Modulation of Human Serum Glutathione S-Transferase A1/2 Concentration by Cruciferous Vegetables in a Controlled Feeding Study Is Influenced by GSTM1 and GSTT1 Genotypes

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Abstract

Glutathione S-transferases (GST) detoxify a wide range of carcinogens. Isothiocyanates (ITC), from cruciferous vegetables, are substrates for and inducers of GST. GST variants may alter ITC clearance such that response to crucifers varies by genotype. In a randomized cross-over trial, we tested the hypothesis that changes in serum GSTA1/2 concentration in response to cruciferous vegetable feeding depends on GSTM1/GSTT1 genotype. Thirty-three men and 34 women (age 20–40 years) ate four 14-day controlled diets—basal (vegetable-free), basal supplemented with two different doses of crucifiers (“single dose” and “double dose”), and single-dose cruciferous-plus-apiaceous vegetables—fed per kilogram of body weight. Fasting bloods from days 0, 7, 11, and 14 of each diet period were analyzed for serum GSTA1/2 by ELISA. GSTA1/2 increased with single- and double-dose cruciferous compared with basal diet (10% and 13%, respectively; P = 0.02 and 0.004), but cruciferous-plus-apiaceous did not differ from basal (P = 0.59). Overall, GSTA1/2 was higher in GSTM1-null/GSTT1-null than GSTM1+/GSTT1+ individuals (4,198 ± 338 and 3,372 ± 183 pg/mL; P = 0.03). The formal interaction of genotype-by-diet was not statistically significant, but the GSTA1/2 increase during the single-dose cruciferous diet was among GSTM1-null/GSTT1-null individuals (by 28%; P = 0.008), largely explained by GSTM1-null/GSTT1-null men (by 41%; P = 0.01). GSTA1/2 increased during the double-dose cruciferous diet in both GSTM1-null/ GSTT1-null men (by 35%; P = 0.04) and GSTM1+/ GSTT1+ men (by 26%; P = 0.01) but not in women. In summary, cruciferous vegetable supplementation increased GSTA1/2, but the effect was most marked in GSTM1-null/GSTT1-null men. (Cancer Epidemiol Biomarkers Prev 2009;18(11):2974–8)

Introduction

Cruciferous vegetables contain high amounts of glucosinolates (1), which, upon hydrolysis, form biologically active compounds such as indoles and isothiocyanates (ITC). These compounds may exert chemoprotective effects through several mechanisms, including induction of detoxification enzymes. Glutathione S-transferases (GST) are enzymes that detoxify a broad range of electrophiles by conjugation with glutathione. ITCs are also substrates for GST, particularly GSTM1 (2). Null genotypes for GSTM1 and GSTT1 result in the absence of their respective enzymes; thus, among GSTM1-null and GSTT1-null individuals, ITC may be metabolized more slowly and thus increase the likelihood of upregulation of other GST isoenzymes (3, 4). GSTA1 is the major hepatic GST (5). Despite overlap in substrate specificity, GSTA1 has a higher affinity than other GSTs for many carcinogens, particularly polycyclic aromatic hydrocarbons, including the activated heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, produced in well-cooked meats and implicated in the etiology of colorectal cancer (6).

We previously reported that, compared with a diet devoid of fruit and vegetables, a cruciferous vegetable diet fed for 7 days statistically significantly increased serum GSTA1/2 concentrations, particularly in GSTM1-null women (7). We also found that GSTA1/2 concentrations measured at day 7 were significantly higher than on day 6, suggesting that the response to diet had not reached a steady state after 1 week. Our objectives in this follow-up study were to test (a) the combined effect of GSTM1/GSTT1 genotypes on serum GSTA1/2 concentrations in response to three defined vegetable diets compared with a vegetable-free diet and (b) whether there was a dose-response effect. Secondary aims were to (a) evaluate the difference in serum GST-α concentrations between 1 and 2 weeks of cruciferous vegetable feeding and (b) determine the additional effect of GSTT1 genotype on serum GSTA1/2 response to diet among GSTM1-null individuals.
Materials and Methods

We used a randomized, controlled, crossover design with four experimental diets as described previously (8). Participants were recruited based on sex and GSTM1/GSTT1 and CYP1A2 genotypes; each participant received the four diets in computer-generated random sequence, blocked on genotype and sex. Each diet was consumed for 14 d with a 3-wk washout period between the diets. Exclusion criteria included factors known to influence biotransformation-enzyme induction, e.g., medications, alcohol, and smoking.

