Metabolism of isothiocyanates in individuals with positive and null *GSTT1* and *M1* genotypes after drinking watercress juice

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Abstract

Background & Aims—Isothiocyanates (ITCs) derived from cruciferous vegetables have been shown to be promising agents against cancer in human cell culture, animal models, and in epidemiological studies. Several epidemiological studies have demonstrated an inverse relationship between intake of dietary isothiocyanates and the risk of cancers, particularly lung, colon, and breast. More importantly, the protective effects of dietary ITCs appear to be influenced by *glutathione S-transferase* (*GST*) genotype; specifically, individuals with *glutathione S-transferase theta 1* (*GSTT1*) and *glutathione S-transferase Mu 1* (*GSTM1*) null are better protected than those with *GSTT1* and *M1* positive. Although majority of studies, especially those conducted in populations exposed to ITCs rich diet, demonstrated such effects there are a few studies showed opposite or no association. While evidence for the interactions of dietary ITCs with *GST* genes is relatively strong, the reasons for the differential effects remain unclear. In this study, we examined one possible mechanism whether subjects with the null genotypes excrete ITCs at a slower rate than those with the positive genotypes after drinking watercress juice, a rich source of ITCs.

Methods—A total of 48 subjects, 28 *GSTT1* and *M1* positive and 20 null genotypes were enrolled in the study. The rates of excretion were determined using five urine samples collected over a period of 24 hours after drinking watercress juice.

Results—No statistically significant differences in the rates of isothiocyanate excretion and the time of peak excretion were observed between the two tested groups with positive and null genotypes.

Conclusions—*GSTT1* and *M1* genotypes are not likely to be involved in the rate of excretion of ITCs in watercress. The demonstrated differences in protection among subjects with the two

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genotypes are not likely due to differences in overall ITC excretion rates, however, excretion rates of ITCs other than PEITC need to be investigated. Other yet to be identified mechanism(s) may underlie the diet and gene interactions between dietary ITCs and *GST* genotypes in human cancer prevention. Further research is needed to evaluate the protective mechanisms of isothiocyanates against cancer.

Keywords

isothiocyanates; watercress; PEITC; *GST* genotype; cancer prevention

Introduction

Isothiocyanates (ITCs) are a family of compounds found in cruciferous vegetables that have been shown to have promising chemopreventive activities (1). In animal and human cell culture studies, these compounds inhibit carcinogenesis in a number of tissues, including lung, breast, colon, and bladder epithelium (2–6). Epidemiological studies have also shown that intake of dietary ITCs are associated with reduced risks of certain human cancers (1,7–15). In a cohort study in Shanghai, dietary ITC intakes were shown to be inversely associated with the risk of lung cancer (10). In that study, the intake of dietary ITCs was estimated by measuring the total ITC metabolites excreted in the urine as a validated biomarker (16,17). More importantly, it showed that *GSTM1* and *T1* genotypes modulate the protective effects of dietary ITCs against lung cancer. The strongest protection was seen in individuals with *GSTM1* and *T1* null, whereas no protective effect was seen in *GSTM1* and *T1* positive individuals. Although the majority of studies support such observation, others found no differences in the protective effects of cruciferous vegetables between *GSTM1/T1* positive and null individuals (14,18). We address this disparity in greater details in the discussion.

Subsequent studies based on either urinary ITC levels or questionnaires not only confirmed the roles of *GSTM1* and *GSTT1* null genotypes in the effect of dietary ITCs against lung cancer (7,15), but further showed that dietary ITC intake is associated with reduced risks of other cancers, such as breast and colon (8,9,11,19). These studies demonstrated the importance of diet-gene interactions in protection against cancer. While the importance of *GSTM1* and *T1* null polymorphism in cancer prevention by dietary ITCs has been supported by a number of independent studies (9–11,13–15,18–25), the mechanisms for the differences related to *GST* null and positive genotypes are still not understood.

The metabolism of ITCs in humans has been investigated. A major route of metabolism is the conjugation of ITC with glutathione (GSH) followed by enzymatic degradation and N-acetylation via the mercapturic acid pathway (26). Because *GSTs* have been shown to mediate conjugation of GSH with ITC (12,27), a plausible hypothesis for the *GST* gene-diet interaction is that individuals with the *GSTT1* and *M1* null genotypes have a slower overall rate of excretion of dietary ITCs and, consequently, more sustained bioavailability as compared with those who are *GSTT1* and *M1* positive genotypes (28).

