CYP1A2, GSTM1, and *GSTT1* Polymorphisms and Diet Effects on CYP1A2 Activity in a Crossover Feeding Trial

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Abstract

Cytochrome P-450 1A2 (CYP1A2) is a biotransformation enzyme that activates several procarcinogens. CYP1A2 is induced by cruciferous and inhibited by apiaceous vegetable intake. Using a randomized, crossover feeding trial in humans, we investigated the dose effects of cruciferous vegetables and the effects of any interaction between cruciferous and apiaceous vegetables on CYP1A2 activity. We also investigated whether response varied by *CYP1A2*1F, GSTM1*, and *GSTT1* genotypes (glutathione *S*-transferases that metabolize crucifer constituents) and whether CYP1A2 activity rebounds after apiaceous vegetables are removed from the diet. Participants (*N* = 73), recruited based on genotypes, consumed four diets for two weeks each: low-phytochemical diet (basal), basal plus single dose of cruciferous (1C), basal plus double dose of cruciferous (2C), and basal plus single dose of cruciferous and apiaceous vegetables (1C+A). CYP1A2 activity was determined by urine caffeine tests administered at baseline and the end of each feeding period. Compared with basal diet, the 1C diet increased CYP1A2 activity ($P < 0.0001$) and the 2C diet resulted in further increases ($P < 0.0001$), with men experiencing greater dose-response than women. The 1C+A diet decreased CYP1A2 activity compared with the 1C and 2C diets ($P < 0.0001$ for both). Although there was no overall effect of *CYP1A2*1F* or *GSTM1-null/GSTT1-null* genotypes or genotype-by-diet interactions, there were significant diet response differences within each genotype. Additionally, CYP1A2 activity recovered modestly one day after the removal of apiaceous vegetables. These results suggest complex interactions among dietary patterns, genetic variation, and modulation of biotransformation that may not be apparent in observational studies. (Cancer Epidemiol Biomarkers Prev 2009;18(11):3118–25)

Introduction

Cytochrome P-450 1A2 (CYP1A2) is a biotransformation enzyme that mediates the activation of several procarcinogens (1-3). Due to the major role of biotransformation enzymes in the metabolism of carcinogens and other xenobiotics, variation in biotransformation-enzyme activity may influence carcinogenesis. CYP1A2 activity in humans is influenced by genetic variation and environmental factors resulting in wide interindividual variability (reviewed in ref. 4). *CYP1A2*1F* (rs762551) is a polymorphism in intron 1 (−164A>C) that confers decreased inducibility (5), thus variation in CYP1A2 activity by *CYP1A2*1F* genotype may be most pronounced in the presence of inducers.

Cruciferous (i.e., broccoli-family) vegetable consumption increases CYP1A2 activity in humans (6, 7). Isothiocyanates and indoles (both metabolites of glucosinolates in cruciferous vegetables) have been evaluated for CYP1A2 induction in many test systems. The results have been inconsistent. In primary human-derived test systems, however, phenethyl isothiocyanate and the indole derivative 3,3′-diindolylmethane consistently induce CYP1A2 (8, 9), whereas sulforaphane, another isothiocyanate, has no effect (8).

Isothiocyanate metabolism involves conjugation with glutathione by glutathione *S*-transferases (GST) M1 and T1 (GSTM1 and GSTT1; ref. 10). In Caucasians, approximately 50% and 20% have null genotypes for *GSTM1* and *GSTT1*, respectively, resulting in a lack of the enzymes; hence *GSTM1-null* and *GSTT1-null* individuals have been hypothesized to experience a longer exposure to glucosinolate metabolites due to slower metabolism (11, 12). Epidemiologic studies in Asia and Europe show that the chemopreventive association of cruciferous intake is more pronounced among individuals with the *GSTM1/GSTT1* null genotypes (12-14), but studies in the United States show the opposite relationship (15, 16) or no relationship (17, 18). Recent feeding studies with broccoli indicate that individuals with the null genotypes had greater isothiocyanate excretion, supporting the US findings that *GST*-positive individuals have greater protection (19, 20) but not supporting the hypothesis that null-genotype individuals have delayed clearance. Moreover, indole metabolism may involve glucuronidation (21), a process mediated by isothiocyanate- and indole-inducible UDP-glucuronosyltransferases, further suggesting a potential influence on CYP1A2 activity by *GSTM1* and *GSTT1* genotype.

