Serum vitamin C and other biomarkers differ by genotype of phase 2 enzyme genes GSTM1 and GSTT1*1–3

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ABSTRACT

Background: Glutathione S-transferases (GSTs) detoxify environmental chemicals and are involved in oxidative stress pathways. Deletion polymorphisms affect enzyme activities and have been associated with risk of disease.

Objective: The objective was to clarify whether biomarkers of oxidation, antioxidation, inflammation, and nutritional factors differ by GST genotype in healthy adults.

Design: Subjects (n = 383) consisted of nonsmokers and nonusers of antinflammatory drugs and antioxidant vitamin supplements. Deletion polymorphisms of GSTM1 and GSTT1 were genotyped. F2-isoprostanes, malondialdehyde, C-reactive protein, serum vitamin C, carotenoids, tocopherols, and other nutritional factors were assessed.

Results: The concentration of serum vitamin C was higher in persons with the inactive GSTM1-0 genotype (P = 0.006). This relation was unchanged after adjustment for age, sex, BMI, or dietary vitamin C. F2-isoprostanes and malondialdehyde were lower in the GSTM1-0 and GSTT1-0 groups, respectively, but significance was lost after control for serum vitamin C. The dual deletion, GSTM1-0/GSTT1-0 (n = 37), was associated with higher serum iron and total and LDL-cholesterol concentrations (all P < 0.01) and lower malondialdehyde concentrations, which persisted after adjustment for age, sex, BMI, and serum vitamin C. Carotenoids and α- and γ-tocopherols were not associated with either genotype.

Conclusions: Oxidative stress and inflammation biomarkers differ by GST genotype, but serum vitamin C appears to be the most consistent factor. Examination of other relevant genes may be needed to understand the concentration and function of ascorbic acid in the GST enzyme system. This trial is registered at clinicaltrials.gov as NCT00079963.


INTRODUCTION

GSTs*4 are a family of phase 2 enzymes found in all eukaryotic species. They play a critical role in detoxifying both naturally occurring and xenobiotic compounds, including carcinogens, environmental toxins, and reactive oxygen species, by catalyzing the transfer and conjugation of glutathione (1, 2). Two major isoforms are GSTT1 and GSTM1. GSTM1, the gene encoding the GSTM1 enzyme, is deleted in ~50% of the human population; GSTT1 is deleted in ~20% of the human population (1, 3). Ethnic differences exist (3, 4). Summarizing several studies, Ginsberg et al (3) reported that GSTM1 deletion was seen in 53% of whites, in 40–60% of Asians, and 21% of African Americans. GSTT1 deletion was seen in 18% of whites, 22% of African Americans, and 45–60% of Asians.

Because of the role of GSTs in detoxifying xenobiotics and products of oxidative stress, the effect of GST deletion has been investigated for numerous conditions. Meta-analyses have indicated that deletion of either GSTM1 or GSTT1 is associated with a significant increased risk of coronary heart disease (5), asthma (6), breast cancer (7–9), colon cancer (10–12), bladder cancer (13, 14), liver cancer (15), lung cancer (16, 17), esophagus cancer (18), several forms of leukemia (19–21), and prostate cancer (22).

However, in most cases the meta-analysis reflected very weak associations and extreme heterogeneity, with some studies providing estimates of no increase or even a decrease in risk with GST deletion. Thus, whereas these analyses implicate loss of GST activity in increasing disease risk, the heterogeneity and the fact that all estimates of increased risk have been modest suggest that unexamined covariates or effect modifiers may be important.

Such covariates might include biomarkers of inflammation, oxidative stress, and nutrient status. Few researchers have examined serum nutrient concentrations by GSTM1 and GSTT1 genotypes. Some have examined the associations of GSTM1 or GSTT1 genotype with markers of inflammation and oxidative stress, but results have been inconsistent (23–28). Such inconsistency may result in part from the populations studied, which frequently included smokers and persons with diseases related to oxidative stress or inflammation. Smoking alters the status of numerous biomarkers, including antioxidants and biomarkers of oxidative stress, and has been shown to alter the association between GST genotype and disease (23–25).

We hypothesized that, in a healthy population without adverse lifestyle and disease characteristics, it might be possible to obtain a more objective picture of the association of GST genotype with biomarkers. In addition, it might be possible to identify potentially

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*4 Abbreviations used: CRP, C-reactive protein; DHA, dehydroascorbic acid; DNPH, 2,4 dinitrophenylhydrazine; GST, glutathione S-transferase; PCR, polymerase chain reaction.

