Modulation of Human Glutathione *S*-Transferases by Botanically Defined Vegetable Diets[1]

Johanna W. Lampe,[2] Chu Chen, Sue Li, JoAnn Prunty, Margaret T. Grate, Diane E. Meehan, Karen V. Barale, Douglas A. Dightman, Ziding Feng, and John D. Potter

Cancer Prevention Research Program [J. W. L., S. L., J. P., M. T. G., K. V. B., Z. F., J. D. P.] and Program in Epidemiology [C. C., D. A. D.], Fred Hutchinson Cancer Research Center , Seattle, Washington 98109, and Bastyr University, Kenmore, Washington 98011 [D. E. M.]

Abstract

Glutathione *S*-transferases (GSTs) conjugate activated xenobiotics with glutathione; thus, GST induction may improve detoxification and excretion of potentially harmful compounds. Using a randomized cross-over design, we tested the hypothesis that, in humans, serum GST-α concentration (GST-α) and GST activity increase with vegetable consumption and that this effect is *GSTM1* genotype dependent. Twenty-one men (10 *GSTM1*-null and 11 *GSTM1*+) and 22 women (15 *GSTM1*-null and 7 *GSTM1*+), nonsmokers, 20–40 years of age and not on medications, ate four 6-day controlled diets: basal (vegetable-free), and basal supplemented with three botanically defined groups of vegetables (*i.e.,* brassica, allium, and apiaceous). Fasting blood samples, collected on the last 2 days of each feeding period, were analyzed for GST-α, serum GST activity [against 1-chloro-2,4-dinitrobenzene (CDNB) and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)] and peripheral-lymphocyte GST-μ activity (against *trans*-stilbene oxide). The brassica, but not allium or apiaceous, vegetable diets (relative to the basal diet) increased GST-α by 26% ($P = 0.005$) and GST (NBD-Cl) activity by 7% ($P = 0.02$) in the *GSTM1*-null individuals, particularly the women. Apiaceous vegetable supplementation decreased GST-α in the *GSTM1*+ men ($P = 0.03$). Among the *GSTM1*+ women, both brassica and the allium diets increased GST-μ activity by 18% ($P = 0.02$) and 26% ($P = 0.001$), respectively. The vegetable diets had no effect on GST (CDNB) activity, irrespective of *GSTM1* genotype or sex. These results demonstrate that *GSTM1* genotype has a significant effect on GST responses to diet and that brassica vegetables are most effective at inducing GST-α, whereas both brassica and allium vegetables induce GST-μ. GST responses were more pronounced in women than men, but it is not clear from this study whether this is a dose-per-body-weight or a sex-specific effect.

Introduction

The GSTs[3] constitute a complex multigene family that, in most instances, deactivates carcinogens, environmental pollutants, drugs, and a broad spectrum of other xenobiotics through conjugation with glutathione (1). The major classes of GSTs in the liver are GST-α and GST-μ (2). A homozygous deletion of the *GSTM1* gene results in no GSTM1 activity in ~50% of Caucasians; however, because of their broad substrate specificity, other GST isozymes (*e.g.,* GST-α) may be able to compensate for the lack of GSTM1 activity. GSTM1 also metabolizes constituents of plant foods, *e.g.,* isothiocyanates (3). *GSTM1*-null individuals may experience greater exposure to dietary chemoprotective agents that are typically deactivated by GSTM1, such that the interaction of *GSTM1* genotype and dietary patterns may be more important than either factor alone (4).

GST-α protein and GST activity can be measured in serum; in healthy individuals, it most probably reflects enzyme release during normal hepatic cell turnover (5, 6). GST-μ protein and activity can be measured in peripheral lymphocytes (7, 8). Thus, these measures provide noninvasive approaches to monitoring the effect of interventions on GST protein levels and activities in healthy individuals (5, 9).

The primary aims of our study were: (*a*) to test the effect of consumption of three botanically defined vegetable diets, compared with a vegetable-free diet, on serum GST-α concentration and GST activity in serum and peripheral lymphocytes; and (*b*) to test whether the effect depends on *GSTM1* genotype or sex. The secondary aims were to examine the day-to-day variability in these measures and the relationships between them under controlled dietary conditions.

Materials and Methods

Participants. Healthy, nonsmoking men and women, ages 20–40, were recruited from the greater Seattle area to take part in a controlled feeding study, reviewed and approved by The Institutional Review Board at Fred Hutchinson Cancer Research Center. Participants were screened using a written survey and were ineligible if they reported any of the following: a medical history of gastrointestinal disorders; food allergies or intolerances; a weight loss or gain of >4.5 kg in the past year; major diet changes within the past year; exercise regimens requiring significant short-term dietary changes; oral antibiotic use within the past 3 months; body weight >150% of ideal;

Received 11/2/99; revised 5/10/00; accepted 5/24/00.
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[1] Supported by National Cancer Institute Grant R01 CA70913 (to J. D. P.) and Fred Hutchinson Cancer Research Center.
[2] To whom requests for reprints should be addressed, at Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., MP-900, Seattle, WA 98109-1024. Phone: (206) 667-6580; Fax: (206) 667-7850; E-mail: jlampe@fhcrc.org.

