GSTT1 genotype modifies the association between cruciferous vegetable intake and the risk of myocardial infarction[1–3]

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ABSTRACT

Background: Cruciferous vegetables are a major dietary source of isothiocyanates that may protect against coronary heart disease. Isothiocyanates induce glutathione *S*-transferases (GSTs), polymorphic genes that code for enzymes that conjugate isothiocyanates, as well as mutagens and reactive oxygen species, to make them more readily excretable.

Objective: The objective of the study was to determine whether GST genotypes modify the association between cruciferous vegetable intake and the risk of myocardial infarction (MI).

Design: Cases (n = 2042) with a first acute nonfatal MI and population-based controls (n = 2042) living in Costa Rica, who were matched for age, sex, and area of residence, were genotyped for a deletion polymorphism in *GSTM1* and *GSTT1* and an Ile105Val substitution in *GSTP1*. Cruciferous vegetable intake and smoking status were determined by questionnaire. Odds ratios (ORs) and 95% CIs for MI were estimated by unconditional logistic regression.

Results: Compared with the lowest tertile of cruciferous vegetable intake, the highest tertile was associated with a lower risk of MI among persons with the functional *GSTT1*1* allele (OR: 0.70; 95% CI: 0.58, 0.84) but not among those with the *GSTT1*0*0* genotype (OR: 1.23; 95% CI: 0.83, 1.82) (P = 0.006 for interaction). This protective effect among those with the *GSTT1*1* allele was greater for current smokers (OR: 0.54; 95% CI: 0.36, 0.79) than for nonsmokers. *GSTP1* and *GSTM1* did not modify the association between cruciferous vegetable intake and MI.

Conclusions: Consumption of cruciferous vegetables was associated with a lower risk of MI among those with a functional *GSTT1*1* allele, which suggests that compounds that are detoxified by this enzyme contribute to the risk of MI. *Am J Clin Nutr* 2007;86:752–8.

KEY WORDS Cruciferous vegetables, isothiocyanate, glutathione *S*-transferase, genotype, myocardial infarction

INTRODUCTION

Atherosclerosis is a major cause of myocardial infarction (MI), one of the leading causes of cardiovascular deaths in the world (1). The American Heart Association recommends ≥5 servings of fruit and vegetables/d to reduce the risk of chronic diseases such as coronary heart disease (CHD) (2). Because fruit and vegetables represent a large group of foods with varied nutrient and nonnutrient profiles, it is unclear which components of this food group provide protection against CHD. Isothiocyanates are a group of naturally occurring compounds that occur as glucosinolates in cruciferous vegetables, primarily those of the *Brassica* genus. Isothiocyanates may have beneficial effects on the cardiovascular system, such as inducing detoxifying enzymes and reducing oxidative stress (3–6). Two studies have reported an inverse association between cruciferous vegetable consumption and serum homocysteine concentrations (7, 8), a risk factor for CHD.

Isothiocyanates are rapidly conjugated by glutathione *S*-transferases (GSTs) and excreted in the urine (9, 10). GSTs are a super-family of xenobiotic-metabolizing enzymes that generally "detoxify" reactive metabolites to more water-soluble and readily excretable forms (11). These enzymes are expressed in several tissues, including the heart and blood vessels (12, 13). GSTs are grouped into several distinct classes with partially overlapping substrate specificities. GSTM1, GSTT1, and GSTP1 are isoforms of the mu, theta, and pi class, respectively. Homozygosity for a common deletion of the *GSTM1* gene (*GSTM1*0*) results in a lack of GSTM1 activity (14). *GSTT1* has 2 alleles, denoted *GSTT1*0* for the nonfunctional allele and *GSTT1*1* for the functional allele (15). An A→G polymorphism at nucleotide 313 of *GSTP1* results in an amino acid substitution (Ile105Val) in the substrate-binding site of the enzyme. The substitution of the less bulky and more hydrophobic valine results in a substrate-dependent alteration in the catalytic activity of GSTP1 (16, 17). GSTM1 and GSTP1 have been shown to efficiently conjugate various isothiocyanates (9, 18, 19), but less is known about the conjugating capacity or efficiency of the GSTT1 isoform (10, 20).