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important covariates or effect modifiers of the relation of GST deletions and disease. To address this hypothesis we examined the GSTM1 and GSTT1 genotypes and biomarkers associated with them in a sample of healthy nonsmokers and nonusers of antiinflammatory agents or antioxidant vitamins.

SUBJECTS AND METHODS

Participants

Healthy nonsmokers aged 18–85 y were recruited in 2005 and 2006 from the communities of San Francisco, Berkeley, and Oakland, CA, for a randomized controlled trial of the effect of vitamin C or E on inflammation and oxidative stress. In addition to smoking, other exclusion criteria included passive smoke exposure, alcohol consumption ≥2 drinks/d, disease conditions (including cancer, stroke, and diabetes mellitus), use of certain prescription medications (antiinflammatory, statin, lipid-lowering, blood-thinning, hormone replacement therapy, or steroid medications), consumption of single iron supplements or of vitamin E supplements in amounts >400 IU/d. Those taking lower-dose vitamin E supplements, multivitamin supplements, vitamin C, or over-the-counter nonsteroidal antiinflammatory drugs were included only after a 30–45-d washout period with no use of those agents. The study design was approved by the institutional review boards of the University of California at Berkeley and Children’s Hospital and Research Center of Oakland, CA. Signed informed consent was obtained from all participants.

These inclusion and exclusion criteria resulted in a sample with unusual health and socioeconomic characteristics in addition to their non–smoke-exposed, nondiseased status: 35% had college degrees and another 35% had advanced degrees beyond college; the median Poverty Index Ratio in our sample was >4, reflecting incomes ≥4 times the poverty level for their household size; 61% were white; and 65.5% were women. All of these characteristics are associated with higher serum antioxidant status (29, 30) and with better health status.

Procedures

Details of the parent randomized trial design, data collection, and procedures were published elsewhere (31, 32) and are repeated here only as relevant to the current analysis. The current analysis focused primarily on the preintervention blood draw. Anthropometric measures were obtained by nurses and technicians according to the National Heart, Lung, and Blood Institute’s published guidelines (33). Information on dietary intake and prior supplement use was obtained by using the Block questionnaire (NutritionQuest; www.nutritionquest.com). The questionnaire was validated in several studies (34–38).

Fasting venous blood was drawn into Vacutainer tubes (Becton-Dickinson) without anticoagulant, protected from light, maintained at <15°C, and processed within 6 h. Blood was centrifuged at 5°C for 10 min at 1200 × g and portioned into serum, buffy coat and clot. Serum aliquots for ascorbic acid were mixed 1:1 with freshly prepared 10% (wt:vol) meta-phosphoric acid to stabilize the ascorbic acid. All aliquots were protected from light and stored at −80°C. All sample batches included masked duplicates and replicated internal control samples.

Laboratory methods

Serum analytes

The laboratory methods for serum analytes were described in detail previously (31, 32). Briefly, serum lipids were measured by using timed-endpoint, coupled enzymatic methodology. High-sensitivity CRP concentrations were measured by latex-enhanced nephelometry with a Hitachi 917 Analyzer (39). Serum F2-isoprostanes were quantitated after purification and derivatization by selected ion-monitoring gas chromatography/negative ion chemical ionization mass spectrometry. Serum malondialdehyde was measured by using lipid peroxidation analysis kits (Oxis International Inc).

Serum vitamin C was measured spectrophotometrically by using DNPH as chromogen (40). The assay was conducted twice, before and after an oxidation step, which produced estimates of ascorbic acid (the reduced form), DHA (the oxidized component), and total serum vitamin C. The DNPH method correlates highly with HPLC analysis (41) and is the method used in the second NHANES (40). DNPH cross-reacts with large doses of erythorbic acid (41), a stereoisomer of ascorbic acid (D-ascorbic acid) used as a food additive, and therefore can overestimate serum vitamin C. Sauberlich et al (42) concluded, however, “To avoid falsely high plasma/serum vitamin C values as a result of erythorbic acid ingestion, the analyses should be conducted on overnight fasting blood specimens or with the use of an HPLC-amperometric method.” Erythorbic acid, like ascorbic acid, is an antioxidant and also enhances nonheme-iron absorption (43), but does not have antiscorbutic properties. To evaluate potential overestimates of serum vitamin C, we compared our concentrations with those measured by HPLC in NHANES 2003–2004 (29).

