44)	16.8 (0.42)	<0.01
β-Cryptoxanthin	20.0 (0.53)	19.2 (0.47)	18.2 (0.44)	18.7 (0.45)	17.2 (0.43)	<0.01
Zeaxanthin/lutein	19.7 (0.50)	18.1 (0.45)	18.6 (0.46)	18.9 (0.46)	17.4 (0.44)	0.08
Sum of carotenoids[c]	19.9 (0.53)	19.4 (0.49)	18.9 (0.46)	17.8 (0.43)	17.3 (0.44)	<0.01
Lycopene	18.8 (0.47)	18.8 (0.47)	18.2 (0.44)	18.7 (0.46)	18.5 (0.47)	0.66
α-Tocopherol	20.1 (0.58)	18.4 (0.46)	18.7 (0.45)	17.7 (0.44)	18.3 (0.49)	0.03
γ-Tocopherol	18.9 (0.45)	18.6 (0.45)	17.7 (0.43)	18.9 (0.47)	19.1 (0.52)	0.83

[a] Adjusted for study center, race, sex, age, BMI, alcohol consumption, smoking, physical activity, LDL-cholesterol, HDL-cholesterol, and triglycerides; dependent variable was lnGGT.
[b] Q1–Q5 are quintiles of the row variables.
[c] Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.

Table 5. Adjusted[a] geometric means (SE) of year 7 serum antioxidants by quintiles of year 0 serum GGT.

	Geometric mean (SE) of serum antioxidants at year 7[b]					
	Q1	Q2	Q3	Q4	Q5	*P* for trend
α-Carotene, μg/L	29.0 (0.9)	27.6 (0.8)	27.4 (0.9)	28.4 (0.8)	26.6 (0.9)	0.25
β-Carotene, μg/L	144 (4.2)	143 (3.6)	144 (4.1)	145 (3.7)	133 (4.1)	0.21
β-Cryptoxanthin, μg/L	77 (1.7)	74 (1.5)	75 (1.7)	76 (1.5)	70 (1.7)	0.12
Zeaxanthin, μg/L	175 (3.3)	174 (2.9)	180 (3.4)	180 (3.0)	170 (3.5)	0.98
Sum of carotenoids,[c] μg/L	449 (8.7)	440 (7.4)	450 (8.8)	452 (7.7)	423 (8.8)	0.30
Lycopene, μg/L	292 (6.0)	288 (5.1)	304 (6.3)	308 (5.4)	280 (6.1)	0.77
α-Tocopherol, mg/L	9.9 (0.1)	10.0 (0.1)	9.9 (0.1)	10.1 (0.1)	9.8 (0.1)	0.95
γ-Tocopherol, mg/L	2.2 (0.04)	2.2 (0.03)	2.2 (0.04)	2.1 (0.03)	2.1 (0.04)	0.13

[a] Adjusted for study center, race, sex, age, BMI, alcohol consumption, smoking, physical activity, LDL-cholesterol, HDL-cholesterol, and triglycerides; dependent variables were serum antioxidants.
[b] Q1–Q5 are quintiles of serum GGT at year 0.
[c] Sum of α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein.

unclear. In our large, diverse sample, circulating concentrations of the carotenoids β-carotene, α-carotene, β-cryptoxanthin, and zeaxanthin/lutein were inversely related to serum GGT both cross-sectionally and longitudinally. α-Tocopherol was also a significant inverse predictor of serum GGT, whereas γ-tocopherol showed an inverse association with GGT cross-sectionally, but higher year 0 concentrations of γ-tocopherol were associated with inconsistent year 10 serum GGT concentrations.

Our present and previous studies with CARDIA participants *(9, 14)* consistently suggest that serum GGT might be one of the enzymes related to oxidative stress. One reason for this conclusion is that dietary heme iron positively predicted future serum GGT concentration; free iron, which would cause oxidative stress *(26)*, might mediate this association, although free iron was not measured. Another reason is that most dietary antioxidants, especially vitamin C and β-carotene, inversely predicted future serum GGT. A third reason is that baseline serum GGT predicted C-reactive protein, a marker of inflammation, and F2-isoprostanes, a marker of oxidative damage to arachidonic acid, measured 15 years later. Finally, in the present study serum antioxidants inversely predicted future serum GGT concentrations.

In agreement with our findings, experimental studies have shown that GGT plays an important role in antiox-

Table 6. Adjusted[a] geometric means (SE) of year 10 serum GGT by quintiles of year 0 sum of carotenoids after stratification by race, sex, alcohol consumption (year 0), BMI (year 0), smoking (year 0), and vitamin supplement use (year 0).