Despite the evidence suggesting a beneficial effect of cruciferous vegetables or isothiocyanates on the cardiovascular system (3–8), only 2 studies have examined the association between cruciferous vegetable intake and the risk of CHD (21, 22).

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Neither study found an association between cruciferous vegetables and the risk of CHD. However, an association between isothiocyanates and CHD may have been masked by genetic differences affecting the biotransformation of bioactive compounds such as isothiocyanates. Persons with a *GST* genotype corresponding to low activity may benefit from the protective effects of isothiocyanates more than would persons with a higher-activity genotype, because these compounds would have a slower rate of biotransformation and subsequent excretion. Alternatively, persons with a functional *GST* gene may benefit more, because the ability of cruciferous vegetables to induce detoxifying enzymes could facilitate the biotransformation and excretion of harmful substrates such as those found in tobacco smoke. The purpose of the present study was to determine whether *GST* genotypes modify the association between cruciferous vegetable intake and the risk of MI.

SUBJECTS AND METHODS

Study design and participants

The catchment area for this study comprised 7071 km^2 (2) and 2 057 000 persons living in Costa Rica who self-report as being Hispanic (23). This area included 36 counties in the Central Valley of Costa Rica; the population has a full range of socioeconomic levels and urban, peri-urban, and rural lifestyles. Medical services in this area were covered by 6 large hospitals, which are part of the National Social Security System. Eligible cases were men and women who were survivors of a first acute MI as diagnosed by a cardiologist at any of the 6 recruiting hospitals in the catchment area between 1994 and 2004. To achieve 100% ascertainment of cases, the hospitals were visited daily by the study fieldworkers. All cases were confirmed by 2 independent cardiologists according to the World Health Organization criteria for MI, which require typical symptoms plus either an elevation in cardiac enzyme concentrations or a diagnostic change on electrocardiogram (24). Enrollment was carried out while cases were in their hospital's step-down unit. Cases were ineligible if they died during hospitalization, were ≥75 y old on the day of their first MI, were physically or mentally unable to answer the questionnaire, or had a previous hospital admission related to cardiovascular disease.

One control for each case, matched for age (± 5 y), sex, and area of residence (county), was randomly selected by using information available at the National Census and Statistics Bureau of Costa Rica. Eligible controls were identified within 1 wk of the case selection. On average, complete data collection took 27 d for cases and 31 d for controls. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment areas had access to medical care without regard for income. Therefore, controls came from the same source population that gave rise to the cases and are not likely to have had cardiovascular disease that went undiagnosed because of poor access to medical care. Controls were ineligible if they were physically or mentally unable to answer the questionnaires or if they had a previous hospital admission related to cardiovascular disease.

The participation rate for eligible cases and controls was 98% and 88%, respectively. Information on diet was collected, the medical history and anthropometric measurements were collected, and biological specimens were obtained at the subjects' homes.

All cases and controls gave written informed consent. The study was approved by the Ethics Committees of the Harvard School of Public Health and the University of Costa Rica, the Office of Protection from Research Risk at the National Institutes of Health, and the Ethics Review Committee at the University of Toronto.

All data were collected by trained fieldworkers during an interview using 2 questionnaires consisting of closed-ended questions regarding smoking, sociodemographic characteristics, socioeconomic status, physical activity, diet, and medical history, including any use of medication and personal history of diabetes and hypertension. For cases, all data collected represented the year preceding their MI. Dietary intake was collected by using a 135-item semiquantitative food-frequency questionnaire (FFQ) specifically developed and validated to assess dietary intake during the previous year in the Costa Rican population (25). Intakes of nutrients were calculated by using the US Department of Agriculture food composition data file. A commonly used unit or portion size (eg, 1/2 cup broccoli) was specified for each food item, and subjects were asked to choose 1 of 9 categories of intake: never or <1 serving/mo, 1–3 servings/mo, 1 serving/wk, 2–4 servings/wk, 5–6 servings/wk, 1 serving/d, 2–3 servings/d, 4–5 servings/d, or ≥6 servings/d. Cruciferous vegetable intake was defined as the sum of broccoli, cauliflower, kale, and cabbage intakes and was recalculated relative to 1 serving/d.