[3] The abbreviations used are: GST, glutathione *S*-transferase; FSA, Food Services of America; CV, coefficient of variation; GSH, reduced glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; NBD-Cl, 7-chloro-4-nitrobenz-2-oxa 1,3 diaxole; LS, least squares; TSO, *trans*-stilbene oxide.

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current drug therapy for a diagnosed disease; regular nonsteroidal anti-inflammatory drug use; alcohol intake >2 drinks/day (750 ml of beer, 240 ml of wine, or 90 ml of hard liquor); job-related exposure to smoke and/or organic solvents; regular overexposure to secondhand smoke; current or planned pregnancy; or no interest in participating in a controlled feeding study. Participants were requested to maintain their usual physical activity and not to take any type of medication (prescription or over-the-counter) or nutritional supplements during each feeding period. Participants who met the eligibility criteria attended an informational session detailing the study protocol, provided informed consent, and completed a baseline visit to provide a blood sample for DNA analysis, specifically to genotype for *GSTM1*.

Our intention was to recruit 40 participants, including an equal number of *GSTM1*+ and *GSTM1*-null individuals and an equal number of men and women. In all, we randomized 57 individuals (12 *GSTM1*+ men, 11 *GSTM1*+ women, 16 *GSTM1*-null men, and 18 *GSTM1*-null women). Of these, 6 failed to appear for their first feeding period, 3 dropped out after 1–2 days on the diet, and 5 dropped out after one feeding period. Their reasons for quitting included: difficulty with protocol adherence (time commitment, coming to the center, the study food, and others), illness, wanting to get pregnant (women were advised not to plan a pregnancy during the study), and travel. As these individuals dropped out, new recruits were selected and randomized into the appropriate treatment orders to maintain the blocks. Approximately twice as many *GSTM1*-null, relative to *GSTM1*+, women volunteered; the overall prevalence of *GSTM1*-null genotypes in our recruitment population was 56%.

Study Design. We used a four-period cross-over design with different experimental diets in each period. Eligible participants were grouped according to sex and *GSTM1* genotype. Within each group, each participant was assigned to receive the four diets in an order drawn randomly from a four-sequence Latin Square. This approach minimizes the impact of possible carryover effects (10). Each diet was consumed for 6 days, and we used a 1-day diet rotation (*i.e.,* the same food daily) to minimize day-to-day variability. The feeding periods began with the dinner meal on a Saturday evening and ended with blood sample collection on the following Friday (day 6) and Saturday (day 7) mornings. There was at least a 2-week washout period between each diet period, a sufficient length of time according to data published previously (11).

Study Diets. During the four feeding periods, participants consumed four different diets: a basal diet devoid of fruits and vegetables (12); and the basal diet supplemented with brassica, allium, or apiaceous vegetables. The brassica supplement comprised: 16 g (1/2 cup United States household measure) of fresh radish sprouts, 150 g (1 cup) of frozen cauliflower (FSA Signature), 200 g (2 cup) of frozen broccoli (FSA Signature), and 70 g (1 cup) of fresh shredded cabbage. The allium supplement comprised: 10 g (3 tablespoons) of fresh chopped chives, 100 g (1 1/3 cup) of fresh chopped leeks, 5 g (1 teaspoon) of fresh minced garlic, and 75 g (1/2 cup) of fresh chopped onion. The apiaceous supplement comprised: 0.50 g (1 teaspoon) of fresh dill weed, 50 g (1/2cup) of fresh celery, 5 g (3 tablespoons) of fresh chopped parsley, 100 g (1 1/4 cup) of fresh grated parsnips, and 110 g (3/4 cup) of frozen carrot coins (FSA Signature). All prepackaged foods were purchased in case lots, and fresh foods were purchased from the same vendor. The Food Services Department at Fred Hutchinson Cancer Research Center prepared the study diets. Nutrient content of the diets was determined using the Nutrition Data System software program (Food Database version 12A, release date November 1996; Nutrient Database version 27, release date November 1996), developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. Components of the basal diet were adjusted to accommodate the added vegetables, such that all diets provided a similar percentage of energy from carbohydrate, protein, and fat: 60, 12, and 29%, respectively. All four study diets were designed to provide 2000 kcal. Participants whose daily energy needs exceeded 2000 kcal were provided with "unit" foods, a combination of foods that were already part of the basal diet and had the same macronutrient composition as the basal diet (12).

Participants were instructed to consume only the foods and beverages provided to them during the diet periods. The dinner meal was served at the study center each evening, and food for the following days' breakfast, lunch, and snacks was distributed at that time. The major portion of the vegetables was provided as part of the dinner meal, and study staff monitored participants' intakes at that meal. Participants were encouraged to report any deviations from the study diets. Overall compliance with the study diet was assessed using a daily checklist. Each checklist listed all foods on the study diets as well as space to record any additional (nonstudy) foods consumed.