Intake of apiaceous (i.e., carrot-family) vegetables decreases CYP1A2 activity in humans (6). Apiaceous

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vegetables are rich in furanocoumarins (22, 23), which inhibit human CYP1A2 *in vitro* and *in vivo* (24-26). Furanocoumarins undergo extensive first-pass metabolism (27, 28); within 24 hours of ingestion, 95% of two predominant furanocoumarins are excreted as a series of glucuronic acid conjugates in the urine (28).

Our primary objectives were: (*a*) to test whether there is a dose-response effect of cruciferous vegetables on CYP1A2 activity; (*b*) to test whether concurrent consumption of cruciferous and apiaceous vegetables reduces CYP1A2 activity; and (*c*) to establish the effect of *CYP1A2*1F* genotype on induction or inhibition of CYP1A2 activity in response to vegetable supplementation. Our secondary aims were to measure the combined effect of *GSTM1* and *GSTT1* genotypes on CYP1A2 activity and to test whether CYP1A2 activity rebounds after apiaceous vegetables are removed from a diet with concurrent exposure to CYP1A2 inducers.

Subjects and Methods

Subjects. Participants were recruited from the Seattle area through media advertisements, flyers, and direct mailings. Subjects were 20 to 40 y old, healthy, and nonsmokers. Initial eligibility was determined by a self-administered screening survey. Exclusion criteria were history of gastrointestinal, hepatic, or renal disorders; pregnancy or lactation; allergies or intolerances to any food used in the feeding trial; weight change >4.5 kg within the past year; major changes in eating habits within the past year; antibiotic use within the past 3 mo; body weight >150% of desirable; exercise patterns that result in major changes in diet; current use of prescription medication, including contraception; current use of over-the-counter medications; regular exposure to passive or second-hand smoke; occupational exposure to smoke or organic solvents; dislike of any food in the trial that would preclude participation; alcohol intake >2 drinks/d (2 drinks is equivalent to 720 mL beer, 240 mL wine, or 90 mL spirits); and no interest in participating in a controlled feeding trial. Final eligibility was based on *CYP1A2*1F, GSTM1*, and *GSTT1* genotypes. The goal was to recruit participants into a 3 × 3 design according to the three groups of *CYP1A2*1F* genotypes (A/A, A/C, and C/C) and three groups of *GST* genotypes (*GSTM1+/GSTT1+*, *GSTM1-null/GSTT1+*, and *GSTM1-null/GSTT1-null*), with an equal number of men and women in each group. We randomized 73 individuals: 14 A/A men, 17 A/A women, 15 A/C men, 15 A/C women, 6 C/C men, and 6 C/C women. Of the 73, 14 men were *GSTM1+/GSTT1+*, 12 women were *GSTM1+/GSTT1+*, 16 men were *GSTM1-null/GSTT1+*, 15 women were *GSTM1-null/GSTT1+*, 5 men were *GSTM1-null/GSTT1-null*, 9 women were *GSTM1-null/GSTT1-null*, and 2 women were *GSTM1+/GSTT1-null* (recruited for their *CYP1A2* genotype only). Nine initially randomized participants dropped out of the study after baseline data were collected but before completing one feeding period. New recruits were selected and randomized into the appropriate treatment orders to maintain the initial blocks. The study activities were approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center. Informed written consent was obtained from all participants prior to the start of the study.

Determination of Genotypes. After initial eligibility was determined, participants were mailed buccal cell collection kits. Genotypes were determined as buccal cells and consents for genotyping were returned. Buccal cell DNA was extracted using the QIAamp DNA Blood Midi kit (Qiagen). The *CYP1A2*1F* polymorphism was analyzed using a PCR/restriction fragment length polymorphism method. At pH 8.3, a 20 μL reaction contained 100 ng genomic DNA, 10 mmol/L Tris HCL, 1.25 mmol/L $MgCl_2$, 50 mmol/L KCl, 200 μmol/L dNTPs, 1.25 U Taq DNA polymerase, and 0.2 μmol/L each primer (5′ CAA CCC TGC CAA TCT CAA GCA C 3′ and 5′ AGA AGC TCT GTG GCC GAG AAG G 3′). Thermal cycling included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 94°C for 1 min, 62°C for 30 s, 72°C for 1 min, and 1 cycle of 72°C for 5 min. The amplicon was digested with 5 Units Apa I for 2 h and resolved by a 3% agarose gel. The wild-type A allele was cleaved into 708 and 212 bp fragments, whereas the variant C allele remained intact. *GSTM1* and *GSTT1* genotyping was conducted using PCR as previously described (29).