Of the 73 participants randomized, two had GSTM1+ /GSTT1-null (versus GSTM1-null/GSTT1+ ) genotypes because they were recruited for their CYP1A2(C148A) genotype and were not included in this analysis. Three additional participants were not included in the analysis due to an insufficient serum sample or extreme GSTA1/2 values (>20,000 pg/mL). Four participants dropped out after the first feeding period, five after the second, and three after the third. Data for all completed diet periods were included in the analysis, even if a participant did not complete all four diet periods, except for one individual who completed only the basal diet. Sixty-seven participants were included in the final analysis.

Participants consumed four different diets with vegetable doses based on a per kilogram of body weight (BW) calculation to minimize confounding by BW between sexes: a basal, fruit- and vegetable-free diet; basal diet supplemented with ~7 g cruciferous vegetables (a mixture of broccoli, cabbage, cauliflower, and radish sprouts) per kilogram of BW (“single dose”); basal diet supplemented with ~14 g cruciferous vegetables per kilogram of BW (“double dose”); and basal diet supplemented with ~7 g cruciferous vegetables plus ~4 g apiaceous vegetables (a mixture of carrots, celery, dill weed, parsley, and parsnips) per kilogram of BW. Study diet details have been published previously (8).

Biological samples were collected at baseline and during each 2-wk feeding period at days 0, 7, 11, and 14 in the morning after a 12-h overnight fast (8). Buccal cells, collected before randomization, were isolated and DNA was extracted for determination of GSTM1/GSTT1 genotype and participant eligibility.

GSTM1 and GSTT1 genotyping (present versus null) was conducted on buccal cell DNA (8), using primers outlined by Arand et al. (9). GSTA1 was amplified using primer sequences 5′-TGTTGATTTGTTGGCTGAATTACAC-3′ and 5′-GTAAACGCCTGTACACCCGTC-3′ under the following PCR conditions: 1 cycle at 95°C for 5 min, 40 cycles at 94°C for 1 min, 63°C for 1 min, 72°C for 2 min, 1 cycle at 72°C for 5 min. The resulting PCR fragment was digested with the restriction enzyme EarI for 2 h at 37°C. The reaction was then run on a 2% agarose gel and the genotype was determined by fragments of different sizes (10).

Serum GSTA1/2 concentrations were measured using a commercially available, enzyme-linked immunoassay kit (High Sensitivity Alpha GST EIA Hepkit, Biotrin International), which measures a mixture of GSTA1 and GSTA2 subunits (7). Intra- and inter-assay coefficients of variation on quality control serum (mean 3,510 pg/mL) were 2.7% and 16.2%, respectively. Using high performance liquid chromatography (11), we measured urinary total ITC in 24-h urines collected on day 13 to assess diet adherence.

Statistical Analysis. Before analysis, natural logarithmic transformations were performed on GSTA1/2 concentrations to normalize distributions. A linear mixed model was used, including sex, GSTM1/GSTT1 genotypes, feeding periods, diet treatments, feeding order, sampling day, and interaction terms as fixed effects and participants as a random effect. Observations at day 0 and habitual diet were covariates adjusted in the model. Analyses by GSTA1 genotype were carried out using the same model. Pearson correlation was used to evaluate the correlations between GSTA1/2 concentrations and 24-h total ITC. All statistical analyses were done using the Statistical Analysis System Program (version 8.2; SAS Institute). Data are presented as back-transformed least squares (LS) means + SEMs, unless otherwise indicated. Because there were no statistically significant differences between analyses with and without adjustment for vegetable amount, the data are presented without adjustment. The two-sided P value for statistical significance was set at <0.05.

Results

Of the 67 participants, two completed only three diet periods, five completed two, and three completed one diet period. There were no differences in demographic and baseline characteristics across genotypes (Table 1). Eighty-seven percent or more of the prescribed dose of study vegetables was consumed on each vegetable-supplemented diet. Based on daily food check-off forms, participants consumed nonstudy food items <3% of study days. Total vegetable intake ranged from 284 to 662 g for the single-dose cruciferous, 568 to 1,324 g for the double-dose cruciferous, and 458 to 1,065 g for the single-dose cruciferous-plus-apiaceous diet.