***GSTM1* and *GSTT1* Genotyping: Identification of Homozygous Null (*GSTM1*0/0*).** *GSTM1* genotyping on all potential study participants was conducted prior to randomization into the feeding trial. DNA from participants was extracted from peripheral lymphocytes for determination of *GSTM1* and *GSTT1* genotype by PCR. The primers used for identifying *GSTM1* genotypes were 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ and 5′-GTT GGG CTC AAA TAT ACG GTG G-3′ (13). Those for the identification of *GSTT1* were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′ (14). β-globin was coamplified to ensure that the *GSTM1*-null and *GSTT1*-null were attributable to the deletion of the *GSTM1* or *GSTT1* alleles and not to failure of the PCR. PCR results, generated from the DNA samples of each participant, show a 215-bp band for individuals that are homozygous or heterozygous for *GSTM1* phenotypes (15). *GSTM1*-null individuals do not show this band. A 480-bp band is detected for individuals who carry at least one *GSTT1* allele.

***GSTM1*1/1* Genotyping: Homozygosity Determination by Long PCR Amplification of *GSTM1*.** We determined *post hoc* the homozygosity (*GSTM1*1/1*) of *GSTM1*+ individuals by performing long-range PCR using the Expand long template PCR system (Boehringer Mannheim) and primers M2F10, 5′-AAG ACA GAG GAA GGG TGC ATT TGA TA-3′ and M5R16, 5′-ACA GAC ATT CAT TCC CAA AGC GAC CA-3′ (16). A 9-kb human tissue plasminogen activator gene fragment was coamplified using primers 5′-GGA AGT ACA GCT CAG AGT TCT GCA GCA CCC CTG-3′ and 5′-CAA AGT CAT GCG GCC ATC GTT CAG ACA CAC C-3′, to serve as an internal control. Fifty μl of reaction mixture contained 500 ng of genomic DNA, 5 pmol of *GSTM1* primers, 10 pmol of tissue plasminogen activator primers, 500 μM of deoxynucleotide triphosphates, 1× PCR reaction buffer containing 2.25 mM $MgCl_2$, and 2.5 units of Expand long template enzyme mixture. The thermal cycling conditions were: denaturation at 94°C for 2 min; 5 cycles of 93°C for 10 s and 68°C for 8 min; 5 cycles of 93°C for 10 s, 64°C for 30 s, and 68°C for 8 min; 25 cycles of 93°C for 10 s, 64°C for 30 s, and 68°C for 8 min, plus elongation of 15 s for each cycle; 1 cycle at 68°C for

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12 min; and cooling at 4°C. The lack of the 4748-bp amplicon is indicative of homozygosity (1/1) of *GSTM1*.

Serum GST-α. The concentration of GST-α in serum was measured using a commercially available, enzyme-linked immunoassay kit (High sensitivity Hepkit; Biotrin International, Dublin, Ireland). Serum was diluted 1:5 using the assay buffer provided; 100 μl of diluted sample and control were added in duplicate to a microtiter plate coated with antibody to GST-α and incubated at room temperature for 1 h with shaking. After washing to remove the unbound fraction, 100 μl of anti-GST IgG conjugated to horseradish peroxidase were added to each well, incubated for 60 min at room temperature and washed, and 100 μl of tetramethylbenzidine substrate were added. After a final 15-min incubation at room temperature, the reaction was stopped by adding 100 μl of 1 N H_2SO_4. Absorbances were read at 450 nm with a 630-nm reference filter on a Dynatech MR5000 MicroPlate Reader (Dynex Corp., Chantilly, VA). Standards of known concentrations were included in every run, and the concentrations of the samples and controls were calculated from a standard curve. The assay was linear from 200 to 2000 pg/ml. Intra-assay CVs were 6.8 and 3.3% at 262 and 1618 pg/ml, respectively; inter-assay CVs were 13.1 and 9.4% at 433 and 1502 pg/ml, respectively.

Peripheral Lymphocyte GST-μ Activity. Frozen buffy-coat fractions were thawed on ice and resuspended in PBS (pH 7.3). While on ice and in a cold room (4–8°C), the cells were sonicated twice with a Fisher Model 50 Sonic Dismembrator (Fisher Scientific, Pittsburg, PA) for 10 s each at a setting of 2 W. The disrupted cells were centrifuged in an Eppendorf 5402C centrifuge at 3800 × *g* for 30 min at 2–8°C, and the supernatant was separated into three aliquots. Protein concentration of the cytosol was measured using the Bradford Coomassie Blue method. Cytosol containing 100 μg of protein was incubated in the presence of 100 μM ^{3}[H]TSO (specific activity, 15 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) and 5 mM GSH in a final volume of 100 μl. The enzymatic reaction was initiated by the addition of GSH, and the reaction continued for 30 min at 37°C in a water bath, after which time it was stopped by the addition of 200 μl of hexanol. After vortexing, the phases were allowed to separate overnight and then were centrifuged in an Eppendorf 5402C centrifuge at 3800 × *g* for 10 min. Ten μl of the aqueous phase was added to 4 ml of Sigmafluor and counted in a Wallac 1409 scintillation counter for 1 min. Samples were assayed in duplicate. A sample control consisting of cytosol that was heat-inactivated for 15 min at 100°C was included as a blank. Activity of the blank was subtracted from the corresponding sample result to correct for nonenzymatic conjugation. Results were expressed as pmol product formed/mg protein/min. The intra- and inter-assay CVs were 6 and 10%, respectively.