GSTM1 and GSTT1 genotyping

DNA for genotyping was isolated from buffy coats by using the QiAamp DNA Blood kits from Qiagen according to product specifications. DNA normalized to 10 μg/mL was stored at −20°C until the analysis. Subjects were genotyped for GSTM1 and GSTT1 deletion polymorphisms by using the Qiagen Multiplex Polymerase Chain Reaction kit with some modifications. DNA primer pairs for GSTT1 and GSTM1 amplification are as follows: GSTT1 F: 5'-CTTACTGGTCCTCACATCTC-3', GSTT1 R: 5'-CAGGGCATCCGCTCTGTGCTTT-3', GSTM1 F: 5'-CTTACTGGTCCTCACATCTC-3', GSTM1 R: 5'-CAGGGCATCACGCTCGCTTC-3'. As an internal positive control to verify DNA amplification in double null subjects, a 212-bp section of the albumin gene was co-amplified by using the primers: ALB F: 5'-GACACGACC-GACCACATT-3', ALB R: 5'-AAACGGGAATGGGCAAA-C-3'. The 50-L PCR reaction included 10 pmol of each of the primers, 25 L of 2x Qiagen Multiplex PCR Master Mix, which contained HotStarTaq DNA Polymerase, Qiagen Multiplex PCR Buffer, and a deoxyribonucleotide triphosphate mix. Gene fragments were simultaneously amplified by using 96-Well GeneAmp PCR System 9700 from Applied BioSystems with the following cycling conditions: 15 min at 95°C (30 s at 94°C, 90 s at 57–63°C, and 90 s at 72°C) for 32 cycles and 10 min at 72°C. The null GSTM1 and GSTT1 genotypes were detected by the absence of a band at 267 and 434 bp, respectively, after electrophoresis and visualization on a 3.5% agarose gel stained with ethidium bromide.
For quality assurance, 2 positive and 2 negative controls were used. Laboratory-standard DNA with 4 combinations for the presence and absence of GSTM1 and GSTT1 bands were run with each experiment. Albumin gene was used as another internal positive control for the success of the amplification reaction. Sterilized deionized water was incorporated as negative control. The quality-control procedures also included duplicates and repeats of 5% of randomly selected samples on a separate gel. A minimum of 3 DNA ladders was used to improve precision reading of PCR products.

Statistical analysis

With one exception, described below, only the baseline pre-intervention data were used. The analyses were conducted by using SAS version 9.1 (SAS Institute). Differences in categorical variables such as ethnicity and sex by genotype were assessed by using chi-square tests. Differences in continuous variables by genotype were assessed by using logistic regression, with genotype as the dependent variable and the continuous variable as the independent variable. The following variables were examined for their association with genotype: serum variables (F₂-isoprostanes, malondialdehyde, CRP, superoxide dismutase, glutathione peroxidase, total thiols, ascorbic acid, DHA, total serum vitamin C, CRP, superoxide dismutase, glutathione peroxidase, total thiols, ascorbic acid, DHA, total serum vitamin C, F₂-isoprostanes, malondialdehyde, CRP, superoxide dismutase, glutathione peroxidase, total thiols, ascorbic acid, DHA, total serum vitamin C, CRP, superoxide dismutase, glutathione peroxidase, total thiols, ascorbic acid, DHA, total serum vitamin C). Stratification by age categories, including one category for BMI (Table 1), weight, height, waist circumference, waist-hip ratio, sagittal abdominal diameter, or percentage body fat.

Ascorbic acid

The mean serum vitamin C concentration was higher in those with the GSTM1-0 genotype than in those with the GSTM1-1 genotype (P = 0.007; Table 1). Serum vitamin C was also higher in those (n = 88) with the GSTT1-0 genotype, but this association was not statistically significant in the population as a whole (P = 0.11). Adjustment for age in Table 1 did not affect the association of serum vitamin C with GSTM1, GSTT1, or the combination, nor did adjustment for sex, BMI, or dietary vitamin C. Stratification by age categories, including one category of persons aged ≥60 y, did not provide support for the hypothesis that healthy older survivors were driving the association of serum vitamin C with GSTM1.