Genotyping

Blood samples were collected in the morning at the subject's home after an overnight fast and were centrifuged (1430 × *g*, 4 min, 20 °C) to separate the plasma and leukocytes for DNA isolation by standard procedures. *GSTM1*, *GSTT1*, and *GSTP1* genotypes were assayed without knowledge of case or control status by using a previously described multiplex polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method that simultaneously detects the polymorphisms of all 3 genes in a single reaction (26). Approximately 1 ng DNA was amplified by thermal cycling with the use of the HotStar DNA polymerase kit (Qiagen, Mississauga, Canada) with PCR buffer containing 1.5 mmol $MgCl_2$/L, 0.2 mmol of each dNTP/L, 0.5 U Taq, and 8 pmol of each primer set. *GSTM1* primers included forward 5′-CTGCCCTACTTGATTGATC-3′ and reverse 5′-CTGGATTGTAGCAGATCATGC-3′. *GSTT1* primers included forward 5′-TTCCTTACTGGTCCTCACATCTC-3′ and reverse 5′-TCACCGGATCATGGCCAGCA-3′. *GSTP1* primers included forward 5′-TCCTTCCACGCACATC CTCT-3′ and reverse 5′-AGCCCCTTTCTTTGTTCAGC-3′ (26, 27). All primers were synthesized by ACGT (Toronto, Canada).

After an initial denaturation at 95 °C for 15 min, amplification was achieved by using a touchdown PCR protocol with 20 cycles of denaturation at 94 °C for 30 s, at 68–48 °C for 30 s (with a reduction of 1 °C in each cycle), and at 72 °C for 30 s, which were followed by 20 cycles with the annealing temperature set at 51 °C and a final extension at 72 °C for 10 min. After overnight restriction enzyme digestion with 2 U *Alw26I*, PCR products were resolved by 2% agarose gel electrophoresis and stained with ethidium bromide. Bands were visualized by using an ultraviolet imaging system (FluorChem; Alpha Innotech Corp, San Leandro, CA). *GSTM1* and *GSTT1* genotypes were determined by the presence or absence of a 275-bp (195 + 80 after digestion) or

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480-bp band, respectively. Because the *GSTM1* fragment contains a nonpolymorphic *Alw26I* restriction site, the 275-bp band that is amplified is digested into 195-bp and 80-bp fragments in all samples, providing a positive control for complete digestion. The 294-bp band represents *GSTP1*, and the Ile105Val (A→G substitution) polymorphism in this gene introduces an *Alw26I* restriction site that produces 234-bp and 60-bp bands after digestion. Amplification of *GSTP1* also serves as an internal control for the PCRs that have both the homozygous *GSTM1*0* and *GSTT1*0* genotypes. The *GSTP1* genotype distribution among controls was in Hardy-Weinberg equilibrium ($P = 0.94$). Deviations from Hardy-Weinberg equilibrium were not tested for distributions of *GSTM1* and *GSTT1* genotypes because the PCR assay does not discriminate heterozygotes from homozygotes for the functional allele. However, frequencies for the *GSTM1*0/*0* and *GSTT1*0/*0* genotypes were similar to frequencies previously reported in other populations (28–31).

Statistical analysis

All data were analyzed by using SAS software (version 8.2; SAS Institute, Cary, NC). DNA was available from 4369 subjects (2113 cases and 2256 controls). A total of 285 subjects were excluded because they had missing data on confounders (31 cases and 29 controls), they could not be genotyped (39 cases and 47 controls), or they became unmatched because of missing data (1 case and 138 controls); these exclusions left 2042 matched case-control pairs for the final analysis. Dietary variables were adjusted for total energy as described elsewhere (25, 32). Because of the matched design, significant differences in the distribution of categorical variables between cases and controls were tested by using McNemar's test, and significant differences in the distribution of continuous variables were tested by using either a paired *t* test or Wilcoxon's signed-rank test. Subjects were categorized into low, medium, and high cruciferous vegetable intake categories according to energy-adjusted tertiles of cruciferous vegetable intake created on the basis of the distribution of intake among control subjects. Chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables were used to determine significant differences across tertiles of cruciferous vegetable intake among controls.