Serum GST (CDNB) Activity. Serum GST activity using CDNB as a substrate was determined according to a modification of the method of Habig *et al.* (17) on a Roche Cobas Mira Plus (Branchburg, NJ) centrifugal analyzer. Serum and controls were incubated at 37°C in the presence of 1.22 μM CDNB in 0.1 M potassium phosphate buffer (pH 6.25) and 10.85 mM GSH in a final volume of 365 μl. The rate of formation of a colored product was measured at 340 nm. Enzyme activity (units/l) was calculated based on the extinction coefficient for the substrate CDNB of 9.2 mM^{-1} cm^{-1}, with a unit defined as that amount of enzyme that catalyzes formation of 1 μmol of product/min. Aliquots of control serum, frozen at −80°C, were thawed and run at the beginning and end of each batch of 60 samples. Intra- and inter-assay CVs were 7.7 and 11.8%, respectively (mean activity, 12.55 units/l).

Serum GST (NBD-Cl) Activity. Serum GST activity using NBD-Cl as a substrate was determined according to a modification of the method of Ricci *et al.* (18) on a Roche Cobas Mira Plus (Branchburg, NJ) centrifugal analyzer. Serum and controls were incubated at 37°C in the presence of 200 μM NBD-Cl in 0.1 M acetate buffer (pH 5.0) and 500 μM GSH in a final volume of 360 μl. The rate of formation of a colored product was measured at 340 nm. Enzyme activity (units/l) was calculated based on the extinction coefficient for the substrate NBD-Cl of 14.5 mM^{-1} cm^{-1}. Aliquots of control serum, frozen at −80°C, were thawed and run at the beginning and end of each batch of 60 samples. Intra- and inter-assay CVs were 5.2 and 7.6%, respectively (mean activity, 61.32 units/l).

Statistical Analysis. GST measures were made on blood samples collected on the last 2 consecutive days of each feeding period. GST (CDNB) and GST-μ activities were approximately normally distributed, although the distribution of GST-μ was bimodal. Serum GST-α concentration and GST activity (NBD-Cl), as well as the macronutrient intake data, were skewed and were log-transformed (natural log) prior to analysis.

This study was a randomized cross-over trial, a type of longitudinal study in which participants receive different treatments at different times. For the purpose of analysis, this design can be thought of as a randomized block design with subjects as blocks; sex, *GSTM1* genotype, and their interaction as between-block factors; and feeding periods, diet treatments, carry-over, sampling day (day 6 or 7), and their interactions with between-block factors as within-block factors. We analyzed the data using a linear mixed model (Ref. 19; PROC MIXED in SAS, Release 6.12), with study participants as a random effect and all other factors as fixed effects. For comparisons between vegetable-supplemented and basal diets, stratified by *GSTM1* genotype and sex, the corresponding contrasts were used. For GST-μ and GST (CDNB) activities, we present LS-means and standard errors for the difference between the vegetable diets and the basal diet by genotype and sex, adjusted for other effects in the model. Because the statistical analyses for GST-α and GST (NBD-Cl) activity were done on the log-transformed variables, the difference of the log-transformed means after back-transformation is presented as the ratio of the LS-means on the original scale. Pearson correlation was used to evaluate the correlations between GST values on days 6 and 7 and between different GST measures.

Results

Of the participants recruited, 40 completed all four feeding periods, 1 woman completed three periods, and 2 men completed two periods. These 43 individuals were included in the statistical analyses; their characteristics are presented in Table 1. Only two individuals (a *GSTM1*-null female and a *GSTM1*+ male) were *GSTT1*-null. Energy and macronutrient intakes on the different diets are presented in Table 2. A similar carbohydrate, protein, and fat distribution was maintained across the four diets; however, we did not attempt to adjust for the additional dietary fiber in the vegetables. Participant compliance to the study diets was excellent. On the basis of the participants' self-reported deviation from the study diets, only 1.6% (19 of 1204) "person feeding days" included consumption of nonstudy foods. The majority of these were attributable to participants having to make food substitutions (about which they consulted the study staff), because a snow storm prevented travel to the feeding center.

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Table 1 Characteristics of study participants consuming defined vegetable diets

	Men		Women	
	GSTM1-null $n = 10$	*GSTM1*+ $n = 11$	*GSTM1*-null $n = 15$	*GSTM1*+ $n = 7$
Age, yr	29.2 ± 5.2[a]	30.3 ± 6.3	29.1 ± 7.1	29.4 ± 6.1
Height, m	1.75 ± 0.07	1.73 ± 0.08	1.66 ± 0.06	1.65 ± 0.06
Weight, kg	74.7 ± 12.4	77.1 ± 16.0	62.7 ± 6.0	63.0 ± 7.6
BMI, kg/m^2	24.3 ± 2.6	25.5 ± 4.1	22.7 ± 1.4	23.1 ± 1.9
Race				
Caucasian, %	80	64	80	86
Non-Caucasian, %	20	36	20	14

[a] Mean ± SD.