In the analyses stratified by race-ethnicity and adjusted for age, sex, BMI, and dietary vitamin C, serum vitamin C was significantly associated with both GSTT1 and GSTM1 in non-Hispanic whites. In Asians and African Americans, the point estimates of association were similar to those in whites, but were not significant.

Because several other variables in Table 1 were significantly associated with genotype, we examined whether serum vitamin C or these other significant variables remained significant when adjusted for one another and for age, sex, and BMI (Table 2). Adjustment for these factors weakened but did not remove the significance of the association of serum vitamin C with GSTM1, and in some cases even increased the OR for serum vitamin C slightly (Table 2). The robustness of this association between vitamin C and GSTM1 was also seen in exploratory stepwise multiple logistic regression in which all other serum variables mentioned in Subjects and Methods were eligible to enter; only serum vitamin C entered the model as being associated with the GSTM1 genotype (P = 0.005).

The correlation (r) between dietary and serum estimates of vitamin C was 0.23 in women and 0.30 in men (P < 0.0001). The multivariate-adjusted OR (95% CI) for having a suboptimal ascorbic acid concentration (<28 µmol/L) was 3.58 (1.35, 9.48) for those whose reported dietary vitamin C intake less than the Estimated Average Requirement, compared with those with a higher intake. However, only 19 subjects had an intake that low.
TABLE 1
Subject characteristics by glutathione S-transferase (GST) genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>GSTM1 genotype</th>
<th>GSTTI genotype</th>
<th>Combined M/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+/+ and +/−</td>
<td>−/−</td>
<td>M and/or T+</td>
</tr>
<tr>
<td>Subjects [n (%)]</td>
<td>383 (100)</td>
<td>202 (52.74)</td>
<td>181 (47.26)</td>
<td>295 (77.02)</td>
</tr>
<tr>
<td>Sex [n (% female)]</td>
<td>251 (65.54)</td>
<td>132 (65.35)</td>
<td>119 (65.75)</td>
<td>193 (65.42)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>44.15 ± 15.09</td>
<td>43.14 ± 14.62</td>
<td>45.29 ± 15.56</td>
<td>44.31 ± 14.87</td>
</tr>
<tr>
<td>Race-ethnicity [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>232</td>
<td>118 (50.86)</td>
<td>114 (49.14)</td>
<td>182 (78.45)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>35</td>
<td>20 (57.14)</td>
<td>15 (42.86)</td>
<td>30 (85.71)</td>
</tr>
<tr>
<td>African American</td>
<td>38</td>
<td>22 (57.89)</td>
<td>16 (42.11)</td>
<td>28 (73.68)</td>
</tr>
<tr>
<td>Asian</td>
<td>56</td>
<td>28 (50.00)</td>
<td>28 (50.00)</td>
<td>38 (67.86)</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>13 (65.00)</td>
<td>7 (35.00)</td>
<td>16 (80.00)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.59 ± 5.46</td>
<td>26.41 ± 5.49</td>
<td>26.78 ± 5.43</td>
<td>26.75 ± 5.50</td>
</tr>
<tr>
<td>Serum ascorbic acid (μmol/L)</td>
<td>58.04 ± 17.25</td>
<td>55.90 ± 17.16</td>
<td>60.42 ± 17.07</td>
<td>57.26 ± 17.34</td>
</tr>
<tr>
<td>Serum vitamin C (μmol/L)</td>
<td>63.18 ± 18.22</td>
<td>60.79 ± 18.16</td>
<td>65.85 ± 17.97</td>
<td>62.37 ± 18.28</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>28.56 ± 6.97</td>
<td>28.10 ± 6.04</td>
<td>29.03 ± 7.89</td>
<td>28.79 ± 7.43</td>
</tr>
<tr>
<td>Serum iron (μg/dL)</td>
<td>0.53 ± 0.36</td>
<td>0.51 ± 0.37</td>
<td>0.54 ± 0.34</td>
<td>0.53 ± 0.36</td>
</tr>
<tr>
<td>Total thiols (μmol/L)</td>
<td>96.35 ± 18.76</td>
<td>96.70 ± 18.65</td>
<td>95.94 ± 18.92</td>
<td>95.63 ± 19.31</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>1012.60 (260.10)</td>
<td>1022.50 (301.40)</td>
<td>1019.40 (204.60)</td>
<td>1013.70 (260.90)</td>
</tr>
<tr>
<td>F2-isoprostanes (μg/mL)</td>
<td>47.00 (30.00)</td>
<td>50.00 (29.00)</td>
<td>44.00 (28.00)</td>
<td>47.00 (31.00)</td>
</tr>
<tr>
<td>Malondialdehyde (μmol/L)</td>
<td>0.85 (0.30)</td>
<td>0.85 (0.31)</td>
<td>0.84 (0.27)</td>
<td>0.86 (0.33)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.85 (1.25)</td>
<td>0.92 (1.30)</td>
<td>0.75 (1.23)</td>
<td>0.91 (1.51)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>193.96 ± 37.43</td>
<td>194.97 ± 37.07</td>
<td>192.80 ± 37.92</td>
<td>192.52 ± 38.50</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>121.88 ± 31.98</td>
<td>122.32 ± 32.07</td>
<td>121.37 ± 31.96</td>
<td>120.53 ± 32.99</td>
</tr>
<tr>
<td>Vitamin C intake, total (mg/dL)</td>
<td>154.40 (230.00)</td>
<td>154.25 (183.90)</td>
<td>154.65 (266.70)</td>
<td>160.10 (237.50)</td>
</tr>
<tr>
<td>Vitamin C intake from food (mg/dL)</td>
<td>96.00 (84.00)</td>
<td>90.70 (80.40)</td>
<td>99.25 (90.65)</td>
<td>94.05 (83.70)</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/mg protein)</td>
<td>38.61 ± 8.90</td>
<td>38.54 ± 9.49</td>
<td>38.68 ± 8.22</td>
<td>38.43 ± 8.97</td>
</tr>
</tbody>
</table>

