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Functional genetic variants of glutathione *S*-transferase protect against serum ascorbic acid deficiency[1–3]

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ABSTRACT

Background: Glutathione *S*-transferases (GSTs) are detoxifying enzymes that contribute to the glutathione-ascorbic acid (vitamin C) antioxidant cycle.

Objective: The objective was to determine whether *GST* genotypes modify the association between dietary vitamin C and serum ascorbic acid.

Design: Nonsmoking men and women (n = 905) between 20 and 29 y of age were participants in the Toronto Nutrigenomics and Health Study. Overnight fasting blood samples were collected to determine serum ascorbic acid concentrations by HPLC and to genotype for deletion polymorphisms in *GSTM1* and *GSTT1* and an Ile105Val substitution in *GSTP1*. A 196-item food-frequency questionnaire was used to estimate vitamin C intake.

Results: A gene-diet interaction on serum ascorbic acid was observed for *GSTM1* (P = 0.04) and *GSTT1* (P = 0.01) but not for *GSTP1* (P = 0.83). The odds ratio (95% CI) for serum ascorbic acid deficiency (<11 μmol/L) was 3.20 (1.88, 5.44) for subjects who did not meet the Recommended Dietary Allowance of vitamin C compared with those who did. The corresponding odds ratios (95% CIs) were 2.17 (1.10, 4.28) and 12.28 (4.26, 33.42), respectively, for individuals with the *GSTT1*1/*1 +*1/*0* (functional) and *GSTT1*0/*0* (null) genotypes and 2.29 (0.96, 5.45) and 4.03 (2.01, 8.09), respectively, for the *GSTM1*1/*1+GSTM1*1/*0* and *GSTM1*0/*0* genotypes.

Conclusions: The recommended intake of vitamin C protects against serum ascorbic acid deficiency, regardless of genotype. Individuals with *GST* null genotypes had an increased risk of deficiency if they did not meet the Recommended Dietary Allowance for vitamin C, which suggests that the GST enzymes protect against serum ascorbic acid deficiency when dietary vitamin C is insufficient. *Am J Clin Nutr* 2009;90:1411–7.

INTRODUCTION

Vitamin C (ascorbic acid) is an essential nutrient involved in the synthesis of carnitine, collagen, norepinephrine, and adrenaline (1), recycling other antioxidants, facilitating iron absorption and the conversion of cholesterol to bile acids, and inhibiting oxidative damage (2). An inverse relation has been observed between serum ascorbic acid concentrations and several markers of chronic disease, including glucose homeostasis (3), blood pressure (4, 5), oxidative stress (6, 7), high-sensitivity C-reactive protein (8), and indicators of obesity such as body mass index (BMI) and waist-to-hip ratio (9, 10). Serum ascorbic acid is also inversely associated with risk of cardiovascular disease (11, 12), diabetes (3, 13), cancer (14) and all-cause mortality (15).

Serum ascorbic acid concentrations are considered to be adequate if >28 μmol/L, suboptimal if between 11 and 28 μmol/L, and deficient if <11 μmol/L (16, 17), because symptoms of scurvy have been observed just below this level (18). Although vitamin C deficiency is not currently viewed as a major health concern in North America, recent reports from the third National Health and Nutrition Examination Survey (NHANES) and the Toronto Nutrigenomics and Health Study showed that deficient serum ascorbic acid concentrations are common, occurring in 11–17% of young adults (19, 20). The Recommended Dietary Allowance (RDA) for vitamin C has been set at 75 mg/d for nonsmoking, nonpregnant women and at 90 mg/d for nonsmoking men to attain adequate serum ascorbic acid concentrations (18). However, there is substantial variability in the serum ascorbic acid response when the same amount of dietary vitamin C is consumed (21), even when known determinants of serum ascorbic acid such as age (22), sex (23, 24), smoking (23, 25), body weight (21, 26), physical activity (27), and season (25) are controlled for.