To test this hypothesis and further define the role of dietary ITCs in cancer prevention, we conducted a trial to determine whether individuals with both *GSTT1* and *GSTM1* null genotypes have lower rates of ITC excretion than those with positive genotypes by quantifying total ITC metabolites in the urine over a period of 24 h after drinking watercress juice which is rich in phenethyl isothiocyanate (PEITC).

Materials and Methods

Chemicals

1,2-Benzenedithiol was purchased from Fluka (Sigma-Aldrich Co., St. Louis, MO). L-Ascorbic acid was purchased from Aldrich (Sigma-Aldrich Co., St. Louis, MO). The *N*-acetylcysteine conjugate of phenethyl isothiocyanate (PEITC-NAC) used was synthesized in our laboratory (29). All other chemicals used were HPLC or ACS reagent grade.

Watercress juice

Watercress juice was prepared by blending watercress from a local grocery store and tap water in proportion of 1:4 (w/w). After blending in a mechanical blender for 1 min. at high speed, the resulting suspension was filtered through two layers of cheesecloth. The filtered juice was kept at 4° C until use. Fresh juice was prepared for each treatment and 18 separated preparations were made during the study period. The juice preparations for most of the treatments were stored for approximately 2 h before treatment. However, for reasons of convenience, preparations were made late Fridays or during weekends for those treatments that occurred early morning of the following Monday.

Study subjects

Recruitment and screening criteria—All subjects participating in this study gave informed consent before treatment. The study protocol was approved by MedStar Research Institute-Georgetown University Oncology Institutional Review Board. Subjects who responded to the on-campus flyers were contacted for initial questionnaire-based screening for eligibility. The criteria of eligibility were as follows: age between 18 and 60 years old, HIV negative, no active prescription medications, non-tobacco users, non-diabetic, not currently pregnant or breast feeding, and having *GSTM1* and *GSTT1* positive or *GSTM1* and *GSTT1* null genotypes. In the first phase of the study, genotyping of *GSTM1* and *GSTT1* was done on eligible subjects. Those who had both *GSTM1* and *GSTT1* positive or *GSTM1* and *GSTT1* null genotypes were contacted. The subjects who agreed to participate in the second phase of the study were scheduled to come to the General Clinical Research Center at Georgetown University Hospital to be treated with watercress juice and donate urine samples for the next 24 hours.

Treatment protocol

Watercress juice drinking—The majority of urinary ITCs are excreted within the first 16 h after ingestion and the excretion is nearly complete 24 h after, although the total ITC excretion can last as long as 72 h (12,30,31). Subjects were instructed to avoid any food containing ITCs for 72 h before treatment. A detailed list of foods containing ITCs was provided to each subject (please see supplementary materials section). The night before treatment the subjects were asked to avoid any food and drink after midnight. In the morning of treatment day, subjects drank 200 ml of watercress juice. Immediately after drinking the juice, a breakfast consisting of 8 ounces of orange juice, one bagel with a pat of butter, a bowl of cornflakes with skim milk, and coffee, if desired, was served. During the remaining 24 h of urine collection period, subjects were free to eat whatever they chose as long as they avoided the ITC-containing foods on the list.

Blood and Urine collections—Blood samples were drawn at Georgetown University General Clinical Research Center by trained nurses. After processing and separation of blood components, buffy coat samples were stored at −80° C for further assays. Blood was drawn once after the initial screening phase purely for genotyping purposes.

A morning urine sample was collected prior to treatment (baseline). After drinking watercress juice, five samples of total urine were collected from 0–2, 2–4, 4–8, 8–12 and 12–24 hours. Each sample was collected in separate jars containing 0.5 g of ascorbic acid as a preservative as previously described (9). The total volume of each collection was recorded and small aliquots (3×1.8 ml) of urine were taken and kept at $-80°$ C until analysis.

Genotyping

GSTM1 and *GSTT1* genotypes were determined by a multiplex polymerase chain reaction (PCR) using genomic DNA as previously described (32). Genotyping was performed by the 5' nuclease assay (TaqMan) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the constitutively present gene BRCA1 as an internal positive control. Genotyping was performed by laboratory personnel blinded to the treatment arm, and a random 10% of the samples were repeated for validation. Two lab personnel independently reviewed all genotyping results, and samples with unclear results were rerun until 100% concordance was achieved.