Categorical and continuous nondietary and energy-adjusted dietary variables were assessed for potential confounding by measuring their effect on the model variable estimates by using the likelihood ratio test. These variables included smoking status (never, past, 1–9 cigarettes/d, or ≥10 cigarettes/d), alcohol consumption (never, past, or current by tertiles of intake), coffee consumption (≤1 cup/d, 1 cup/d, 2–3 cups/d, or ≥4 cups/d), history of diabetes (yes or no), history of hypertension (yes or no), and quintiles of the continuous variables waist-to-hip ratio, physical activity, income, and energy-adjusted intakes of saturated fat, *trans* fat, polyunsaturated fat, cholesterol, protein and folate. Odds ratios (ORs) and Wald 95% CIs were estimated by using conditional logistic regression to determine the effect of cruciferous vegetable intake and *GST* genotype on the risk of MI. Genotypes and tertiles of intake were modeled by using indicator variables in logistic models, with the wild-type of each gene or the lowest tertile of intake as the reference. Tests of linear trend across increasing tertiles of cruciferous vegetable intakes were conducted by assigning the medians of intakes in tertiles (servings/d) as a continuous variable. Confounders included in the final models were smoking, alcohol, history of diabetes, history of hypertension, waist-to-hip ratio, physical activity, income, and folate and saturated fat intakes. We evaluated potential gene-diet interactions by determining the relation between tertiles of cruciferous vegetable intake and the risk of MI for each genotype with the use of conditional and unconditional logistic regression (with matching variables in the model) and by comparing −2 log (likelihood) ratios from a model with cruciferous vegetable intakes and gene main effects only with those from another model that included their interaction term. Because results for conditional and unconditional analyses were similar, we report only the data from unconditional analyses to maximize the number of subjects. All statistical analyses were 2-sided, and *P* values < 0.05 were considered significant.

RESULTS

Demographic and risk factor characteristics of subjects based on case-control status and data on cruciferous vegetable intake among controls are presented in **Table 1**. Median cruciferous vegetable intakes for the first (low), second (medium), and third (high) tertiles were 0.08, 0.43, and 0.86 servings/d, respectively. Only high consumption of cruciferous vegetables was associated with a significant reduction in risk of MI. Compared with low cruciferous vegetable intake, the OR (95% CI) of MI associated with medium and high intakes was 0.92 (0.79,1.06) and 0.69 (0.59,0.80), respectively ($P < 0.001$ for trend). Corresponding multivariate-adjusted ORs (95% CI) were 1.05 (0.88, 1.25) and 0.83 (0.69,0.99) ($P = 0.04$ for trend).

The *GSTM1*0/*0* genotype occurred in 48% of cases and 51% of controls, and the *GSTT1*0/*0* genotype occurred in 19% of cases and 20% of controls. The frequency of the *GSTP1 Val* allele was 40% for both cases and controls. Compared with the *GSTM1*1* allele, the multivariate adjusted OR (95% CI) of MI associated with the *GSTM1*0/*0* genotype was 0.86 (0.74, 0.99). Compared with the *GSTT1*1* allele, the adjusted OR (95% CI) of MI associated with the *GSTT1*0/*0* genotype was 0.99 (0.83, 1.18; NS). Compared with persons with the *GSTP1 Ile/Ile* genotype, the adjusted OR (95% CI) of MI was 0.99 (0.85, 1.15; NS) for those with the *Ile/Val* genotype and 0.96 (0.77, 1.18; NS) for those with the *Val/Val* genotype ($P = 0.89$ for trend). Similar results were obtained when nonsmokers and current smokers were examined separately (data not shown). The effect of combined genotypes was also examined, but no significant gene × gene interactions were observed (data not shown).