The glutathione *S*-transferases (GSTs) are a family of enzymes that catalyze the transfer of glutathione to a variety of substrates. GSTs are able to reduce dehydroascorbic acid back to ascorbic acid through enzymatic reactions with glutathione (28). GSTM1, GSTT1, and GSTP1 are isoforms of the mu, theta, and pi class of GSTs, respectively. Homozygosity for a common deletion of the *GSTM1* gene (*GSTM1*0*) results in the absence of GSTM1 activity and is considered to be a nonfunctional genotype (29). Similarly, a deletion polymorphism in *GSTT1* leads to lack of enzyme activity and has 2 alleles: *GSTT1*0* (nonfunctional) and *GSTT1*1* (functional) (30). An A to G polymorphism at nucleotide 313 of *GSTP1* results in an amino acid substitution (Ile105Val) that alters the catalytic activity of GSTP1 (31, 32).

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Glutathione and ascorbic acid show a strong functional interdependence, because both are electron donors and have the ability to protect each other from oxidation (33). Together they form a compensatory network by which oxidative stress can be decreased (34). Serum ascorbic acid concentrations have been shown to differ between individuals with different GST genotypes, although dietary vitamin C was not considered (35). The purpose of the present study was to determine whether GST genotypes modify the association between dietary vitamin C and serum ascorbic acid deficiency.

SUBJECTS AND METHODS

Study design and participants

Subjects (n = 1090; 756 women and 334 men) were recruited from the University of Toronto campus to be participants in the Toronto Nutrigenomics and Health Study, which is a cross-sectional examination of free-living adults between 20 and 29 y of age. Subjects were recruited between October 2004 and July 2008. Individuals did not participate in the study if they could not provide a venous blood sample or if they were pregnant or breastfeeding. Smokers (n = 77) were excluded because of the known ascorbic acid–depleting effects of smoking (23, 25). Individuals who may have underreported (<800 kcal/d) or overreported (>3500 kcal/d for women, >4000 kcal/d for men) their energy intakes (n = 79) were excluded. Subjects were also excluded if they had any missing data (n = 29). After the exclusions, 905 subjects (633 women and 272 men) remained. Vitamin C supplement users (n = 333) were identified as anyone who took a vitamin C–containing multivitamin (n = 63), a supplement containing vitamin C exclusively (n = 192), or both (n = 78). Three major ethnocultural groups were present within the sample: white (n = 452), East Asian (n = 311), and others (n = 160), which included those with a mix of ≥2 ethnocultural groups. The date that each subject provided the blood sample was used to classify the subjects by the 4 seasons of spring (March, April, and May), summer (June, July, and August), autumn (September, October, and November), and winter (December, January, and February). The study protocol was approved by the Research Ethics Board at the University of Toronto, and all subjects provided written informed consent.

Dietary assessment

A 196-item Toronto-modified Willett food-frequency questionnaire (FFQ) was used to assess habitual food intake over the past month. Each subject was given instructions on how to complete the FFQ by using visual aids of portion sizes to improve the measurement of self-reported food intake. Subject responses to the individual foods were converted to daily number of servings for each item. A vitamin C content value was assigned to a serving of each item based on the nutrient contents of the food in the US Department of Agriculture database. Vitamin C content values were combined to compute a total daily vitamin C intake for each subject.

Anthropometric measurements and physical activity

Anthropometric measurements, including height, weight, and waist circumference were measured, and BMI (in kg/m^2) was calculated. Modifiable physical activity was measured by questionnaire and expressed as metabolic equivalent (MET)-hours per week, which represents both leisure and occupational activity, but does not include sedentary hours of sleeping or sitting. One MET is equal to 1 kcal expended per kilogram body weight per hour sitting at rest (36).

Serum ascorbic acid and other biochemical measurements

After a minimum 12-h overnight fast, blood samples were collected at LifeLabs medical laboratory services (Toronto, Canada), where all of the biochemical measures were performed. Serum ascorbic acid concentrations were measured as previously described by using HPLC (20) with salicylsalicylic acid as a deproteinizing agent, metaphosphoric acid as a stabilizer, and amber tubes to protect against light.

