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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[1]

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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 yr), ate four 14-day controlled diets: basal (vegetable-free), basal supplemented with 2 different doses of crucifers, (single-"dose" and double-"dose") and single-dose cruciferous-plus-apiaceous vegetables, fed per kg 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 to 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 (4198 ± 338 and 3372 ± 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.

Keywords

Isothiocyanates; cruciferous vegetables; glutathione *S*-transferase (GST)

Introduction

Cruciferous vegetables contain high amounts of glucosinolates (1) which, upon hydrolysis, form biologically active compounds such as indoles and isothiocyanates (ITC). These

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*Both authors contributed equally to this work compounds may exert chemo-protective 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 up-regulation 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-phenylimidazaol[4,5-*b*]pyridine (PhIP), produced in well-cooked meats and implicated in the etiology of colorectal cancer (6).

Previously we reported that, compared to 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 to 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 one and two 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.

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* genotype and each participant received the four diets in computer-generated random sequence, blocked on genotype and sex. Each diet was consumed for 14 days with a 3-week 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*(*C*^{734} *A*) 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-kg-body weight (BW) calculation to minimize confounding by BW between sexes: a basal, fruit- and vegetable-free diet; the basal diet supplemented with ~7 g cruciferous vegetables (a mixture of broccoli, cabbage, cauliflower and radish sprouts) per kg BW ("single-dose"); the basal diet supplemented with ~14 g cruciferous vegetables per kg BW ("double-dose"); and the basal diet supplemented with ~7 g cruciferous vegetables plus ~4 g apiaceous vegetables (a mixture of carrots, celery, dill weed, parsley and parsnips) per kg BW. Study diet details have been published previously (8).

Biologic samples were collected at baseline and during each two-week feeding period at day 0, 7, 11, and 14 in the morning after a 12-hour overnight fast (8). Buccal cells, collected prior to randomization, were isolated and DNA 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′ TGTTGATTGTTTGCCTGAAATTCAC 3′ and 5′ GTTAAACGCTGTCACCGTCCTG3′ 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 restriction enzyme *EarI* for 2 hours at 37°C. The reaction was then run on a 2% agarose gel and genotype determined by fragments of different size (10).

Serum GSTA1/2 concentrations were measured using a commercially available, enzyme-linked immunoassay kit (High Sensitivity Alpha GST EIA Hepkit, Biotrin International, Dublin, Ireland), which measures a mixture of GSTA1 and GSTA2 subunits (7). Intra- and inter-assay CVs on quality-control serum (mean 3510 pg/ml) were 2.7 and 16.2%, respectively. Using HPLC (11), we measured urinary total ITC in 24-hour urines collected on day 13 to assess diet adherence.

Statistical analysis

Prior to 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 performed using the Statistical Analysis System Program (version 8.2; SAS Institute). Data are presented as back-transformed least squares (LS) – means ± standard errors (SE), 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. 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 non-study food items <3% of study days. Total vegetable intake ranged from 284 – 662 g for the single-dose cruciferous, 568 – 1324 g for the double-dose cruciferous, and 458 – 1065 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 (4198 ± 338 pg/ml and 3372 ± 183 pg/ml, respectively; $P = 0.03$), but did not differ between men and women ($P = 0.4$; Table 2). Among *GSTM1*-null individuals, there was no additional effect of *GSTT1*-null genotype (3573 ± 190 pg/ml *versus* 4198 ± 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 to 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%; $P = 0.008$), largely explained by *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 of cruciferous-plus-apiaceous vegetables compared to the basal diet, increases in GSTA1/2 concentrations were observed in *GSTM1+/GSTT1+* men (by 20%; $P = 0.03$; Table 2), but was related to lower GSTA1/2 concentrations during the basal diet. Compared to the single- and double-dose cruciferous diets, the cruciferous-plus-apiaceous diet decreased GSTA1/2 concentration in *GSTM1*-null/*GSTT1*-null men (by 35% and 33%, respectively; $P = 0.003$ and 0.009).