Table 2 Average daily energy and macronutrient intake on study diets

	Basal	Brassica	Allium	Apiaceous
Energy, kcal	2383 ± 72[a]	2307 ± 72	2336 ± 72	2361 ± 72
Carbohydrate, g[b]	353 ± 10	344 ± 9	348 ± 9	355 ± 10
% energy from carbohydrate	60 ± 0.1	60 ± 0.1	61 ± 0.1	61 ± 0.1
Fat, g[b]	75 ± 2	72 ± 2	73 ± 2	73 ± 2
% energy from fat	29 ± 0.1	29 ± 0.1	29 ± 0.1	28 ± 0.1
Protein, g[b]	67 ± 2	69 ± 2	67 ± 2	65 ± 2
% energy from protein	12 ± 0.03	12 ± 0.03	12 ± 0.03	11 ± 0.03
Dietary fiber, g[b]	14 ± 0.3	21 ± 0.4	17 ± 0.3	21 ± 0.4

[a] LS-means ± SE, adjusted for subject effect.
[b] Analyses performed on log-transformed data; LS-means and SE are back-transformed.

LS-means of the GST measures (adjusted for sex, *GSTM1* genotype, feeding period, carry-over effects, and sampling day) for the basal diet and the ratio or difference of LS-means between the three vegetable-supplemented diets and the basal diet are presented in Tables 3 and 4. There was no carry-over effect for any of the GST measures. Overall, for the four controlled diets, serum GST-α concentrations did not differ between the *GSTM1*-null and *GSTM1*+ genotypes (3283 ± 415 *versus* 4124 ± 617; $P = 0.3$) but were higher in men than women (4634 ± 628 *versus* 2922 ± 414; $P = 0.02$). GST-α concentrations increased significantly with consumption of the brassica diet relative to the basal, vegetable-free diet (13%; $P = 0.05$; Table 3). This increase was exclusively among the *GSTM1*-null individuals (26%; $P = 0.005$), particularly the *GSTM1*-null women (38%; $P = 0.002$). Among *GSTM1*+ men, GST-α concentrations decreased by 22% with the addition of apiaceous vegetables ($P = 0.03$).

Serum GST activity, as measured by conjugation of CDNB and NBD-Cl, did not differ by *GSTM1* genotype. Mean GST activity as measured by CDNB, but not NBD-Cl, was higher in men than women ($P = 0.01$) and reflected a similar, but statistically nonsignificant, pattern in response to dietary treatment as did GST-α concentration. Among *GSTM1*-null women, there was a small (8%; $P = 0.04$) increase in serum GST (NBD-Cl) activity with addition of brassica vegetables.

As expected, LS-mean peripheral lymphocyte GST-μ activity was significantly higher in *GSTM1*+ than in the *GSTM1*-null individuals (257 ± 14 *versus* 17 ± 12; $P = 0.0001$) for the four experimental diets. GST-μ activity was higher in *GSTM1*+ men than *GSTM1*+ women (290 ± 18 *versus* 224 ± 22; $P = 0.03$). Among the *GSTM1*+ women, GST-μ activity increased by 52 ± 16 ($P = 0.001$) and 36 ± 16 pmol mg^{-1} min^{-1} ($P = 0.02$), with the addition of allium and brassica vegetables, respectively (Table 4). Of the *GSTM1*+ individuals, 12 (5 women and 7 men) were *GSTM1*1/0* and 6 (2 women and 4 men) were *GSTM1*1/1*. Further evaluation of GST-μ activity in *GSTM1*1/0* and *GSTM1*1/1* participants showed no significant ($P = 0.8$) effect of the interaction of homozygosity-by-diet on GST-μ activity, and on each diet, mean GST-μ activity was similar in both groups (Fig. 1). With allium vegetable supplementation specifically, GST-μ activity increased 19% among the *GSTM1* heterozygotes ($P = 0.02$), whereas it increased only 5% among the homozygotes ($P = 0.7$). The study size was not large enough to examine the combined effect of *GSTM1*+ homozygosity and sex.

Vegetable dose-per-body-weight was different between men and women. On the study diets, all participants were provided the same amount of vegetables daily. As a result, the doses differed by sex (*e.g.,* women received ~7 g of brassica vegetables per kg body weight, whereas the men received a significantly ($P = 0.001$) lower dose of 6 g per kg). Among the *GSTM1*-null individuals (*i.e.,* the group in which GST-α responded to the brassica diet), women who weighed ≤62 kg (median weight for women) had a similar GST-α response as did women who weighed >62 kg (an increase of 1162 ± 876 and 1329 ± 776 pg/ml, respectively). On average, men who weighed ≤70 kg (median weight for men), relative to those who weighed >70 kg, had a greater but statistically nonsignificant ($P = 0.6$) increase in serum GST-α (1199 ± 1533 and 234 ± 267 pg/ml, respectively).