Genotyping

DNA was isolated from peripheral white blood cells. Subjects were genotyped for *GSTM1*, *GSTT1*, and *GSTP1* by using a multiplex restriction fragment length polymorphism (RFLP) polymerase chain reaction method as previously described (37). Plates (96-well) that contained controls and a 10% random replication of samples were genotyped consecutively. All subjects were successfully genotyped for each of the 3 polymorphisms.

Statistical analysis

All statistical analyses were performed by using Statistical Analysis Systems software (SAS version 9.1; SAS Institute Inc, Cary, NC). Significant P values are 2-sided and <0.05. Subject characteristics were compared between genotypes by using chi-square tests for categorical variables and unpaired t tests for continuous variables. A few of the independent variables were skewed, but transformation of these variables did not alter the results, so they remained untransformed for analyses. Potential gene-diet interactions were evaluated by using a general linear model because the dependent variable (serum ascorbic acid) had a normal distribution. Polytomous logistic regression was used to compute odds ratios (ORs) and 95% CIs. Covariate-adjusted mean serum ascorbic acid concentrations were compared between genotypes in an analysis of variance stratified by dietary vitamin C adequacy. A secondary analysis that combined the *GSTM1* and *GSTT1* genotypes into a *GSTM1/T1* genotype was also conducted.

The adjusted model used in the analyses included sex, BMI, ethnocultural group, energy intake, high-sensitivity C-reactive protein, oral contraceptive use (women only), and season, as determined by stepwise linear regression and an analysis of covariance at the 0.05 significance level. No interactions between these covariates and dietary vitamin C on serum ascorbic acid concentrations were observed. Many other covariates were considered as potential confounders, including serum lipids, blood pressure, physical activity, and intakes of carotenoids, tocopherols, flavonoids, iron, fiber, and alcohol. However, none was statistically significant or materially altered the results; therefore, these variables were not included in the final model.

Analyses were conducted including and excluding supplement users and yielded similar results. Therefore, the results reported include supplement users to maximize the number of subjects.

The results were not materially altered whether the *Val/Val* genotype of the *GSTP1* polymorphism was grouped with the *Ile/Val* genotype (dominant model) or left ungrouped, so the results are presented for the *GSTP1 Ile/Val* and *Val/Val* genotypes combined to maximize the sample size of the groups being compared.

RESULTS

Genotype frequencies and subject characteristics are summarized in **Table 1**. All 3 of the polymorphisms were common, with 52% of the subjects having the nonfunctional (**0/*0*) *GSTM1* genotype, 31% of the subjects having the *GSTT1*0/*0* genotype, and 50% of the subjects carrying the *GSTP1 Val* allele. The *GSTP1* genotype frequencies were in Hardy-Weinberg equilibrium ($P = 0.15$). Deviations from Hardy-Weinberg equilibrium were not tested for distributions of *GSTM1* and *GSTT1* genotypes because the polymerase chain reaction assay does not discriminate heterozygotes from homozygotes for the functional allele.

Genotypes had similar subject characteristics apart from different frequencies between ethnocultural groups, although no

TABLE 1
Subject characteristics by glutathione *S*-transferase (*GST*) *M1*, *T1*, and *P1* genotypes[1]