When the association between cruciferous vegetable intake and MI was evaluated by *GSTM1*, *GSTT1*, or *GSTP1* genotypes (**Table 2**), a significant *GSTT1* × diet interaction was observed. Compared with the lowest level of cruciferous vegetable intake, the adjusted OR (95% CI) for the highest level of intake was 0.70 (0.58, 0.84) in persons with the *GSTT1*1* allele and 1.23 (0.83, 1.82; NS) in persons with the *GSTT1*0/*0* genotype. No significant interaction between cruciferous vegetable intake and *GSTM1* or *GSTP1* genotype was observed.

In addition to being substrates of GSTs, isothiocyanates are inducers of these enzymes, which enhance the biotransformation and subsequent elimination of mutagens (3). Therefore, we examined whether *GST* genotypes modify the risk of MI associated with cruciferous vegetable intake separately for nonsmokers and current smokers (**Table 3**). The protective effect of cruciferous vegetables was greater among current smokers who had the *GSTT1*1* allele (OR: 0.54; 95% CI: 0.36, 0.79) than among

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TABLE 1
Demographic and risk factor characteristics by case or control status and cruciferous vegetable intake among controls[1]

Characteristic	Cases ($n = 2042$)	Controls ($n = 2042$)	Cruciferous vegetable intake (servings/d, median) Low (0.08)	Medium (0.43)	High (0.86)	P^2
Age (y)[3]	58.4 ± 11.1[4]	58.1 ± 11.3	58.7 ± 11.6	56.8 ± 11.6	58.8 ± 10.7	<0.001
Male (%)[3]	74	74	80	77	65	<0.001
Urban residence (%)[3]	74	74	71	74	76	
Secondary education or higher (%)	36	40[5]	33	45	43	<0.001
Household income (US $/mo)	499 ± 389	572 ± 426[5]	497 ± 378	589 ± 413	625 ± 470	<0.001
Waist-to-hip ratio	0.97 ± 0.07	0.95 ± 0.07[5]	0.96 ± 0.07	0.95 ± 0.07	0.95 ± 0.07	0.003
Physical activity (METS)	1.51 ± 0.70	1.56 ± 0.69[5]	1.61 ± 0.75	1.56 ± 0.67	1.50 ± 0.63	0.008
History of hypertension (%)	39	30[5]	29	26	34	0.002
History of diabetes (%)	24	14[5]	14	11	15	
Current smokers (%)[6]	40	21[5]	24	23	17	<0.001
Current alcohol drinker (%)	49	53[5]	51	56	51	
Total energy (kcal)	2710 ± 948	2453 ± 761[5]	2641 ± 930	2565 ± 692	2161 ± 516	<0.001
Carbohydrate (% of energy)	54.3 ± 7.6	55.4 ± 7.3[5]	55.7 ± 7.7	55.0 ± 7.0	55.6 ± 7.2	
Protein (% of energy)	13.2 ± 2.2	12.9 ± 2.1[5]	12.7 ± 2.2	12.9 ± 1.9	13.2 ± 2.2	<0.001
Fat (% of energy)	32.4 ± 5.9	31.9 ± 5.8[5]	31.6 ± 6.2	32.2 ± 5.6	31.7 ± 5.7	
Saturated fat (% of energy)	12.5 ± 3.2	11.7 ± 2.9[5]	12.0 ± 3.2	11.8 ± 2.8	11.2 ± 2.7	<0.001
Polyunsaturated fat (% of energy)	6.9 ± 2.3	7.1 ± 2.3[5]	6.7 ± 2.5	7.3 ± 2.3	7.3 ± 2.2	<0.001
Monounsaturated fat (% of energy)	11.2 ± 3.5	11.2 ± 4.0	11.0 ± 4.0	11.3 ± 3.9	11.3 ± 4.2	
trans Fat (% of energy)	1.3 ± 0.6	1.3 ± 0.6	1.3 ± 6.4	1.3 ± 0.6	1.3 ± 0.6	
Cholesterol (mg/1000 kcal)	126.8 ± 58.3	117.4 ± 51.8[5]	121.9 ± 59.5	117.2 ± 46.7	113.4 ± 48.0	0.01
Sucrose (g/d)	80.1 ± 50.7	75.2 ± 43.3[5]	83.4 ± 53.3	77.9 ± 39.5	64.7 ± 32.7	<0.001
Fiber (g/1000 kcal)	9.5 ± 2.4	10.0 ± 2.5[5]	9.5 ± 2.4	9.7 ± 2.4	10.7 ± 2.4	<0.001
Folate (μg/1000 kcal)	169.2 ± 46.1	174.7 ± 46.3[5]	168.3 ± 48.0	171.3 ± 44.1	184.2 ± 45.2	<0.001
*GSTM1*0/*0* (%)	48	51	50	52	51	
*GSTT1*0/*0* (%)	19	20	22	19	19	
GSTP1 Val (%)	40	40	38	40	40	0.02