Correlations between GST measures on days 6 and 7 were 0.87, 0.93, 0.47, and 0.66 for GST-α concentration and GST-μ, GST (CDNB), and GST (NBD-Cl) activities, respectively; however, the scatter for the serum GST activities was large. Mean GST-α concentrations and GST-μ activities, but not serum GST activities, on day 7 were statistically significantly higher than on day 6 ($P < 0.004$); day 7 values were 9 and 11% higher for GST-α concentrations and GST-μ activities, respectively. Overall, the correlations between serum GST-α concentration and the serum GST activities were not strong; GST-α concentration was weakly correlated with GST (NBD-Cl) activity ($r = 0.18$; $P = 0.02$) but not GST (CDNB) activity ($r = 0.048$; $P = 0.54$). The correlation between GST (CDNB) and GST (NBD-Cl) activity was 0.23 ($P = 0.0024$).

Discussion

In experimental studies in animals, a variety of plant-food constituents have been identified as inducers of GSTs (20) and as potential chemopreventive agents (21). In experimental studies in humans, only the effect of Brussels sprouts (300 g) on GSTs has been tested to date (5, 9). One of those studies included both men and women (5) and neither controlled for *GSTM1* genotype. However, it is becoming apparent that the chemoprotective effects of certain vegetables may be influenced, in part, by genotype profiles in the biotransformation enzymes. For example, Lin *et al.* (4) showed that *GSTM1*-null individuals consuming broccoli were less susceptible to colon cancer than were *GSTM1*+ individuals consuming the same amount of broccoli. Our study tested the effect of three botanically defined vegetable diets on several measures of GST in *GSTM1*+ and *GSTM1*-null individuals and examined the relationships within and between these measures in blood samples collected on 2 consecutive days.

We had postulated that lack of GSTM1 enzyme activity would result in a greater effect of vegetable diets on GST-α, potentially as a result of decreased inactivation of biologically active dietary constituents. Isothiocyanates, produced from glu-

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Table 3 Serum GST-α concentration and serum GST (NBD-C1) activity: The ratio between response to basal and vegetable-supplemented diets

	Basal[a]	Brassica/Basal[b]	Allium/Basal[b]	Apiaceous/Basal[b]
GST-α, pg/ml	3,605 ± 378	1.13 ± 0.07[c]	1.02 ± 0.06	0.95 ± 0.06
GSTM1-null	3,041 ± 417	1.26 ± 0.10[c]	1.07 ± 0.09	1.00 ± 0.08
Men (n = 10) [d]	3,498 ± 735	1.16 ± 0.14	1.05 ± 0.14	0.93 ± 0.12
Women (n = 15)[d]	2,644 ± 449	1.38 ± 0.14[c]	1.09 ± 0.11	1.08 ± 0.11
GSTM1+	4,316 ± 691	0.96 ± 0.09	0.95 ± 0.09	0.90 ± 0.08
Men (n = 11)[d]	6,568 ± 1,314	0.90 ± 0.11	0.95 ± 0.11	0.78 ± 0.09*
Women (n = 7)	2,836 ± 709	1.02 ± 0.15	0.96 ± 0.14	1.04 ± 0.15
GST (NBD-C1), units/l	75.9 ± 2.7	1.03 ± 0.02	1.02 ± 0.02	1.01 ± 0.02
GSTM1-null	70.1 ± 3.2	1.07 ± 0.03[c]	1.04 ± 0.03	1.05 ± 0.03
Men	71.5 ± 5.0	1.06 ± 0.05	1.08 ± 0.05	1.08 ± 0.05
Women	68.7 ± 3.9	1.08 ± 0.04[c]	1.00 ± 0.04	1.03 ± 0.04
GSTM1+	82.3 ± 4.1	0.98 ± 0.04	0.99 ± 0.03	0.97 ± 0.03
Men	87.4 ± 5.8	1.00 ± 0.04	1.01 ± 0.04	0.94 ± 0.04
Women	77.5 ± 6.2	0.96 ± 0.05	0.97 ± 0.05	0.99 ± 0.05

[a] LS-mean ± SE, adjusted for sex, *GSTM1* genotype, feeding period, and sampling day.
[b] The statistical analyses for GST-α and GST (NBD-C1) activity were done on the log-transformed variables. The results presented are the values after back-transformation. The difference of the log-transformed means after back-transformation is the ratio of the LS-means on the original scale.
[c] Significant comparison between vegetable diet and basal diet ($P < 0.05$).
[d] One *GSTM1*-null male and one *GSTM1*-null female did not complete the basal diet; one *GSTM1+* male did not complete the brassica and allium diets; and one *GSTM1*-null male did not complete the apiaceous diet.