	GSTM1 Genotype		*GSTT1* Genotype		*GSTP1* Genotype	
Characteristic	**1/*1+*1/*0*	**0/*0*	**1/*1+*1/*0*	**0/*0*	*Ile/Ile*	*Ile/Val + Val/Val*
Subjects [*n* (% of total)]	431 (48)	474 (52)	627 (69)	278 (31)	456 (50)	449 (50)
Sex [*n* (%)]						
Women	305 (48)	328 (52)	430 (68)	203 (32)	316 (50)	317 (50)
Men	126 (46)	146 (54)	197 (72)	75 (28)	140 (51)	132 (49)
Age (y)	22.7 ± 0.1[2]	22.7 ± 0.1	22.8 ± 0.1	22.4 ± 0.1	22.5 ± 0.1	22.9 ± 0.1
Ethnocultural group [*n* (%)]						
White	213 (49)	221 (51)[a]	333 (77)	101 (23)[a]	193 (44)	241 (56)[a]
East Asian	130 (42)	181 (58)[b]	171 (55)	140 (45)[b]	189 (61)	122 (39)[b]
Other	88 (55)	72 (45)[a]	123 (77)	37 (23)[a]	74 (46)	86 (54)[a]
Season [*n* (%)]						
Spring	132 (51)	125 (49)	180 (70)	77 (30)	138 (54)	119 (46)
Summer	101 (44)	131 (56)	162 (70)	70 (30)	114 (49)	118 (51)
Autumn	112 (47)	126 (53)	168 (71)	70 (29)	119 (50)	119 (50)
Winter	86 (48)	92 (52)	117 (66)	61 (34)	85 (48)	93 (52)
Activity (MET-h/wk)	7.6 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.8 ± 0.2	7.6 ± 0.1	7.7 ± 0.1
BMI (kg/m^2)	22.7 ± 0.2	22.6 ± 0.2	22.7 ± 0.1	22.7 ± 0.2	22.4 ± 0.2	23.0 ± 0.2[3]
Waist circumference (cm)	74.0 ± 0.4	73.6 ± 0.4	73.9 ± 0.4	73.6 ± 0.5	73.1 ± 0.4	74.3 ± 0.4
Oral contraceptive use, women only [*n* (%)]						
No	336 (48)	360 (52)	485 (70)	211 (30)	359 (52)	337 (48)
Yes	95 (45)	114 (55)	142 (68)	67 (32)	97 (46)	112 (54)
Systolic blood pressure (mm Hg)	113.9 ± 0.5	114.2 ± 0.5	114.2 ± 0.5	113.6 ± 0.7	114.1 ± 0.6	114.0 ± 0.5
Diastolic blood pressure (mm Hg)	68.7 ± 0.4	69.0 ± 0.4	68.7 ± 0.3	69.3 ± 0.5	68.8 ± 0.4	69.0 ± 0.4
Total cholesterol (mmol/L)	4.18 ± 0.04	4.26 ± 0.03	4.20 ± 0.03	4.28 ± 0.05	4.26 ± 0.04	4.18 ± 0.03
Total:HDL cholesterol	2.78 ± 0.04	2.78 ± 0.03	2.80 ± 0.03	2.72 ± 0.04	2.80 ± 0.04	2.74 ± 0.03
hs-CRP (mg/L)	1.32 ± 0.14	1.36 ± 0.12	1.34 ± 0.12	1.34 ± 0.15	1.26 ± 0.13	1.42 ± 0.14
Serum ascorbic acid [*n* (%)]	31.8 ± 0.8	30.2 ± 0.8	31.4 ± 0.7	30.1 ± 1.1	30.9 ± 0.8	31.1 ± 0.8
Adequate, >28 μmol/L	243 (49)	248 (51)	348 (71)	143 (29)	247 (50)	244 (50)
Suboptimal, 11–28 μmol/L	143 (48)	153 (52)	202 (68)	94 (32)	153 (52)	143 (48)
Deficient, <11 μmol/L	45 (38)	73 (62)	77 (65)	41 (35)	56 (47)	62 (53)
Dietary vitamin C (mg/d)						
All subjects	251.8 ± 13.4	232.9 ± 11.4	249.9 ± 11.0	223.8 ± 14.0	218.2 ± 10.5	266.0 ± 14.0[3]
No supplement users	133.7 ± 5.0	146.2 ± 5.5	142.1 ± 4.4	122.9 ± 7.0	140.0 ± 5.5	140.9 ± 5.0
Dietary vitamin C adequacy [*n* (%)][4]						
<RDA	80 (52)	73 (48)	110 (72)	43 (28)	83 (54)	70 (45)
≥RDA	351 (47)	401 (53)	517 (69)	235 (31)	373 (50)	379 (50)
Supplement use [*n* (%)][5]						
No	265 (46)	307 (54)	396 (69)	176 (31)	295 (52)	277 (48)
Yes	166 (39)	167 (35)	231 (69)	102 (31)	161 (48)	172 (52)
Energy (kcal/d)	1923 ± 31	1983 ± 29	1982 ± 26	1892 ± 38[6]	1917 ± 31	1992 ± 29

[1] hs-CRP, high-sensitivity C-reactive protein; RDA, Recommended Dietary Allowance; MET-h, metabolic equivalent task hours. Differences between genotypes were assessed by using a *t* test for continuous variables and a chi-square test for categorical variables. Values with different superscript letters are significantly different after a Bonferroni correction for multiple comparisons ($P < 0.0167$).