[1] METS, metabolic equivalent tasks.
[2] ANOVA for continuous variables and chi-square test for categorical variables.
[3] Matching variable.
[4] $\bar{x}$ ± SD (all such values).
[5] Significantly different from cases ($P < 0.05$), based on McNemar's test for categorical variables and paired *t* tests or Wilcoxon signed-ranked tests for continuous variables.
[6] ≥1 Cigarette/d.

nonsmokers who had this allele (OR: 0.78; 95% CI: 0.63, 0.98; $P = 0.008$ for GSTT1 × diet × smoking interaction). Regardless of smoking status, no significant interactions were observed between *GSTM1* or *GSTP1* genotype and the risk of MI (data not shown). Similar results were observed when data were analyzed separately for men and women (data not shown).

DISCUSSION

Cruciferous vegetables are a rich source of dietary glucosinolates, which are hydrolyzed to biologically active compounds including isothiocyanates. Despite evidence suggesting a protective effect of these compounds on the cardiovascular system (3–6), only 2 studies have examined the association between cruciferous vegetable intake and risk of CHD (21, 22), and neither of those studies found an association. To our knowledge, the present study is the first to examine the association between cruciferous vegetable intake and the risk of MI. Isothiocyanates derived from glucosinolates present in cruciferous vegetables induce GSTs and other enzymes to enhance detoxifying capacity (3–6). After consumption, isothiocyanates are conjugated by GSTs and eliminated. The effect of cruciferous vegetables on CHD may, therefore, be modified by GST genotype.

Because of the biological interaction between GST and isothiocyanates, persons with a functional *GSTM1* or *GSTT1* allele may benefit more from the detoxifying enzyme–inducing properties of cruciferous vegetables than may persons who lack these alleles. Alternatively, persons with a GST genotype corresponding to low activity may benefit from the protective effects of isothiocyanates more than may those with a higher activity genotype because these compounds could remain in the body longer as a result of a slower rate of biotransformation and subsequent excretion. Our results show a protective effect of cruciferous vegetables only among those with the functional *GSTT1*1* allele, which is consistent with the former hypothesis. As a result of cruciferous vegetables inducing the functional *GSTT1*1* allele, *GSTT1*1* carriers may be more protected against oxidative stress or DNA damage. There is growing evidence that reactive oxygen species and DNA damage caused by mutagens present in the environment or diet play a role in the development of CHD (33, 34). Thus, the importance of *GSTT1* in detoxifying mutagenic compounds may be more important than its role in eliminating beneficial compounds such as isothiocyanates. The more pronounced protective effect of cruciferous vegetables that we observed among current smokers is consistent

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TABLE 2
Cruciferous vegetable intake and risk of myocardial infarction by *GST* genotype