Overall (days 7, 11, and 14, and all diets combined), GSTA1/2 concentrations were higher among GSTM1-null/GSTT1-null individuals than GSTM1+ /GSTT1+ individuals (4,198 ± 338 and 3,372 ± 183 pg/mL, respectively; P = 0.03), but did not differ between men and women (P = 0.4; Table 2). GSTM1-null individuals, there was no additional effect of GSTT1-null genotype (3,573 ± 190 pg/mL versus 4,198 ± 338 pg/mL for GSTM1-null/GSTT1-null; P = 0.1).

GSTA1/2 concentrations were higher on the single-dose and double-dose cruciferous diets than on the basal diet (by 10% and 13%, respectively; P = 0.02 and 0.004); however, there was no dose-response effect (P = 0.5). Consumption of the single-dose cruciferous-plus-apiaceous diet did not increase GSTA1/2 concentrations compared with the basal diet.

When evaluating response to diet stratified by genotype and sex, increases in GSTA1/2 concentrations during the single-dose cruciferous diet were exclusively among GSTM1-null/GSTT1-null individuals (by 28%); GSTM1-null/GSTT1-null men (by 41%; P = 0.01). During the double-dose cruciferous diet, GSTA1/2 concentrations increased in both GSTM1-null/GSTT1-null men (by 35%; P = 0.04) and GSTM1+/GSTT1+ men (by 26%; P = 0.01), but not in women (Table 2). Although there was no overall effect...
Table 1. Characteristics of study participants stratified by sex and GSTM1/GSTT1 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1+/GSTT1+</td>
<td>(n = 14)</td>
<td>GSTM1+/GSTT1+</td>
</tr>
<tr>
<td>Age (y)</td>
<td>33.9 ± 6.1</td>
<td>30.4 ± 7.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>177 ± 0.07</td>
<td>178 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.6 ± 12.1</td>
<td>77.6 ± 11.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 3.3</td>
<td>24.4 ± 2.3</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>11 (79%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (14%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>Baseline GST-α (pg/mL)</td>
<td>5,070 ± 374</td>
<td>8,379 ± 1,106</td>
</tr>
</tbody>
</table>

NOTE: There were no significant differences in baseline characteristic means ± SD across genotypes.

Table 2. Serum GST-α concentrations by GSTM1/GSTT1 genotype, sex, and diet: the ratio between response to basal and vegetable diets

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet periods*</th>
<th>Ratios†</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-α, pg/mL</td>
<td>Basal§</td>
<td>Single/basal</td>
</tr>
<tr>
<td>Overall (n = 67)</td>
<td>3,480 ± 355</td>
<td>1.2 ± 0.05§</td>
</tr>
<tr>
<td>GSTM1+/GSTT1+ (n = 26)</td>
<td>3,161 ± 212</td>
<td>1.02 ± 0.04</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1 + (n = 31)</td>
<td>3,500 ± 223</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1-null (n = 14)</td>
<td>3,811 ± 371</td>
<td>1.28 ± 0.12§</td>
</tr>
<tr>
<td>GSTM1+/GSTT1+ Men (n = 14)</td>
<td>2,863 ± 272</td>
<td>1.06 ± 0.09</td>
</tr>
<tr>
<td>GSTM1+/GSTT1+ Women (n = 12)</td>
<td>3,490 ± 356</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1+ Men (n = 16)</td>
<td>3,836 ± 342</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1+ Women (n = 15)</td>
<td>3,192 ± 303</td>
<td>1.08 ± 0.09</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1-null Men (n = 5)</td>
<td>3,923 ± 578</td>
<td>1.41 ± 0.15§</td>
</tr>
<tr>
<td>GSTM1-null/GSTT1-null Women (n = 9)</td>
<td>3,701 ± 468</td>
<td>1.16 ± 0.14</td>
</tr>
</tbody>
</table>

*Basal, fruit/vegetable-free; single, single-dose cruciferous; double, double-dose cruciferous; all vegetable diets adjusted per kilogram of BW.
†The difference of the back-transformed LS means between diets as indicated.
‡The difference of the back-transformed LS means between diets as indicated.
§Significantly different at P < 0.05.
differences in potency and function of ITC between humans; however, several laboratories have shown that GSTM1+/GSTT1+ individuals respond to cruciferous vegetable feeding more than GSTM1−null individuals. In our prior feeding trial, all participants received the same amount of vegetables daily. Consequently, the dose of broccoli may also lead to changes in gut microbial communities and altered ITC exposure (18).