[2] Mean ± SE (all such values).

[3] Significantly different from *Ile/Ile*, $P < 0.05$.

[4] The RDA for vitamin C is 75 mg/d for nonsmoking women and 90 mg/d for nonsmoking men.

[5] Supplement use includes the use of vitamin C supplements and vitamin C–containing multivitamins.

[6] Significantly different from **1/*1 + *1/*0*, $P < 0.05$.

interactions between ethnocultural group and the polymorphisms on serum ascorbic acid were observed. The prevalence of deficient, suboptimal, and adequate serum ascorbic acid concentrations did not differ between genotypes and neither did the mean unadjusted serum ascorbic acid concentrations. However, a significant diet-gene interaction was observed with dietary vitamin C and *GSTM1* ($P = 0.04$) and *GSTT1* ($P = 0.01$) on serum ascorbic acid. These interactions remained when the subjects were grouped into the 2 main ethnocultural groups (*GSTM1*: $P = 0.008$ for whites and $P = 0.01$ for East Asians; *GSTT1*: $P = 0.001$ for whites and $P = 0.02$ for East Asians) (data not shown), which indicated that the interaction effect was not due to population admixture. A diet-gene interaction was also present for the GSTM1/T1 combination of genotypes ($P = 0.03$). No significant diet-gene interaction was observed for *GSTP1* ($P = 0.83$). Although dietary vitamin C varied among the *GSTP1* genotypes, further analyses indicated that this difference did not mask an effect of *GSTP1* genotype on serum ascorbic acid (data not shown).

Overall, the multivariate-adjusted OR (95% CI) for serum ascorbic acid deficiency was 3.20 (1.88, 5.44) for subjects who reported not meeting the RDA for vitamin C compared with those who met the requirement (**Table 2**). This risk of serum ascorbic acid deficiency was ≈12-fold for the *GSTT1*0/*0* genotype, but was only ≈2-fold for carriers of the *GSTT1*1* allele. A similar, although less pronounced, pattern was observed for the *GSTM1* polymorphism, where the corresponding ORs (95% CI) were 4.03 (2.01, 8.09) and 2.29 (0.96, 5.45) for the *GSTM1*0/*0* and *GSTM1*1/*1+ GSTM1*1/*0* genotypes, respectively. The OR (95% CI) for serum ascorbic acid deficiency was 15.35 (3.64, 64.61) for subjects with both nonfunctional (**0/*0*) genotypes ($n = 150$), and 2.19 (0.77, 6.25) for subjects with both functional genotypes ($n = 303$) (data not shown).

Among subjects who met the RDA, serum ascorbic acid concentrations were not different between any of the *GST* genotypes, and all mean values were in the adequate range of serum ascorbic acid concentrations (>28 μmol/L) (**Figure 1**). Among subjects who reported not meeting the RDA for vitamin C, those with the *GSTT1*0/*0* genotype had significantly lower average (mean ± SE) serum ascorbic acid concentrations than those with the *GSTT1*1/*1 + *1/*0* genotype (15.3 ± 2.9 compared with 26.0 ± 2.6 μmol/L; $P = 0.0002$). When the *GSTT1* and *GSTM1* genotypes were combined, this protective effect of a functional GST enzyme was even more pronounced.