HPLC analysis of total ITC metabolites in urine and ITCs content in watercress juice

Urine analysis—The urine samples were analyzed by HPLC after reacting with 1,2-benzenedithiol (BDT) as previously described (8) with minor revisions: an ITC-free urine sample was used for PEITC-NAC standards (0, 0.5, 1, 2, 5, 10, 15, 20, 25 and 50 mM) instead of 20 mM phosphate buffer (pH 5.0); the 1 mM BDT in 2-propanol was used instead of 10 mM for the cyclocondensation reaction to minimize byproducts formation; in the HPLC assay the mobile phase consisted of a mixture of methanol and water 5:2 (v/v) rather than the previously reported ratio of 7:3 (v/v) due to the technical design of the gradient controller. In every assay samples containing mobile phase and standard in triplicates were interspersed at every nine samples throughout the batch for quality control of the assay.

For each group of samples used for quality control, a different concentration of standard was used (5, 10 and 15 µM). For each set of samples, average ITC concentration, standard deviation and coefficient of variation (CV) were calculated. The triplicate values were then checked for consistency. The criteria used were: 25% or less CV for ITC values between 0.5 to 2 µM and 15% CV for values above 2 µM. Samples that did not fit these criteria were re-analyzed. To ensure assay reproducibility 3% of randomly selected samples were re-analyzed. The average intra- and inter-batch CV for triplicates was 4% and 5%, respectively.

Analysis of watercress juice—Watercress juice was centrifuged for 15 min. ($\times$ 4,500 rpm) on a desktop Eppendorf 5804 centrifuge (Eppendorf, Madison, WI) and the supernatant was diluted 1:2 with water, then processed and analyzed in the same manner as urine samples. Because watercress juice was prepared with water, all standard solutions used in the assay were prepared using water instead of ITC-free urine.

Statistical methods

Demographic characteristics, specifically gender and race, were compared between the *GST* positive and null groups using Fisher's exact test. In addition, t-tests were used to compare age at juice consumption, urinary baseline ITC level, and ITC content in juice between the groups. Normality assumptions were checked using graphical techniques on the residuals. Several approaches were used to examine differences in magnitude of ITC excretion between *GST* positive and null groups.

The primary outcome was the cumulative amount of ITC excreted over time, which was calculated by adding the total amount of ITC excreted by the end of each interval to the

amount excreted in all previous intervals. This resulted in five measurements per subject over the collection period.

Secondary outcomes included the total amount of ITC excreted per two hours and the time at which the peak excretion occurred. Since the time intervals between collections were not equal and we expected more ITC excretion over longer time periods, the total amount of ITC excreted was standardized to a two hour interval. This assumes that ITC was excreted at a uniform rate over the entire interval, but allows the measurements from different collections to be comparable. The ITC content in juice, urine baseline ITC (amount of ITC metabolites in urine collected before the treatment), and demographics were considered as covariates.

Since ITC excretion was measured repeatedly on the same subject over time, we expected that the measurements from the same subject were correlated. In order to account for the dependence, a mixed effects model was used to determine if there was a difference in cumulative ITC excretion between the *GST* positive and null groups over time. The model building began with time, *GST* status, and their interaction in the model. Since cumulative ITC excretion follows a nonlinear trajectory, higher order terms with time and their interactions with *GST* status, urine baseline ITC, and ITC content in juice were introduced to the model. The overall rate of cumulative ITC excretion between *GST* groups was compared using a likelihood ratio test in which all terms involving the interaction between *GST* status and time were tested jointly. A similar modeling approach was used for the total amount of ITC excreted per two hours after consumption. The total amount was transformed using the natural logarithm in order to meet normality assumptions. The results were back-transformed and are presented on the original scale. In addition, Fisher's exact test was used to compare the time interval at which the peak ITC excretion occurred between the *GST* groups. Finally, a sensitivity analysis was performed by excluding the ITC measurements after an unusual spike in total ITC excreted. The same analysis was repeated and compared to determine the impact that these outliers may have had on the results.

The study planned to enroll 25 subjects in each of the two groups in order to have an 80% chance of detecting as significant (at the two-sided 5% level) a 12 unit difference in mean ITC excretion in any time interval, with an assumed standard deviation of 15. Statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC).