Overall, a statistically significant effect of the single-dose cruciferous diet on GSTA1/2 concentrations (compared to basal diet) was observed at day 7 (by 13%; $P = 0.04$) but not at day 11 (by 11%; $P = 0.07$) or day 14 (by 5%; $P = 0.4$ Table 3). The double-dose cruciferous diet increased GSTA1/2 concentrations at day 7 and day 11 (by 14% and 15%, respectively; $P = 0.04$ and 0.03) but only marginally by day 14 (by 11%; $P = 0.08$).

When examining the diet effects measured at different sampling days by genotype, the greatest effect of single-dose cruciferous vegetables was observed among *GSTM1*-null/*GSTT1*-null individuals at day 7 (by 50%; $P = 0.002$), and day 11 (by 31%; $P = 0.04$), but not day 14 (by 6%; $P = 0.6$). Compared to the basal diet, the double-dose cruciferous diet did not differ by genotype at any sampling day, except for an increase in GSTA1/2 concentrations among *GSTM1+/GSTT1+* individuals at day 11 (by 23%; P=0.02), a result of lower GSTA1/2 concentrations during the basal diet for this group.

The −69C>T polymorphism in the promoter region of the *GSTA1* gene has been associated with 3- to 4-fold lower GSTA1/2 enzyme expression (10). We therefore evaluated whether serum GSTA1/2 concentrations differed by *GSTA1* genotype. The overall interaction term for genotype-by-diet was not statistically significant, nor were there any statistically significant differences in GSTA1/2 concentrations by *GSTA1* within diet (data not shown).

Mean ± SD total ITC concentrations for the basal, single and double-dose cruciferous, and cruciferous-plus-apiaceous diets were 7.0 ± 36.2, 130.7 ± 57.1, 270.0 ± 185.3, 107.9 ± 49.8 μmol/24 hours, respectively, indicating a dose-dependent increase in ITC excretion over the basal-diet period. Correlations between GSTA1/2 concentrations and 24-h urinary ITC excretion were not statistically significant (P=0.46).

Discussion

In response to cruciferous vegetable feeding, GSTA1/2 concentrations were increased among individuals with combined *GSTM1*-null/*GSTT1*-null genotypes compared to their wildtype counterparts. Few human intervention trials have evaluated the ability of *GST* genotype to modulate response to cruciferous vegetable intake on biomarkers. In one controlled feeding trial, *GSTM1* genotype-related changes were reported in transforming growth factor-β1 and epidermal growth factor signaling pathways in prostate tissue after 11 men consumed 400 g broccoli/week for six months (12); *GSTM1+* individuals showed greater diet-induced changes in prostate tissue gene expression. In our prior feeding study, the GSTA1/2 response to cruciferous vegetable feeding was greatest among *GSTM1*-null women (7).

Lack of consistent *GSTM1* modulation of crucifer effects across intervention studies is probably due to multiple factors, including tissue-specific responses, differences in endpoints measured, and the type and amount of crucifers fed. In our studies, we used a mixture of crucifers, previously ~ 400 g/day for one week (7) and currently ~300 – 1300 g/day for two weeks, whereas Traka et al. (12) used only broccoli (400 g/week). Glucosinolate composition, both amount and type, varies substantially among different cruciferous vegetables (13,14). It is unknown whether these differences in glucosinolate profiles, and therefore ITC, lead to different biologic effects in humans; however, several laboratories have demonstrated differences in potency and function of ITC *in vitro* (15-17). Longer-term, chronic consumption of cooked broccoli may also lead to changes in gut microbial enzymes 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 (approximately 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 physiological 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 while 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 to 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 not a dose-response between the single- and double-dose cruciferous diets, nor was there a significant difference in response between one and two 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, 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 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 two-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 U.S. is about 25 to 30 g/d (20). Although the cruciferous vegetables used in our study are commonly consumed in the U.S. diet, they are not usually consumed in the amounts fed in this study (e.g., 5 – 10 servings/day or ~300 – 1300 g). Finally, although we had sufficient power to detect overall diet and genotype differences, we were not sufficiently powered to evaluate sexby-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 80-96% power with a sample size of 64. A post-hoc calculation based on our present results indicates that our power was lower, ranging from 60-81% 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.