DISCUSSION

The purpose of the present study was to determine whether GST genotypes interact with dietary vitamin C to affect serum ascorbic acid concentrations and the risk of deficiency. Therefore, we examined whether the genetic subpopulations differ in their risk of serum ascorbic acid deficiency depending on whether they did or did not meet the RDA for dietary vitamin C. Although meeting the RDA for dietary vitamin C protected against serum

TABLE 2
Odds ratios (ORs) (and 95% CIs) for suboptimal and deficient serum ascorbic acid in relation to dietary vitamin C adequacy status by glutathione *S*-transferase (*GST*) genotype[1]

Serum ascorbic acid adequacy	<RDA[2]	≥RDA[2]	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[3]
	n (%)	*n* (%)		
All subjects				
Adequate	63 (41)	428 (57)	1.00	1.00
Suboptimal	53 (35)	243 (32)	1.48 (1.00, 2.21)	1.63 (1.07, 2.51)
Deficient	37 (24)	81 (11)	3.10 (1.94, 4.97)	3.20 (1.88, 5.44)
GSTM1[4]				
**1/*1 + *1/*0*				
Adequate	35 (44)	208 (59)	1.00	1.00
Suboptimal	31 (39)	112 (32)	1.65 (0.96, 2.81)	2.02 (1.10, 3.17)
Deficient	14 (17)	31 (9)	2.68 (1.30, 5.55)	2.29 (0.96, 5.45)
**0/*0*				
Adequate	28 (38)	220 (55)	1.00	1.00
Suboptimal	22 (30)	131 (33)	1.32 (0.73, 2.40)	1.34 (0.71, 2.53)
Deficient	23 (32)	50 (12)	3.61 (1.92, 6.80)	4.03 (2.01, 8.09)
GSTT1[5]				
**1/*1 + *1/*0*				
Adequate	53 (48)	295 (57)	1.00	1.00
Suboptimal	36 (33)	166 (32)	1.21 (0.76, 1.92)	1.49 (0.89, 2.48)
Deficient	21 (19)	56 (11)	2.09 (1.17, 3.75)	2.17 (1.10, 4.28)
**0/*0*				
Adequate	10 (23)	133 (56)	1.00	1.00
Suboptimal	17 (40)	77 (33)	2.94 (1.28, 6.73)	3.13 (1.26, 7.76)
Deficient	16 (37)	25 (11)	8.51 (3.47, 20.89)	12.28 (4.26, 35.42)

[1] Results were determined by polytomous logistic regression. RDA, Recommended Dietary Allowance.
[2] The RDA for vitamin C is 75 mg/d for nonsmoking women and 90 mg/d for nonsmoking men.
[3] Model adjusted for BMI, sex, energy intake, oral contraceptive use (women only), high-sensitivity C-reactive protein, ethnocultural group, and season.
[4] There was a significant *GSTM1*-diet interaction, $P = 0.04$ (general linear model with an interaction term).
[5] There was a significant *GSTT1*-diet interaction, $P = 0.01$ (general linear model with an interaction term).