There were differences in response to crucifers between our prior study and the present one. Previously, we found that GST-α response was greatest among GSTM1-null women. Here, testing GSTM1-null/GSTT1-null genotypes combined, increases in GSTA1/2 concentrations were most marked in men. This may reflect a difference in dose. In our prior feeding trial, all participants received the same amount of vegetables daily. Consequently, the vegetable dose per BW was different between men and women (7 g/kg BW for women and ~6 g/kg BW for men, P = 0.001). In the present study, vegetable amounts were dosed by BW to determine whether our previous observation was due to a dose difference or other sex-related physiologic effects. The lower dose in men relative to that in women in the original study may partially explain why women responded to a greater extent previously whereas men had a greater response here. There were also differences in baseline GSTA1/2 concentrations between sexes, between the studies. GSTM1-null women had lower basal serum GST-α concentrations than men of both genotypes in the initial study, and GSTM1-null/GSTT1-null women had the higher basal serum GSTA1/2 concentrations in the present study.

These differences in concentrations during the control diet influence the comparisons of diets between men and women in both trials. In either case, individuals with one or more null alleles responded to a greater extent than individuals with both intact alleles. These results also suggest that the intact GSTT1 allele may be compensating for the lack of active GSTM1 enzyme activity by playing a larger role in ITC metabolism among GSTM1-null individuals; when both alleles are absent, this compensation is no longer possible. Overlap in substrate specificity has been observed between different GST enzymes (6). Thus, it is possible that other GST enzymes compensate for polymorphic isoforms that result in lower activity.
Supplementation of apiaceous vegetables also affected GSTA1/2 concentrations, decreasing GSTA1/2 concentrations when consumed alone compared with the basal diet among GSTM1+/GSTT1+ men in the first study (7) and attenuating the effects of the cruciferous vegetables in the present study. This underscores the challenge in interpreting the relationship between a complex, mixed diet and phenotype in the context of observational studies.

Contrary to our hypothesis, there was no dose response between the single- and double-dose cruciferous diets nor was there a significant difference in response between 1 and 2 weeks of supplementation. Overall, GSTA1/2 concentrations increased significantly by day 7 relative to the basal diet on both the single- and double-dose cruciferous diets; then, by day 11, GSTA1/2 concentrations were lower for the single-dose cruciferous diet but were still increasing for the double-dose cruciferous diet. These data are consistent with evidence of adaptation to crucifers (19). However, it is not clear why GSTA1/2 concentrations started to decrease after day 11. Perhaps there is an adaptation of hepatic enzymes, as well as gut microbial enzymes, in the presence of chronic crucifer consumption.

The strengths of this study include the controlled feeding-study design, recruitment of participants based on GSTM1 and GSTT1 genotypes, the 2-week duration of each study diet, blood collection at multiple time points during each feeding period, and dosing based on BW. Further, the stringent exclusion criteria minimized potential confounding due to other factors that may influence GST enzyme activity.

A limitation of the study is our reliance on serum GSTA1/2 concentrations. Because GSTA1 is mainly found in the liver, the actual change in hepatic enzyme activity in response to vegetable feeding may be greater than what can be measured using circulating GSTA1/2 concentrations. Another potential limitation is generalizability. The average intake of cruciferous vegetables in the United States is ∼25 to 30 g/d (20). Although the cruciferous vegetables used in our study are commonly consumed in the United States, they are not usually consumed in the amounts fed in this study (e.g., 5-10 servings per day or ∼300-1,300 g). Finally, although we had sufficient power to detect overall diet and genotype differences, we were not sufficiently powered to evaluate sex-by-genotype-by-diet interactions. We based the sample size estimate for the current study on results from our previous GST study, which included a similar study population (7), and determined that we would have 80% to 96% power with a sample size of 64. A post hoc calculation based on our present results indicates that our power was lower, ranging from 60% to 81% for overall effects. Therefore, it is possible that significant results may also be explained by chance.

In summary, cruciferous vegetable supplementation increased serum GSTA1/2 concentrations, but the effect was most marked in GSTM1-null/GSTT1-null men. In addition, the combination of apiaceous vegetables and cruciferous vegetables attenuated the effects of cruciferous vegetables alone.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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We thank Karen Noar, Kara Breymeyer, and their staff in the Human Nutrition Laboratory for their dedicated work, and JoAnn Prunty, Sherianne Fish, and Maureen Downey for their technical support.

References
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