Results

Out of 417 subjects who responded to the posted flyers, 174 were eligible for the study. After genotyping, 52 were found to have both *GSTM1* and *GSTT1* positive and 26 were negative for both. Of the 78 subjects with the desired genotypes, 48 subjects participated in the trial, 28 *GST1* and *M1* positive and 20 *GST1* and *M1* null. Of the *GST* positive subjects 39% were males compared to 50% in the *GST* null group (Table 1). Among those reporting race (63%), 70% were white, 20% Asian, 3% black and 7% defined themselves as "other". There was no statistically significant difference between the two groups on gender or race (p=0.56 and 0.99, respectively). The average age was 26 years and there were no significant differences between *GST* positive and null subjects (p=0.07) in age. The concentration of ITC in the juice had an overall average of 72 µM and ranged between 15 and 206 µM. This wide range of ITC content in the juice was caused by a small number of subjects consuming juices containing relatively high levels of ITCs (91 – 206 µM) possibly due to batch differences in watercress purchased from the store. In addition, the significant differences of ITC content in the juice ingested by the subjects may also be caused by the unexpected, rapid decomposition of ITCs at 4°C during the first few hours after preparation (data not shown). Subsequently, to minimize the variability, we collected and immediately froze the

juice samples used at the time of sampling. Both factors may contribute to the statistically significant difference in the ITC content between *GST* positive and *GST* null subjects (p=0.02) (Table 1). There was no statistically significant difference in baseline ITC in the urine between the two groups (p=0.22).

The final model to estimate the trend of cumulative ITC excretion over the 24 h follow-up period included fixed effects for time, *GST* status, a quadratic time term to model the curvature in the cumulative ITC trajectory, and the interactions between *GST* status and time and ITC content in juice. The *GST* positive group consumed juice with a higher concentration; juice concentration remained in the final model to adjust for this difference. Results did not change when other functional forms for juice concentration were considered such as log-transformed concentration or adding a quadratic term into the model. In addition, age was removed from the final model since it was not a significant predictor (p=0.21). Also, random effects for intercept, time and quadratic time were included in order to account for the correlation between measurements of ITC excretion on the same subject. The likelihood ratio test for the overall difference in rate of the excretion between the groups was not statistically significant after controlling for ITC content in juice (p=0.43). The estimated mean cumulative ITC excretion from the model among the *GST* null group was higher at the end of each collection interval except the first, after controlling for ITC content in juice. However, these differences were not statistically significant (Table 2). Figure 1 shows cumulative ITC excretion over time for subjects after consuming watercress juice.

The mixed model for log-transformed total amount of ITC excretion included fixed effects for *GST* status, time, their interaction, juice content, and random effects for the intercept and slope. There was no statistically significant difference in the rate of excretion (slope) between the *GST* null and positive groups after controlling for ITC content in juice (p=0.53, Figure 2). Finally, there was no statistically significant difference in the time of peak excretion between the two groups (p=0.17).

Twenty five subjects were suspected of having consumed ITC-containing foods based on a spike in the total amount of ITC excreted during the follow-up period and had at least some outlying data removed for the sensitivity analysis. However, the results were robust to the apparent protocol violations. Since the conclusion did not change after these exclusions, the results presented here included ITC measurements for all the urine samples collected. The difference in cumulative ITC, the rate of ITC excretion, and the time to peak excretion between the groups remained statistically insignificant (p=0.58, 0.93, and 0.57, respectively).

Discussion

This study was designed to examine whether metabolic rates of dietary ITCs may explain the observations that *GST1* and *M1* null genotypes are better protected by cruciferous vegetables. The study showed that there are no significant differences in overall rate, total amount of excretion, and the time of peak excretion of ITCs after drinking watercress juice between the null and positive *GSTT1* and *M1* genotypes and the results point to possibilities that mechanisms other than PEITC metabolism may be involved.

The major route of metabolism of ITCs in humans involves the initial conjugation with GSH followed by enzymatic degradation via the mercapturic acid pathway (12,27,30). In human urine, the N-acetylcysteine conjugates of ITCs have been found to be the predominant metabolite. Excretion peaks between 1–2 h after ingesting broccoli, watercress or its juice and is intake-dependent (12,16,31). An HPLC-based assay has been used to quantify total urinary ITCs, including the N- acetylcysteine conjugates, as a marker of dietary ITC intake

by converting to a cyclocondensation product with 1,2-benzenedithiol (16). Approximately, a total of 75% of all ITC ingested is excreted within 8 h. The urinary marker of dietary ITCs has been validated against questionnaire responses in a Singapore cohort study and then applied to numerous population studies to investigate the role of dietary ITCs in human cancers (8–10,15,17,20).