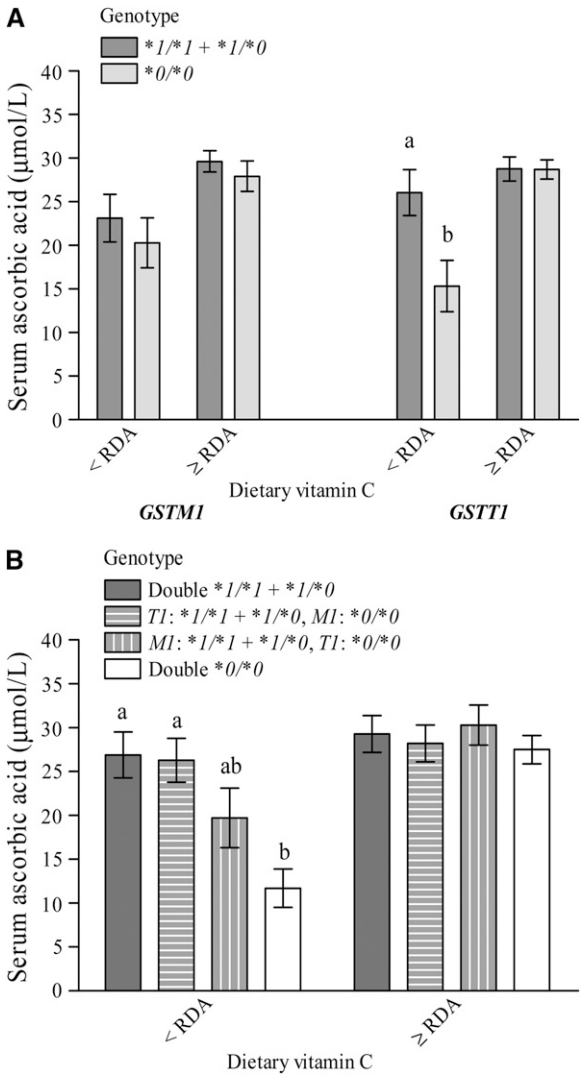

FIGURE 1. Mean (±SE) covariate-adjusted serum ascorbic acid concentrations between glutathione *S*-transferase (*GST*) genotypes by dietary vitamin C adequacy status. The Recommended Dietary Allowance (RDA) of vitamin C is 75 mg/d for nonsmoking women and 90 mg/d for nonsmoking men. The results were obtained by using a general linear model adjusted for BMI, sex, energy intake, oral contraceptive use (women only), high-sensitivity C-reactive protein, ethnocultural group, and season. Values with different superscript letters are significantly different by using Bonferroni correction ($P < 0.0167$) for multiple comparisons. Interactions were assessed by using a general linear model with an interaction term. A: $P = 0.04$ for the *GSTM1*-diet interaction and $P = 0.01$ for the *GSTT1*-diet interaction. Among subjects who reported not meeting the RDA for vitamin C, those with the *GSTT1*0/*0* genotype had lower mean ascorbic acid concentrations than did those with the *GSTT1*1/*1 + *1/*0* genotype ($P = 0.0002$). Number of subjects with dietary vitamin C < RDA: *GSTM1*1/*1 + *1/*0* ($n = 80$), *GSTM1*0/*0* ($n = 73$), *GSTT1*1/*1 + *1/*0* ($n = 110$), and *GSTT1*0/*0* ($n = 43$). Number of subjects with dietary vitamin C ≥ RDA: *GSTM1*1/*1 + *1/*0* ($n = 351$), *GSTM1*0/*0* ($n = 401$), *GSTT1*1/*1 + *1/*0* ($n = 517$), and *GSTT1*0/*0* ($n = 235$). B: $P = 0.03$ for the combined *GSTM1*/*T1* gene-diet interaction. Among subjects who reported not meeting the RDA for vitamin C, mean ascorbic acid concentrations varied between some of the genotypes ($P = 0.0005$). Number of subjects with dietary vitamin C < RDA: double **1/*1 + *1/*0* ($n = 59$); *T1*: **1/*1 + *1/*0 M1*: **0/*0* ($n = 51$); *M1*: **1/*1 + *1/*0 T1*: **0/*0* ($n = 21$); and double**0/*0* ($n = 22$). Number of subjects with dietary vitamin C ≥ RDA: double **1/*1 + *1/*0* ($n = 244$); *T1*: **1/*1 + *1/*0 M1*: **0/*0* ($n = 273$); *M1*: **1/*1 + *1/*0 T1*: **0/*0* ($n = 107$); and double**0/*0* ($n = 128$).

ascorbic acid deficiency for most subjects, it was particularly beneficial for subjects with one or both of the *GSTT1* and *GSTM1* nonfunctional genotypes. These findings are strengthened by the use of data from an ethnoculturally diverse sample of young adults, for whom the diet-gene effects were observed among whites and East Asians separately, even though the frequencies of the polymorphisms differed between these 2 groups. Functional GST, therefore, appears to have a protective effect against serum ascorbic acid deficiency, regardless of ancestral background.