	Geometric mean (SE) serum GGT at year 10,[b] U/L					
	Q1	Q2	Q3	Q4	Q5	*P* for trend
Race (*P* for interaction = 0.83)						
Black (n = 1428)	23.5 (0.94)	22.7 (0.85)	22.9 (0.86)	20.6 (0.80)	21.5 (0.96)	0.04
White (n = 1700)	17.2 (0.62)	16.9 (0.56)	16.0 (0.50)	15.8 (0.48)	14.7 (0.44)	<0.01
Sex (*P* for interaction = 0.60)						
Male (n = 1385)	27.6 (1.11)	25.8 (0.97)	24.5 (0.93)	23.4 (0.91)	22.4 (0.98)	<0.01
Female (n = 1743)	15.3 (0.55)	15.5 (0.52)	15.2 (0.48)	14.2 (0.44)	14.1 (0.43)	0.02
Alcohol (*P* for interaction = 0.07)						
No (n = 1182)	17.3 (0.72)	16.8 (0.64)	17.0 (0.64)	16.1 (0.56)	16.6 (0.59)	0.34
Yes (n = 1931)	21.6 (0.75)	21.1 (0.69)	20.0 (0.63)	19.0 (0.62)	17.8 (0.62)	<0.01
BMI (*P* for interaction = 0.01)						
<25 (n = 2119)	18.7 (0.64)	18.2 (0.56)	17.7 (0.51)	16.7 (0.47)	15.6 (0.44)	<0.01
≥25 (n = 1009)	22.5 (0.97)	22.4 (0.94)	21.6 (0.99)	20.2 (0.95)	22.8 (1.25)	0.47
Smoking (*P* for interaction <0.01)						
No (n = 1835)	18.3 (0.67)	17.8 (0.56)	17.9 (0.53)	17.1 (0.49)	16.7 (0.47)	0.04
Ex or current (n = 1274)	22.0 (0.87)	22.0 (0.90)	20.3 (0.84)	19.1 (0.81)	18.0 (0.90)	<0.01
Supplement (*P* for interaction = 0.49)						
No (n = 2140)	20.2 (0.62)	20.1 (0.60)	19.6 (0.58)	18.5 (0.56)	17.6 (0.59)	<0.01
Yes (n = 988)	20.0 (1.13)	18.1 (0.82)	17.4 (0.74)	16.4 (0.67)	16.4 (0.62)	<0.01

a Adjusted for study center, race, sex, age, BMI, alcohol consumption, smoking, physical activity, LDL-cholesterol, HDL-cholesterol, and triglyceride; dependent variable was lnGGT.
[b] Q1–Q5 are quintiles of sum of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin/lutein).

idant defense systems at a cellular level *(10–13)*. Although serum GGT has been commonly used as a marker of alcohol consumption or liver disease *(1)*, cellular GGT activity is widely distributed in the human body, especially in the kidney and liver *(27)*. Cellular GGT catalyzes the initial step in the degradation of extracellular glutathione, thereby providing a supply of constituent amino acids for uptake and reutilization in intracellular glutathione synthesis. Glutathione plays an important role in protecting cells against oxidants that are produced during normal metabolism. If oxidative stress increases, then so will the requirement for reduced glutathione. Conversely, if glutathione is not available, then the cells will be more vulnerable to development of oxidative stress. The importance of cellular GGT in maintaining adequate concentrations of intracellular glutathione under normal conditions has been demonstrated across several cell types, tissues, and organs *(10)*.

Inverse associations between serum antioxidants and markers of oxidative stress might be interpreted in two ways: low serum antioxidants can cause oxidative stress, but high oxidative stress can also deplete serum antioxidants. However, in this study, although baseline antioxidants predicted future serum GGT concentrations, baseline GGT did not predict future serum antioxidants. If our interpretation of GGT as a marker of oxidative stress is true, it suggests that serum GGT might be an earlier or more sensitive marker of oxidative stress than commonly used markers of oxidative stress or inflammation, such as F2-isoprostanes or CRP. Increased serum GGT within physiologic concentrations might not reflect high enough oxidative stress to cause depletion of antioxidants.

In this study, although the inverse associations between serum antioxidants and serum GGT were consistently observed among most subgroups, the finding tended to be stronger among drinkers and past or current smokers. The ethanol contained in alcoholic beverages and cigarette smoking can increase oxidative stress *(28, 29)*; it therefore might be interpreted that protection against oxidative damage may be greatest among those with an initial oxidative stress burden.

In conclusion, in this prospective study, we found that baseline serum antioxidants were strongly and inversely associated with future serum GGT concentrations. Taken together with our previous studies, serum GGT might be a marker of oxidative stress. Measurement of serum GGT is easy and inexpensive. Further studies on serum GGT as a marker of oxidative stress are needed.

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