Several studies have shown a positive association between *GSTM1* and *GSTT1* null genotypes and cancer prevention by dietary ITCs (8–11,15,19). The Shanghai cohort study was the first to link dietary ITC intake to a decreased risk of lung cancer and showed that the protective effect of ITCs was seen only among individuals with homozygous deletion of either *GSTM1* or *GSTT1*, and that it was particularly strong in individuals with deletion of both *GSTM1* and *GSTT1* (10). This conclusion is supported by a large case-control study of patients with lung cancer conducted among mostly non-smoking Chinese women in Singapore (15). A large prospective study from central and eastern Europe further lends support by confirming a protective effect of cruciferous vegetables against lung cancer, and only individuals with *GSTM1* null or both *GSTM1/T1* null were protected (7). Recently, taking into account results from 6 cohort and 12 case-control studies, a review concluded that, independent of cigarette smoking, there is a modest inverse association between lung cancer risk and high intake of cruciferous vegetables and that the effect is stronger among subjects with null genotype for *GSTM1* and *GSTT1* (33).

Similar conclusions were made in a case-control study of breast cancer conducted in Shanghai and a colon cancer study in Los Angeles (8,9,18,23,24). In another report, low ITC intake and *GSTM1* and *GSTT1* null genotypes were shown to be associated with increased lung cancer risk, whereas subjects with deficient genotypes and high ITC intake did not have increased lung cancer risk (13).

Contrary to those findings, one study conducted in the US showed a protective effect of cruciferous vegetable against lung cancer only in individuals with *GSTM1* positive genotype (14). In a Dutch case-control colorectal adenoma study, no differences were found in the protective effect of cruciferous vegetables between *GSTM1/T1* positive and null individuals; however, less active *GSTP1* and *GSTA1* genotypes seemed associated with the protective effect of ITC intake (18).

The divergent results may be related to substantial differences in dietary habits between populations. Possible explanations in the discrepancy are frequency of consumption, types of cruciferous vegetable consumed, and possibly differences in *GST* specificities towards various ITCs consumed by each populations (22,27). For example, the daily estimated consumption of cruciferous vegetables in North America was reported to be 25–30 g, with a range of 16–40 g, significantly lower compared to those studies conducted in Shanghai, China (more than 100 g per day) and the general Asian region (40–80 g per day) (1). Furthermore, the food questionnaire used in most of those studies is not as reliable as urinary ITCs metabolites, especially when the consumption is relatively low.

Because *GSTs* can mediate the conjugation of ITC with GSH, a plausible explanation for the protective effect in *GSTM1* and *GSTT1* null genotypes is that they have more sustained bioavailability of ITCs due to slower excretion (8,10,15,17). An earlier study showed that, while *GSTM1* null and positive individuals have no differences in urinary ITC levels, *GSTT1* positive is significantly associated with increased total urinary ITC excretion, especially in subjects having high consumption (17). However, Gasper et al. reported that *GSTM1* null subjects excreted sulforaphane (SFN) metabolites at a faster rate during the first 6 h and to a greater extent during 24 h than *GSTM1* positive subjects after consumption of broccoli (22).

Contrary to that report, Steck et al. showed no differences in the average 24 h urinary excretion of ITC between groups with the different *GST* genotypes, except that a greater proportion of individuals with *GSTM1* null genotype had a higher than median urinary ITC level when compared to those with *GSTM1* positive (20). However, as only a 24 h urine sample was collected in that study, it may have failed to capture any possible differences in the rate of excretion between the positive and null *GST* genotypes due to the relatively fast ITC metabolism. Furthermore, the number of subjects with *GSTM1/T1* null vs. positive genotypes seems quite low. It should also be noted that there are significant differences in *GST* activity toward SFN vs. PEITC. SFN has been shown to be a poor substrate for all *GST* enzymes with significantly slower GSH conjugation by *GSTM1* than other ITCs (22,27). While these findings are plausible explanations for the discrepancies from the previous studies, the inconsistent results prompted us to conduct a more detailed study on kinetics of excretion after drinking watercress juice containing known amounts of ITCs. In our study, we determined the overall rates of ITC excretion by collecting multiple urine samples over a 24 h period after consumption. Considering the rapid ITC metabolism (12,31,34), it is possible that the earlier time points may reflect more accurately the relationship between *GST* genotype and the ITC metabolic rate.

Our results showed that there are no significant differences in overall rate, total amount of excretion, and the time of peak excretion of ITCs after drinking watercress juice between the null and positive *GSTT1* and *M1* genotypes, however, this study has some limitations. Even though we used the same quantity of fresh watercress for juice preparation for each subject we did observe significant variations in the amount of ITC in the watercress juice consumed due to unexpected rapid decomposition under the storage conditions, and possibly cultivar differences of watercress batches from store. Also, the watercress juice administrated was not corrected by BMI or other related factors. Although the excretion was based on the amount of ITC measured in the ingested juice, it may still be argued, with these limitations, that a larger sample size may be needed to detect any differences in the ITC metabolism by these two genotype groups. Nevertheless, we believe the differences, if they exist, may be small.