Table 4 Serum GST (CDNB) and peripheral-lymphocyte GST-μ activities: The difference between response to basal and vegetable-supplemented diets

	Basal[a]	Brassica-Basal[b]	Allium-Basal[b]	Apiaceous-Basal[b]
GST (CDNB), units/l	13.53 ± 0.25	0.22 ± 0.21	−0.17 ± 0.21	0.22 ± 0.21
GSTM1-null	13.40 ± 0.33	0.32 ± 0.28	−0.04 ± 0.28	0.28 ± 0.29
Men (n = 10)[c]	13.83 ± 0.52	0.23 ± 0.44	0.03 ± 0.44	0.58 ± 0.45
Women (n = 15)[c]	12.98 ± 0.42	0.40 ± 0.35	−0.10 ± 0.35	−0.04 ± 0.35
GSTM1+	13.66 ± 0.38	0.07 ± 0.33	−0.29 ± 0.33	0.18 ± 0.33
Men (n = 11[c])	14.30 ± 0.48	−0.03 ± 0.42	−0.61 ± 0.42	0.57 ± 0.41
Women (n = 7)	13.01 ± 0.60	0.18 ± 0.51	0.02 ± 0.51	−0.21 ± 0.51
GST-μ, pmol mg^{-1} min^{-1}	131 ± 10	4 ± 7	10 ± 7	4 ± 7
GSTM1-null	18 ± 13	−4 ± 9	−1 ± 9	0 ± 9
Men	23 ± 21	−7 ± 14	−5 ± 14	−3 ± 14
Women	13 ± 16	−0 ± 11	3 ± 11	3 ± 11
GSTM1+	243 ± 16	18 ± 10	29 ± 10[d]	10 ± 10
Men	286 ± 20	−1 ± 13	6 ± 13	9 ± 13
Women	199 ± 24	36 ± 16[d]	52 ± 16[d]	11 ± 16

[a] LS-mean ± SE, adjusted for sex, *GSTM1* genotype, feeding period, and sampling day.
[b] The difference of LS-means between the vegetable diets and the basal diet.
[c] One *GSTM1*-null male and one *GSTM1*-null female did not complete the basal diet; one *GSTM1+* male did not complete the brassica and allium diets; and one *GSTM1*-null male did not complete the apiaceous diet.
[d] Significant comparison between vegetable diet and basal diet ($P < 0.05$).

cosinolates in cruciferous vegetables, are conjugated by GST. *In vitro* studies suggest that several of the isothiocyanates are good substrates for GSTM1 (3), whereas *in vivo* data (an observational study) showed that a greater urinary excretion of total isothiocyanates was associated with only the *GSTT1+*, but not the *GSTM1+*, genotype (22). We were unable to assess the effect of *GSTT1* genotype on GST-α in this study population; only 2 individuals (a *GSTM1*-null woman and a *GSTM1+* man) had the *GSTT1*-null genotype. However, our results suggest that, under controlled dietary conditions, *GSTM1* genotype modulates effects of vegetable supplementation.

Overall, among our participants, LS-mean serum GST-α concentrations and GST (CDNB) activity were higher in men than in women. We observed similar but nonsignificant differences in GST-μ and GST (NBD-Cl) activities. Sex differences in serum concentrations of GST-α (23) and the specific GST-α isoform, GSTA1-1 (24), but not lymphocyte GST-μ concentration (8) or serum GST (CDNB) activity (6), have been reported previously in observational studies.

Differences in vegetable-dose-per-body-weight between men and women may contribute to the observed sex-dependent response to diet in our study; however, body weight and sex were confounded, and this effect could not be tested effectively. In the study of Bogaards *et al.* (9), heavier men (>80 kg) had a smaller change in GST-α than lighter men (<80 kg) in response to consumption of Brussels sprouts. Together, these results suggest that body size may influence response; however, we cannot resolve whether this is specific to men. There may be a threshold dose-per-body-weight, such that most individuals, irrespective of sex, respond similarly and only the heaviest ones have a lower GST-α response. On the other hand, in the study of Nijhoff *et al.* (5), supplementation with 300 g of Brussels sprouts daily increased GST-α in men (n = 5), but not in women (n = 5). Furthermore, in our study, addition of apiaceous vegetables decreased GST-α concentrations among *GSTM1+* men but not women. Thus, taking the literature as a whole, it remains unclear whether the differences in responses to cruciferous feeding in humans are the result of dose-

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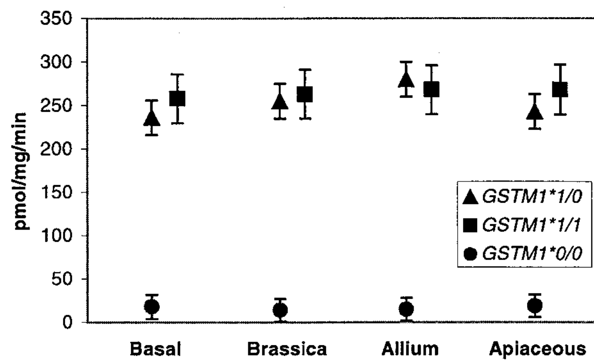

Fig. 1. Peripheral lymphocyte GST-μ activity (LS-means; *bars,* SE) on controlled vegetable diets in *GSTM1+* hetero- (*GSTM1*1/0*), homozygote (*GSTM1*1/1*), and null (*GSTM1*0/0)* individuals.

per-body-weight or physiological effects. It is also unclear whether men or women are better responders.