Cruciferous vegetable intake	Cases	Controls	Model 1[1]	Model 2[2]
		n (%)		
GSTM1				
**1/*1 *1/*0*				
Low	378 (36)	340 (34)	1.00[3]	1.00[4]
Medium	373 (35)	324 (32)	1.03 (0.84, 1.28)	1.06 (0.84, 1.32)
High	303 (29)	343 (34)	0.78 (0.63, 0.97)	0.83 (0.65, 1.05)
**0/*0*				
Low	393 (40)	334 (32)	1.00	1.00
Medium	341 (34)	350 (34)	0.83 (0.67, 1.02)	0.92 (0.73, 1.15)
High	254 (26)	351 (34)	0.61 (0.49, 0.76)	0.75 (0.59, 0.95)
GSTT1				
**1/*1 *1/*0*				
Low	639 (39)	527 (32)	1.00	1.00[5]
Medium	582 (35)	549 (34)	0.88 (0.74, 1.03)	0.94 (0.79, 1.12)
High	428 (26)	560 (34)	0.63 (0.52, 0.74)	0.70 (0.58, 0.84)
P for trend			<0.001	0.10
**0/*0*				
Low	132 (34)	147 (36)	1.00	1.00
Medium	132 (34)	125 (31)	1.16 (0.82, 1.63)	1.23 (0.84, 1.80)
High	129 (32)	134 (33)	1.05 (0.74, 1.48)	1.23 (0.83, 1.82)
P for trend			0.81	0.02
GSTP1				
Ile/Ile				
Low	290 (39)	260 (35)	1.00	1.00[6]
Medium	260 (35)	255 (34)	0.92 (0.72, 1.17)	1.01 (0.78, 1.31)
High	194 (26)	230 (31)	0.77 (0.60, 1.00)	0.84 (0.64, 1.11)
Ile/Val				
Low	362 (37)	311 (32)	1.00	1.00
Medium	351 (35)	304 (31)	0.99 (0.80, 1.23)	1.06 (0.84, 1.34)
High	280 (28)	370 (37)	0.63 (0.50, 0.78)	0.72 (0.57, 0.92)
Val/Val				
Low	119 (39)	103 (33)	1.00	1.00
Medium	103 (34)	115 (37)	0.77 (0.53, 1.12)	0.80 (0.52, 1.23)
High	83 (27)	94 (30)	0.71 (0.47, 1.07)	0.81 (0.51, 1.29)

[1] Results from unconditional logistic regression that included matching variables (age, sex, and area of residence).
[2] Model 1 plus adjustments for smoking, waist-to-hip ratio, income, physical activity, history of diabetes and hypertension, intake of alcohol, and energy-adjusted saturated fat and folate.
[3] OR; 95% CIs in parentheses (all such values).
[4] *GSTM1* × diet interaction, $P = 0.59$.
[5] *GSTT1* × diet interaction, $P = 0.006$.
[6] *GSTP1* × diet interaction, $P = 0.88$.

with this hypothesis. It is possible that *GSTT1* is not the major GST isoform responsible for the biotransformation of the isothiocyanates present in the cruciferous vegetables commonly eaten in this population. Cruciferous vegetables differ in their composition of glucosinolates (35), which could explain the inconsistency of the effect of *GSTT1* genotype on human isothiocyanate metabolism (10, 20). Seow et al (20) reported that persons with the *GSTT1*0/*0* genotype excrete isothiocyanates more slowly than do those with the *GSTT1*1* allele, whereas no differences were observed between *GSTM1* and *GSTP1* genotypes. However, Fowke et al (10) examined the same GST isoforms and found a positive association between cruciferous vegetable intake and urinary isothiocyanate excretion only among persons with the *GSTP1 Ile/Ile* genotype.

In our population, the frequency of the *GSTT1*1* allele is 80%. Because the prevalence of this allele has been reported to vary between 36% and 90% (31), future studies examining the association between cruciferous vegetables and risk of CHD should account for genetic differences in GSTT1. A positive association between cruciferous vegetables and risk of CHD may be more evident in populations in whom the *GSTT1*1* allele is more common. The significant *GSTT1* × diet interaction observed in the current study is not likely to be due to residual confounding. Although variables not accounted for may potentially confound the main effect of cruciferous vegetable consumption on risk of MI, that would not explain the significant protective effect observed in one genotype but not in the other, because a potential confounder is unlikely to be differentially distributed by genotype.