1 Mean ± SD (all such values).
2 Significant difference between functional and nonfunctional variants, \( P \leq 0.01 \) (t test).
3 Values are median; interquartile ranges in parentheses.
4,5 Significant difference between functional and nonfunctional variants (Wilcoxon’s 2-sample test, 2-sided): \( 4P \leq 0.01 \), \( 5P < 0.05 \).
Oxidation and inflammation biomarkers

$F_2$-isoprostane was significantly higher in the $GSTM1-1$ group than in the $GSTM1-0$ group ($P = 0.01$; Table 1). Adjustment for serum vitamin C, BMI, sex, and age weakened the $F_2$-isoprostane association ($P = 0.16$), whereas ascorbic acid remained close to being significant ($P = 0.058$; Table 2). $F_2$-isoprostane was not associated with $GSTT1$.

Malondialdehyde was higher in the $GSTT1-1$ group than in the $GSTT1-0$ group ($P = 0.01$; Table 1). After adjustment for serum vitamin C, BMI, sex, and age, the significance of the malondialdehyde-$GSTT1$ association was reduced to $P = 0.15$ (Table 2). In subjects carrying the double deletion, malondialdehyde was significantly higher in the functional form, even after adjustment for those factors ($P = 0.038$; Table 2). CRP was higher in the $GSTT1-1$ group than in the $GSTT1-0$ group ($P = 0.04$; Table 1).

### TABLE 2

Effect of adjustment for either vitamin C or other variables on their respective association with genotype: ORs for being the null (inactive) form of the gene