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Table 1

Characteristics of study participants stratified by sex and *GSTM1/GSTT1* genotypes*

	Men			Women		
	GSTM1+/GSTT1+ **N=14**	***GSTM1−/GSTT1+*** **N=14**	***GSTM1−/GSTT1−*** **N=5**	***GSTM1+/GSTT1+*** **N=12**	***GSTM1−/GSTT1+*** **N=13**	***GSTM1−/GSTT1−*** **N=9**
Age (yr)	33.9 ± 6.1	30.4 ± 7.0	29.7 ± 6.0	28.2 ± 5.8	30.3 ± 5.3	28.7 ± 3.5
Height (m)	177 ± .07	178 ± .07	174 ± .08	162 ± .07	165 ± .07	162 ± .08
Weight (kg)	83.6 ± 12.1	77.6 ± 11.6	76.3 ± 11.4	59.7 ± 9.1	61.2 ± 9.2	61.9 ± 12.8
BMI (kg/m^2)	26.7 ± 3.3	24.4 ± 2.3	25.3 ± 3.4	22.8 ± 2.6	22.5 ± 2.5	23.3 ± 4.0
Race:						
Caucasian	11 (79%)	9 (64%)	3 (60%)	8 (67%)	9 (69%)	3 (33%)
Asian	2 (14%)	5 (36%)	2 (40%)	4 (33%)	2 (15%)	4 (44%)
Other	1 (7%)	0	0	0	2 (15%)	2 (22%)
Baseline GST-α (pg/ml)	5070 ± 374	8379 ± 1106	5270 ± 587	4923 ± 415	2692 ± 150	4239 ± 416

*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

	Diet Periods[a]						
	GST-α, pg/ml	Ratios[b]					
Genotype	**Basal[c]**	**Single/ Basal**	**Double/ Basal**	**Double/ Single**	**Single + Apiaceous/ Basal**	**Single + Apiaceous/ Single**	**Single + Apiaceous/ Double**
Overall (n=67)	3480 ± 155	1.10 ± 0.05[d]	1.13 ± 0.05[d]	1.03 ± 0.04	1.02 ± 0.04	0.93 ± 0.04	0.90 ± 0.04[d]
GSTM1+/*GSTT1*+ (n=26)	3161 ± 212	1.02 ± 0.06	1.16 ± 0.07[d]	1.14 ± 0.07[d]	1.10 ± 0.07	1.08 ± 0.07	0.95 ± 0.06
GSTM1-null/*GSTT1*+ (n=31)	3500 ± 223	1.02 ± 0.06	1.07 ± 0.06	1.05 ± 0.06	1.00 ± 0.06	0.98 ± 0.06	0.93 ± 0.05
GSTM1-null/*GSTT1*-null (n=14)	3811 ± 371	1.28 ± 0.12[c]	1.18 ± 0.12	0.92 ± 0.09	0.98 ± 0.10	0.77 ±0.08[d]	0.83 ± 0.09
GSTM1+/*GSTT1*+							
Men (n=14)	2863 ± 272	1.06 ± 0.09	1.26 ± 0.11[d]	1.19 ± 0.10[d]	1.20 ± 0.11[d]	1.13 ± 0.10	0.96 ± 0.08
Women (n=12)	3490 ± 336	0.98 ± 0.08	1.06 ± 0.09	1.09 ± 0.10	1.00 ± 0.09	1.02 ± 0.09	0.94 ± 0.08
GSTM1-null/*GSTT1*+							
Men (n=16)	3836 ± 342	0.96 ± 0.08	1.02 ± 0.08	1.06 ± 0.08	1.01 ± 0.08	1.05 ± 0.08	0.99 ± 0.08
Women (n=15)	3192 ± 303	1.08 ± 0.09	1.13 ± 0.09	1.05 ± 0.09	0.98 ± 0.08	0.91 ± 0.08	0.87 ± 0.07
GSTM1-null/*GSTT1*-null							
Men (n=5)	3923 ± 578	1.41 ± 0.19[d]	1.35 ± 0.19[d]	0.96 ± 0.14	0.91 ± 0.13	0.65 ± 0.09[d]	0.67 ± 0.10[d]
Women (n=9)	3701 ± 468	1.16 ± 0.14	1.02 ± 0.14	0.88 ± 0.11	1.05 ± 0.14	0.91 ± 0.12	1.03 ± 0.14

[a] Basal = fruit/vegetable-free; Single = single-dose cruciferous; double = double-dose cruciferous; all vegetable diets adjusted per kg BW.