To our knowledge, only one other study has reported serum ascorbic acid concentrations among individuals with different GST genotypes. Consistent with our findings, Dusinská et al (35) observed that mean serum ascorbic acid concentrations were lower among *GSTT1* null genotypes than among those with the *GSTT1* functional genotype. However, Dusinská et al reported that the *GSTM1* null genotypes had higher serum ascorbic acid concentrations than did those with a functional *GSTM1* allele (35), which was not observed in the present study. Our findings, however, indicate that the association between GST genotype and serum ascorbic acid depends on the levels of dietary vitamin C, which was not reported in the previous study (35).

It is not clear how the presence of a functional GST enzyme protects against serum ascorbic acid deficiency. Administration of glutathione has previously been shown to delay the onset of scurvy in ascorbic acid–deficient guinea pigs (38), which, as in humans, cannot synthesize ascorbic acid de novo. The mechanism proposed is that a sparing effect of glutathione in scurvy occurs through an increase in the reduction of dehydroascorbic acid, which would otherwise be degraded, to ascorbic acid and to shared antioxidant roles of glutathione and ascorbic acid (38). Alternatively, in individuals with a nonfunctional GST genotype, ascorbic acid may have to compensate for the antioxidant role of GST, which puts these individuals at greater risk of serum ascorbic acid deficiency if dietary vitamin C is inadequate. The precise roles of molecules within the glutathione–vitamin C antioxidant cycle in protecting cells from oxidative damage are uncertain, but have been shown to overlap (39). The results of the present study support the conclusion that there is some metabolic redundancy of the functions of glutathione and ascorbic acid. The omega class of GST has been shown to directly reduce dehydroascorbic acid to ascorbic acid (28), but it is not known whether the theta and mu classes can do this as well. Findings from the present study suggest that this might occur with *GSTT1* being more effective than *GSTM1*.

Many studies have examined the effect of *GST* genotype on the risk of heart disease (40, 41) and cancer (42, 43), but the findings have been equivocal, in part, because the polymorphisms interact with environmental factors such as smoking (44–47). Differences in dietary vitamin C could also explain some of the inconsistencies among studies examining *GST* genotype and risk of heart disease or cancer. It has been shown that circulating antioxidant vitamins, such as serum ascorbic acid concentrations, are lower in patients with coronary heart disease (11) or cancer (14) than in healthy control subjects, which indicates that an impairment of antioxidant defenses may contribute to the development of these diseases.

An inverse association between serum ascorbic acid and markers of chronic disease has already been shown to exist among the participants of the present study (20), which suggests

that serum ascorbic acid deficiency early in adulthood could have long-term adverse health consequences. For example, subjects with serum ascorbic acid deficiency had significantly higher mean C-reactive protein, waist circumference, BMI, and blood pressure than did subjects with adequate serum ascorbic acid (20). A recent study of young women between 18 and 21 y of age also reported blood pressure to be inversely associated with serum ascorbic acid (48). Thus, identifying and targeting young individuals at risk of serum ascorbic acid deficiency could have important public health implications.

The validity of the results of the present study depends highly on the reliability of the methods used to measure serum ascorbic acid and dietary vitamin C, as we previously described (20). However, because of the design of the present study, any misclassification of dietary vitamin C status associated with the FFQ or degradation of serum ascorbic acid from the blood samples would be nondifferential between genotypes. Therefore, measurement error would not have led to a false-positive diet-gene interaction effect, but could only have attenuated the effect.

Findings from the present study have several implications. They emphasize the importance of obtaining the RDA, regardless of genotype, to decrease the prevalence of serum ascorbic acid deficiency and to potentially decrease the risk of long-term adverse health effects that are associated with low serum ascorbic acid concentrations. Obtaining the RDA for vitamin C is particularly important for individuals with one or both null GST genotypes. The consistency of studies examining dietary vitamin C and health outcomes could be improved by incorporating GST genotypes into the study design, and genetic association studies involving GST should consider the role of dietary vitamin C. These findings provide further evidence that the functions of glutathione and ascorbic acid overlap and indicate a new biological significance of GST-T and GST-M, which appear to protect against serum ascorbic acid deficiency when dietary vitamin C is insufficient.

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