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GSTM1 genotype</th>
<th></th>
<th>GSTT1 genotype</th>
<th></th>
<th>Combined M/T</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>$P$</td>
<td>OR (95% CI)</td>
<td>$P$</td>
<td>OR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>Serum vitamin C (unadj.)</td>
<td>1.33 (1.08, 1.64)</td>
<td>0.007</td>
<td>1.22 (0.96, 1.55)</td>
<td>0.11</td>
<td>1.18 (0.84, 1.66)</td>
<td>0.34</td>
</tr>
<tr>
<td>Model 1$^1$</td>
<td></td>
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</tr>
<tr>
<td>Serum vitamin C</td>
<td>1.25 (0.99, 1.58)</td>
<td>0.058</td>
<td>1.24 (0.95, 1.62)</td>
<td>0.121</td>
<td>1.29 (0.86, 1.92)</td>
<td>0.214</td>
</tr>
<tr>
<td>$F_2$-isoprostanes</td>
<td>0.84 (0.66, 1.07)</td>
<td>0.164</td>
<td>1.17 (0.89, 1.53)</td>
<td>0.260</td>
<td>1.32 (0.90, 1.94)</td>
<td>0.163</td>
</tr>
<tr>
<td>Model 2$^2$</td>
<td></td>
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<tr>
<td>Serum vitamin C</td>
<td>1.34 (1.07, 1.67)</td>
<td>0.009</td>
<td>1.16 (0.90, 1.50)</td>
<td>0.247</td>
<td>1.16 (0.80, 1.68)</td>
<td>0.438</td>
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<tr>
<td>Malondialdehyde</td>
<td>0.90 (0.71, 1.14)</td>
<td>0.380</td>
<td>0.78 (0.57, 1.09)</td>
<td>0.153</td>
<td>0.43 (0.20, 0.95)</td>
<td>0.038</td>
</tr>
<tr>
<td>Model 3$^3$</td>
<td></td>
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<tr>
<td>Serum vitamin C</td>
<td>1.36 (1.09, 1.70)</td>
<td>0.006</td>
<td>1.16 (0.90, 1.50)</td>
<td>0.244</td>
<td>1.13 (0.33, 1.12)</td>
<td>0.517</td>
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<tr>
<td>C-reactive protein</td>
<td>0.92 (0.72, 1.17)</td>
<td>0.486</td>
<td>0.71 (0.50, 1.01)</td>
<td>0.060</td>
<td>0.61 (0.33, 1.12)</td>
<td>0.113</td>
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<tr>
<td>Model 4$^4$</td>
<td></td>
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<tr>
<td>Serum vitamin C</td>
<td>1.30 (1.05, 1.62)</td>
<td>0.018</td>
<td>1.26 (0.97, 1.64)</td>
<td>0.080</td>
<td>1.27 (0.87, 1.86)</td>
<td>0.218</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.87 (0.69, 1.11)</td>
<td>0.275</td>
<td>1.31 (0.98, 1.75)</td>
<td>0.065</td>
<td>1.76 (1.17, 2.65)</td>
<td>0.006</td>
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<tr>
<td>Model 5$^5$</td>
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<tr>
<td>Serum vitamin C</td>
<td>1.30 (1.05, 1.62)</td>
<td>0.018</td>
<td>1.27 (0.98, 1.65)</td>
<td>0.071</td>
<td>1.28 (0.87, 1.88)</td>
<td>0.205</td>
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<tr>
<td>LDL cholesterol</td>
<td>0.92 (0.72, 1.16)</td>
<td>0.476</td>
<td>1.37 (1.03, 1.82)</td>
<td>0.032</td>
<td>1.73 (1.15, 2.58)</td>
<td>0.008</td>
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<tr>
<td>Model 6$^6$</td>
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<tr>
<td>Serum vitamin C</td>
<td>1.31 (1.05, 1.63)</td>
<td>0.015</td>
<td>1.24 (0.96, 1.60)</td>
<td>0.106</td>
<td>1.27 (0.86, 1.85)</td>
<td>0.229</td>
</tr>
<tr>
<td>Serum iron</td>
<td>0.89 (0.70, 1.13)</td>
<td>0.335</td>
<td>1.31 (0.99, 1.75)</td>
<td>0.062</td>
<td>1.82 (1.22, 2.73)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

$^1$Data derived from a multiple logistic regression analysis.

$^2$ORs (95% CIs) for a 1-SD change in the variable. An OR of 1.3 indicates that the odds of being $GSTM1-0$ increases 1.3-fold with every 1-SD increase in the variable. An OR >1.0 indicates a tendency to be higher in the null form. An OR <1.0 indicates a tendency to be higher in the functional form.

$^3$Models 1–6 included age, sex, BMI, and ascorbic acid. In addition, each model contained a different variable that was significant at $P < 0.05$ for any genotype in bivariate analyses in Table 1.

Although the estimate of dietary intake of vitamin C was associated with serum vitamin C, adjustment for dietary intake of vitamin C or prior use of supplements did not alter the pattern of association with $GSTM1$ genotype. DHA as a percentage of total $GSTM1$ genotype continued to have higher serum vitamin C concentrations ($P < 0.005$).
Adjustment for serum vitamin C, BMI, sex, and age weakened the association somewhat ($P = 0.06$; Table 2).