[b] The difference of the back-transformed LS-means between diets as indicated.

[c] LS-means ± SE, adjusted for baseline and feeding period day 0 serum GST-α concentrations.

[d] Significantly different at $P<0.05$.

Table 3

Serum GST-α concentrations by *GSTM1/GSTT1* genotype, sampling day, and diet: the ratio between response to basal and vegetable diets

	Diet Periods[a]						
	GST-α, pg/ml	Ratios[b]					
Genotype	Basal[c]	Single/ Basal	Double/ Basal	Double/ Single	Single + Apiaceous/ Basal	Single + Apiaceous/ Single	Single + Apiaceous/ Double
Overall							
Day 7	3433 ± 185	1.13 ± 0.07[d]	1.14 ± 0.07[d]	1.00 ± 0.06	1.00 ± 0.06	0.88 ± 0.05[d]	0.88 ± 0.05[d]
Day 11	3520 ± 190	1.11 ± 0.07	1.15 ± 0.07[d]	1.03 ± 0.06	1.07 ± 0.07	0.96 ± 0.06	0.93 ± 0.06
Day 14	3488 ± 188	1.05 ± 0.06	1.11 ± 0.07	1.06 ± 0.06	1.01 ± 0.06	0.96 ± 0.06	0.90 ± 0.06
GSTM1+/*GSTT1*+							
Day 7	3263 ± 265	1.00 ± 0.09	1.12 ± 0.10	1.11 ± 0.10	1.10 ± 0.10	1.09 ± 0.10	0.98 ± 0.09
Day 11	3081 ± 250	1.03 ± 0.09	1.23 ± 0.11[d]	1.20 ± 0.11[c]	1.11 ± 0.10	1.08 ± 0.09	0.90 ± 0.08
Day 14	3143 ± 255	1.03 ± 0.09	1.13 ± 0.10	1.09 ± 0.10	1.09 ± 0.10	1.06 ± 0.09	0.97 ± 0.09
GSTM1-null/*GSTT1*+							
Day 7	3637 ± 278	0.96 ± 0.08	1.08 ± 0.09	1.12 ± 0.10	0.93 ± 0.08	0.97 ± 0.08	0.86 ± 0.07
Day 11	3550 ± 272	1.03 ± 0.09	0.99 ± 0.08	0.96 ± 0.08	1.01 ± 0.08	0.99 ± 0.08	1.03 ± 0.09
Day 14	3319 ± 257	1.07 ± 0.09	1.15 ± 0.10	1.08 ± 0.09	1.05 ± 0.09	0.98 ± 0.08	0.91 ± 0.08
GSTM1-null/*GSTT1*-null							
Day 7	3409 ± 402	1.50 ± 0.19[d]	1.21 ± 0.17	0.81 ± 0.11	0.97 ± 0.14	0.65 ± 0.09[d]	0.80 ± 0.12
Day 11	3989 ± 470	1.31 ± 0.17[d]	1.25 ± 0.17	0.96 ± 0.13	1.09 ± 0.15	0.84 ± 0.12	0.88 ± 0.13
Day 14	4068 ± 480	1.06 ± 0.14	1.07 ± 0.15	1.01 ± 0.14	0.89 ± 0.13	0.84 ± 0.12	0.83 ± 0.12

[a]Basal = fruit/vegetable-free; Single = single-dose cruciferous; double = double-dose cruciferous; all vegetable diets adjusted per kg BW.

[b]The difference of the back-transformed LS-means between diets as indicated.

[c]LS-means ± SE, adjusted for baseline and feeding period day 0 serum GST-α concentrations.

[d]Significantly different at $P<0.05$.