Peripheral lymphocyte GST-μ activity, as determined by conjugation of TSO, increased with allium and brassica supplementation in *GSTM1+* women but not in men. In rodents, both hepatic and intestinal GST-μ have been shown to increase with various dietary and drug treatments (21, 25); however, the capacity to induce GST-μ in humans, particularly in circulating cells (which presumably are not exposed to the same concentrations of phytochemicals as are the intestine and liver) has not been reported previously. In humans, Nijhoff *et al.* (26) found no effect of Brussels sprouts (300 g) on GST-μ protein in duodenum, rectum, or lymphocytes. On the other hand, Pool-Zobel *et al.* (27) reported increased expression of GSTP1 protein in lymphocytes in a subset of individuals fed carotenoid-rich beverages, indicating that certain GST isozymes in circulating cells may be responsive to dietary changes. On this basis, our results suggest that, over a week, at moderate, readily achievable levels of vegetable intake, GST-μ activity can be induced by vegetables in the Brassicaceae and Alliaceae families. With the present diet design, we cannot compare the difference in magnitude of response among vegetable treatments; each botanical grouping was fed as different gram weights, and the vegetables were provided in different forms (*e.g.,* the allium vegetables were fresh, whereas the majority of the brassica vegetables was frozen). The effect of frozen *versus* fresh on differences in response across the botanical groupings is unknown and would depend in part on the potency, stability, and metabolism of the various phytochemicals found in each botanical grouping.

Our study does not support the hypothesis that the variation in peripheral lymphocyte GST-μ activity (against TSO) among *GSTM1+* individuals is attributable to an allelic dose effect (16). We found no difference in GST-μ activity between *GSTM1*1/0* and *GSTM1*1/1* individuals in this selected population, under defined dietary conditions. However, the number of participants was small, especially *GSTM1*1/1* individuals ($n = 6$), and these results may be attributable to chance. This hypothesis needs to be tested further with a larger sample size.

Correlations were strong for GST-α concentration and GST-μ activity measured in blood samples drawn on 2 consecutive days. However, ~10% higher values were obtained on day 7 than on day 6, particularly on the brassica diet, suggesting that response to diet may not have reached a steady state with 6 days of feeding. Typically, inducible biotransformation enzymes respond rapidly to changes in diet. Sreerama *et al.* (11) demonstrated that salivary GST activity increased and reached a plateau within 5 days in response to broccoli feeding. Whether rate of adaptation is affected by individuals' baseline GST levels under habitual diet conditions could not be determined here; we did not collect and test blood samples at the start of each period. Other aspects of dietary adaptation may also need to be considered. Recent studies have shown that intestinal bacteria hydrolyze glucosinolates that have not been cleaved by myrosinase in the cruciferous vegetable (28). In our study, a predominant portion of the brassica diet was fed as cooked vegetables, in which most of the myrosinase has been inactivated. Bacterial hydrolysis could have a major influence on exposure to isothiocyanates in cooked vegetables, and thus, ultimately, GST-α response. The adaptation of intestinal microflora to the diet may result in an increasing dose of isothiocyanates as bacterial enzymes are induced or bacterial populations are altered.

The lack of association between GST-α and serum GST activities, measured using CDNB and NBD-Cl as substrates, is not altogether unexpected. CDNB has been used for decades as a general measure of GST activity; it is a good substrate for many of the GST isozyme classes (18). Therefore, activity against CDNB in serum most probably reflects not only GST-α and -μ (in *GSTM1+* individuals) circulating as a result of hepatic cell turnover (26) but also GSTs from other tissues (2) and leakage from erythrocytes either *in vivo* or *ex vivo* in the collected blood sample (29). NBD-Cl has been identified as a substrate that is more specific for GST-α (18). To our knowledge, this is the first attempt to quantify serum GST activity against NBD-Cl. The low correlation between serum GST (NBD-Cl) activity and GST-α concentration implies that the GST (NBD-Cl) assay is not highly specific for GST-α in serum; however, the small, but statistically significant, increase in GST (NBD-Cl) activity in *GSTM1*-null women supplemented with brassica vegetables follows a similar pattern as observed for GST-α concentrations. Overall, serum GST activities in these healthy individuals were very low and nonenzymatic conjugation in the serum was high, suggesting that these measures are not sensitive enough to monitor effectively responses to dietary interventions, even under controlled conditions.

In summary, brassica, but not allium or apiaceous, vegetable supplementation under controlled dietary conditions significantly increased serum GST-α concentrations and GST (NBD-Cl) activity in *GSTM1*-null individuals, particularly in women. Both brassica and allium vegetables increased peripheral lymphocyte GST-μ activity in *GSTM1+* women. These results suggest that the capacity of moderate intakes of cruciferous vegetables to induce GST-α is dependent in part on the lack of GSTM1 activity. Whether sex differences in GST-α in response to diet are a function of a dose-per-body-weight effect or a genuine difference in physiological response between men and women remains to be clearly established.

Acknowledgments

We thank Annie Lamb and Darlene Lanes for preparing and serving the study diets; Lisa Levy, Lisa Szymura, and Judy Standley for assisting with study operations; and Wilas Nirunsuksiri and Heather Skor, and Shirley Wang, for technical support on the GST-μ activity assay and *GSTM1* genotyping, respectively.

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Johanna W. Lampe, Chu Chen, Sue Li, et al.

Cancer Epidemiol Biomarkers Prev 2000;9:787-793.

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