**Other serum measures**

Serum iron was not significantly different between subjects with the GSTM1 or GSTT1 null and those with the functional genotypes in bivariate analysis (Table 1). However, a significant difference was observed when the dual deletion was examined, with serum iron being higher in those with the dual deletion GSTM1-0/GSTT1-0 than in those who carried at least one functional GSTM1/T1 allele ($P = 0.007$; Table 1). Adjustment for serum vitamin C, BMI, sex, and age did not weaken this association, which remained significant ($P = 0.004$; Table 2), nor did further adjustment for menopause status or dietary iron intake. Transferrin saturation followed the same pattern, with higher transferrin saturation in the dual deletion carriers ($P = 0.02$; data not shown).

Total and LDL-cholesterol concentrations were significantly higher in the double-null GSTM1-0/GSTT1-0 genotype group ($P = 0.01$) and remained significant after adjustment for serum vitamin C, BMI, sex, and age (Table 2) and after further adjustment for percentage body fat and dietary saturated fat (data not shown). HDL cholesterol and triglycerides were not associated with the GSTM1 or GSTT1 genotype, either with or without further adjustment (data not shown).

$\alpha$-Tocopherol and $\beta$-carotene were not associated with GSTM1 or GSTT1 genotype (Table 1). $\gamma$-Tocopherol, other carotenoids, and other serum factors mentioned in Subjects and Methods were not associated with genotype (data not shown.).

**Interaction with vitamin C treatment**

As noted in Subjects and Methods, in previous analyses from the randomized trial component of this study we showed a treatment effect of 1000 mg vitamin C/d on change in CRP and serum F$_2$-isoprostanes (31, 32). In the current analysis, genotype did not modify the treatment effect of vitamin C on CRP or F$_2$-isoprostanes. Furthermore, in the vitamin C treatment group serum vitamin C increased by 18.94 $\mu$mol/L, whereas it decreased slightly in the other groups, and this was not modified by genotype.

**DISCUSSION**

The participant sample in the current study has unique advantages for the examination of the relation between genotype and biomarkers of antioxidants, oxidative stress, and inflammation. In particular, smokers and heavy drinkers were excluded, as were persons with diabetes and other diseases and those consuming nonsteroidal antiinflammatory drugs and other medications with antiinflammatory or lipid-lowering action. Persons who had consumed antioxidant supplements underwent a 30–45-d washout period before providing the blood sample examined here. Thus, we can be reasonably certain that the associations found here were not due to those behaviors or conditions.

Serum iron and total and LDL cholesterol were significantly higher in the double-deletion group (Table 2). The finding for iron was consistent with other research (45). One research group found a similar pattern of LDL by GSTM1 and GSTT1 genotype, but did not examine the dual deletion (24), whereas another group found a significant association of triglycerides but not of LDL or total cholesterol with the double deletion (46).

Malondialdehyde was significantly lower in the combined M/T deletion group. Some researchers have found the opposite association, in persons with kidney disease and in women in labor (47–49). Others have not found significant associations of malondialdehyde with GSTM1-0 or GSTT1-0 or with the combination (26, 27, 50, 51). In the present analysis, the significantly lower F$_2$-isoprostane concentration in the GSTM1-0 group disappeared after control for serum vitamin C, and others found no association (52).

CRP was significantly lower in the GSTT1-0 group and in the combined-deletion group, which became nonsignificant ($P = 0.06$ and 0.11, respectively) after adjustment for vitamin C and other factors. Hayek et al (23) also found a significantly lower CRP concentration in subjects with the GSTT1-0 genotype, whereas Tang et al (53) found a significantly higher CRP concentration in subjects with the GSTM1-0 and GSTT1-0 genotypes. Four other studies found no significant association of CRP with the GSTM1 or GSTT1 genotype (24, 25, 44, 54).

This analysis showed a markedly higher serum vitamin C concentration in persons with the GSTM1-0 genotype. Previous research on this topic has been inconsistent. One study by Dusinska et al (26) found, as we did, a significantly higher vitamin C concentration in persons with the GSTM1-0 genotype. However, a later study by the same research group (55) found a significant association in the opposite direction. Others found no significant association with the GSTM1 genotype (44). Further research in a sample of healthy nonsmokers is needed.

Cahill et al (44) found that the GSTM1 and GSTT1 genotypes modified the association between vitamin C intake and serum concentrations, whereas we did not. One explanation for this discrepancy may be the relatively high proportion of suboptimal and deficient serum vitamin C concentrations (<28 $\mu$mol/L) in the study by Cahill et al: 46% compared with 22.2 ± 2.1% in NHANES in the comparable age group (29). Thus, it is possible that the important interaction observed by Cahill et al is operative only in persons with very low serum vitamin C. We had insufficient subjects with low serum vitamin C concentrations or intakes to evaluate this hypothesis, resulting either from an overestimation by the DNPH method, from the sociodemographic characteristics of our sample, or a combination of those factors.