Several possible explanations may be suggested for the lack of difference in these two genotypes. First, *GSTT1* and *M1* do not play a significant role as a rate determinant step in ITC conjugation; second, PEITC, a major component in watercress, is not a good substrate for *GSTT1* and *M1*; and third, genes other than *GST* in the mercapturic acid pathway may be more important in metabolizing ITCs (35,36). The question is why individuals with *GSTT1* and *M1* null genotypes are better protected.

The results of the present study suggest that mechanism(s) other than ITC metabolism may exist for the observed protection in *GSTT1* and *M1* null genotypes. For example, ITC metabolism is differentially regulated in target tissues such as lung, breast and colon. The lack of *GSTT1* and *M1* genes may influence the expression of other genes that are associated with reduced risk of cancer. Earlier studies have shown that *GSTM1* genotype status may influence other biotransformation enzymes including *GSTA*, *GSTM* and *cytochrome P450 1A2* (28,37). A recent study showed that *GSTT1* activities can be up-regulated by *GSTM1* null genotypes upon exposure to pesticides (38). It is plausible that genes other than *GST* family members or cytochrome *P450s* may be modulated by dietary ITCs in the absence of *GSTM1* and *T1* genes. More studies are needed to examine these possibilities in order to fully understand the proficient protection toward *GSTT1* and *M1* null genotypes by dietary ITCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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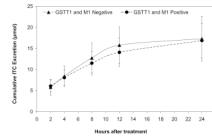
Figure 1.
Fitted mean cumulative ITC (µmol) (and 95% CI) excreted in the urine over 24 hours after drinking watercress juice for *GSTT1* and *M1* positive and null subjects with an ITC concentration of 72 µM estimated with a linear mixed model. The final model included fixed effects for time, *GST* status, a quadratic time term to model the curvature in the cumulative ITC trajectory, and the interactions between *GST* status and time and ITC content in juice and random effects for intercept, time, and quadratic time.
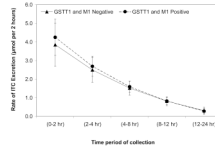
Figure 2.
Fitted mean total ITC (and 95% CI) excreted in the urine for *GSTT1* and *M1* positive and null subjects with an ITC concentration of 72 µM estimated with a linear mixed model. The model was fit to the log-transformed total ITC values to meet modeling assumptions, however, the fitted means presented here have been back-transformed and are on the original scale. The final model included fixed effects for *GST* status, time, their interaction, juice content, and random effects for the intercept and slope.

Table 1

Characteristics of subjects by *GSTT1* and *M1* status (N=48), gender, race, age at the time of study, and juice concentration used.

	GSTT1 and M1		
	Null (N=20) [a]	Positive (N=28)	p-value
	N (%)	N (%)	
Gender			0.56
Male	10 (50%)	11 (39%)	
Female	10 (50%)	17 (61%)	
Race [b]			0.99
White	6 (67%)	15 (71%)	
Non-White	3 (33%)	6 (29%)	
	Mean ± SD	Mean ± SD	
Age, years	29 ± 11	25 ± 5.8	0.07
ITC concentration in juice [μM] [c]	46 ± 45	88 ± 60	0.02
Baseline ITC [μmol] [d]	0.83 ± 1.64	0.42 ± 0.61	0.22

[a] Total number of subjects.

[b] Unknown: 11 *GST* null, 7 *GST* positive.

[c] Unknown: 3 *GST* null.

[d] Amount of ITC in the urine sample collected over 24 h before watercress juice treatment.

Table 2

Fitted mean cumulative ITC (μmol) excreted at each time point based on *GSTT1* and *M1* genotypes from subjects after consuming watercress juice with an ITC concentration of 72 μM from the linear mixed model (N=45).

| Time (h) | *GSTT1* and *M1* | | p-value of difference |
	Null	Positive	
2	5.8 (3.8, 7.7)	6.0 (4.6, 7.5)	0.83
4	8.4 (5.9, 11)	8.1 (6.2, 10)	0.83
8	13 (9.1, 16)	12 (8.7, 14)	0.59
12	16 (11, 20)	14 (11, 17)	0.55
24	17 (12, 23)	17 (13, 21)	0.90