Several studies have examined the effect of *GSTT1* genotype on risk of CHD, but the findings have been equivocal (29, 30, 36, 37). None of these studies, however, included data on cruciferous vegetable intake, and substantial differences in the average consumption of these vegetables exist between countries. For example, Chinese Singaporeans consume ≈1 serving of cruciferous

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TABLE 3
Cruciferous vegetable intake and risk of myocardial infarction by *GSTT1* genotype and smoking status

Cruciferous vegetable intake	Cases	Controls	Model 1[1]	Model 2[2]
		n (%)		
Never + past smokers				
GSTT1[3]				
**1/*1 *1/*0*				
Low	349 (35)	405 (31)	1.00[4]	1.00
Medium	340 (34)	424 (33)	0.95 (0.78, 1.17)	1.00 (0.81, 1.23)
High	305 (31)	468 (36)	0.76 (0.61, 0.93)	0.78 (0.63, 0.98)
P for trend			0.007	0.02
**0/*0*				
Low	63 (27)	105 (34)	1.00	1.00
Medium	84 (35)	93 (30)	1.47 (0.95, 2.26)	1.47 (0.91, 2.37)
High	89 (38)	110 (36)	1.26 (0.82, 1.94)	1.33 (0.82, 2.15)
P for trend			0.36	0.27
Current smokers				
GSTT1				
**1/*1 *1/*0*				
Low	290 (44)	122 (36)	1.00	1.00
Medium	242 (37)	125 (37)	0.79 (0.58, 1.07)	0.89 (0.64, 1.24)
High	123 (19)	92 (27)	0.48 (0.33, 0.68)	0.54 (0.36, 0.79)
P for trend			<0.001	0.001
**0/*0*				
Low	69 (44)	42 (43)	1.00	1.00
Medium	48 (31)	32 (33)	0.89 (0.49, 1.64)	0.83 (0.41, 1.68)
High	40 (25)	24 (24)	1.05 (0.54, 2.02)	1.11 (0.50, 2.50)
P for trend			0.93	0.61

[1] Results from unconditional logistic regression that included matching variables (age, sex, and area of residence).
[2] Model 1 plus adjustments for cigarettes/d (current smokers), waist-to-hip ratio, income, physical activity, history of diabetes and hypertension, intake of alcohol, and energy-adjusted saturated fat and folate.
[3] *GSTT1* × diet × smoking interaction, $P = 0.008$.
[4] OR; 95% CIs in parentheses (all such values).

vegetables/d, which is >3 times the average intake of 2 servings/wk in the United States (20, 38). Differences in cruciferous vegetable intake may explain some of the inconsistencies among studies examining *GSTT1* genotype and risk of CHD.

The lower risk of MI that we observed with the *GSTM1*0/*0* genotype is consistent with 2 previous studies that examined the association between this gene and the risk of MI (30, 36) and with a study reporting a higher proportion of control subjects with a low-activity GSTM1 phenotype than of patients with atherosclerosis (39). Because no *GSTM1* × diet interaction was observed in the present study, the role of *GSTM1* in isothiocyanate metabolism is unlikely to explain the protective effects of the *GSTM1*0/*0* genotype. Furthermore, the role of *GSTM1* appears to be unrelated to mutagen detoxification, because we would have expected the *GSTM1*0/*0* genotype to be associated with a greater risk among smokers than among nonsmokers. Thus, the lower risk of MI associated with the *GSTM1*0/*0* genotype suggests that beneficial compounds—other than isothiocyanates that are metabolized by GSTM1—may protect against MI. In contrast to reports of a protective effect of the *GSTM1*0/*0* genotype on risk of MI, other studies have reported either no effect (40) or a greater risk (29, 37) associated with this genotype. Inconsistencies among these studies may be related to the dual role of *GSTM1* in eliminating both harmful mutagens from the environment and potentially beneficial compounds found in the diet.

In summary, consumption of cruciferous vegetables was associated with a lower risk of MI only among those with a functional *GSTT1*1* allele. These results suggest that cruciferous vegetable intake may protect against MI through the ability of those vegetables to induce detoxifying enzymes. The protective effect of the *GSTM1*0/*0* genotype observed in the present study merits further investigation.

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