The total vitamin C concentrations seen in this study (63.18 ± 18.22 $\mu$mol/L) may be elevated due in part to the DNPH method, but may also be due to the nonsmoking status, sex, and socioeconomic characteristics of our sample and, for 49% of the sample, to the incompletely washed-out effects of prior supplement use. Our concentrations are reasonably consistent with measurements by HPLC in NHANES in comparable subsets: 58.0 $\mu$mol/L (95% CI: 54.9, 61.2 $\mu$mol/L) in nonsmoking women and 50.7 (95% CI: 47.2, 54.3 $\mu$mol/L) in nonsmoking men (29). Note that those NHANES values include subjects with a range of socioeconomic status and disease state. Finally, notwithstanding the possibility of elevated point estimates, it seems unlikely that overestimates by the DNPH method caused the different concentrations of vitamin C by genotype seen in the tables and in Figure 1, because that would require differential overestimates by genotype.

The markedly higher ascorbic acid concentration in persons with the GSTM1-0 genotype could be due to up-regulation of
factors involved in the transport or recycling of vitamin C. In turn, one could hypothesize that such upregulation resulted from coevolution to compensate for the absence of key metabolic detoxification functions. The ascorbic acid transporter SVCT1, which is located in the intestine and kidney, is involved in both ascorbic acid absorption from the intestine and in resorption in the kidney (56). Upregulation of functional forms of this transporter could increase vitamin C concentrations. Alternatively, variants of the gene coding for SVCT1, SLC23A1, have been shown in a large population study to be associated with markedly different circulating concentrations of ascorbic acid (56). Thus, co-evolution of variants of this gene with GSTM1 could explain the observed difference. It would be of interest to examine the SLC23A1 genotypes in our sample. Upregulation or coevolution of factors involved in recycling of vitamin C could also influence concentrations. In many reactions, ascorbic acid is oxidized to DHA, which must be reconstituted back to ascorbic acid to prevent irreversible loss. DHA is non-enzymatically converted to ascorbic acid by glutathione. Enzymatically, conversion is achieved by at least 2 NADPH-dependent enzymes (3-z-hydroxysteroid dehydrogenase and thioeldoxin reductase) and several glutathione-dependent enzymes (glutaredoxin, protein disulfide-isomerase, and glutathione-dependent DHA reductase) (57). Upregulation of NADPH-dependent recycling of ascorbate was shown by Lykkesfeldt (58) in guinea pigs—a species that, like humans, cannot synthesize ascorbic acid and in which, therefore, recycling of DHA is critical. Finally, another class in the GST superfamily, GSTO2, functions as a dehydroascorbate reductase (59, 60), and it has been suggested that the specific role of GSTO2 is in recycling ascorbate (60). Upregulation, or co-evolution of variants with more active forms, of any of these could influence the ascorbic acid concentration in subjects with the GSTM1-0 genotype. We could hypothesize that such regulation of ascorbic acid concentrations in persons with nonfunctional GSTM1 or GSTT1 could be “designed” to compensate in part for the missing functionality. Ascorbic acid has been known since the 1940s to play an important role in the metabolism and detoxification of numerous xenobiotics and products of oxidative stress (61–64). In addition, there is increasing evidence of its role in gene expression (65), which could influence the expression of other genes that may compensate for some of the GSTM1 functions. Epigenetic modifications that can be induced by oxidative stress or antioxidants may be a mechanism for changes in expression (66, 67). Several researchers have described the upregulation by ascorbic acid of genes involved in DNA repair (63), of phase 2 enzymes (68), and of a battery of genes in the cAMP pathway and in cell differentiation (65). Further research is needed to determine whether any of these hypothesized explanations of the observed difference in ascorbic acid by GSTM1 genotype are correct. Several researchers have noted the weakness and heterogeneity of results from studies of the association of GST polymorphisms with disease or biomarkers and have called for the examination of combinations of genes (69–71). GSTO2 and SLC23A1 may be useful to include in such combinations. In addition, this analysis has uncovered an important potential covariate or effect modifier, serum vitamin C, which has not been examined in most previous biomarker/GST polymorphism, research, and which should be included in future research.

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