Study Design. Before starting the study, participants completed a 4-d baseline study wherein they followed their habitual diet, kept 3-d food records (instructions provided by a dietitian), completed a 24-h urine collection from day 3 to day 4, and came to the center on day 4 for the first urine caffeine test (a measure of CYP1A2 activity). After the baseline study, each participant received all four experimental diets using a crossover design in a randomly assigned Latin Square sequence. Randomization of participants was blocked on *CYP1A2*1F*-by-sex and *GSTM1/GSTT1*-by-sex. Each diet was consumed for 2 wk; the menu was a 3-d dinner rotation with the same breakfast and lunch daily to minimize variability. Diet periods began on Sundays with dinner and ended on Saturdays (day 14) with breakfast and measurement of CYP1A2 activity. There was a minimum 3-wk washout period in between each 2-wk feeding period; participants followed their habitual dietary patterns during the washout period.

Study Diets. Participants received four controlled diets and were assessed for adherence as described previously (29). Briefly, the diets were a basal diet low in phytochemicals (devoid of fruits and vegetables), basal plus ~7 g/kg body weight of cruciferous vegetables (1C), basal plus ~14 g/kg body weight of cruciferous vegetables (2C), and basal plus ~7 g/kg body weight of cruciferous vegetables and ~4 g/kg body weight of apiaceous vegetables (1C+A). Vegetable doses were based on our previous data (6), but were modified to provide dosing by body weight. The cruciferous vegetables used were frozen broccoli and cauliflower, fresh daikon radish sprouts, and raw shredded cabbage (red and green). The apiaceous vegetables used were frozen carrots and fresh celery, dill, parsley, and parsnips. All frozen vegetables and other prepackaged foods were purchased in case lots to minimize variability. Fresh foods were purchased from the same local vendor. One week prior to and during each feeding period, subjects were asked to avoid the following: alcoholic beverages, prescription and over-the-counter medications, and nutritional supplements (including herbal products).

To test for rebound in CYP1A2 activity after vegetables were removed from the diet, 36 participants received 1 d

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of the basal diet added to the end of the 1C diet period and to the 1C+A diet period. Thus, participants were exposed to phytochemicals for 13 consecutive d followed by 1 d of foods low in phytochemicals with CYP1A2 activity measured on day 14 and day 15.

The energy needs for each participant were assessed by comparing the results of the Harris-Benedict equation (30), Mifflin-St. Jeor equation (31), and 3-d food records. Physical activity, using metabolic equivalent intensity levels (32), was considered during assessment of calorie needs and was based on a 1-wk activity log that participants turned in on day 4 of the baseline study. Body weight was monitored throughout each diet period; daily energy intakes were adjusted accordingly using basal diet foods to maintain weight.

Urine Collection and Analysis. CYP1A2 is the primary enzyme involved in the first two steps of caffeine metabolism; hence, CYP1A2 activity is typically measured by urine caffeine metabolite ratios (5, 33). Thus, on day 7 and day 14 (and on day 15 of the 1C and the 1C+A diet periods for 36 individuals), participants completed a urine caffeine test for the measurement of CYP1A2 activity. Arriving at the center in the morning after an overnight 10-h fast, they consumed 200 mg caffeine (NoDoz, Bristol-Meyers Squibb) with 30 mL water. Participants ate a light breakfast (42-64 g ready-to-eat cereal, 240 mL milk, 122 g of a commercial fruit drink made with 10% fruit juice) and remained at the center for the next 5 h, during which additional food and fluids were withheld. At hour 4, participants were asked to void and then at hour 5 to void again and collect their urine. The total volume and pH of each urine sample were measured. The pH was adjusted to 3.5 with 1 mol/L HCl, and 1.8 mL aliquots were stored at −70°C until time of analysis.

Acidified urine samples were thawed and centrifuged prior to analysis. Three hundred microliters of ammonium sulfate were added to 500 μL of centrifuged urine containing 50 μg/mL of internal standard, 4-acetamidophenol (APAP). The samples were vortexed and sat for 1 h at room temperature prior to extraction to assure complete dissolution of the ammonium sulfate. The urines were extracted with 2 × 3.0 mL chloroform:isopropanol, 9:1. After each addition of the extraction solvent, the samples were vortexed for 1 min and centrifuged for 15 min at 540× *g*. The organic layers from both extractions were combined and dried under nitrogen in a 45°C water bath. The residue was reconstituted with 500 μL of 0.05% acetic acid, sonicated for 30 s, and vortexed. This solution was filtered through a 0.45-μm Whatman mini-Uni-prep filter device (Whatman Inc.) and placed directly into the sampling tray of the high performance liquid chromatography device for analysis.

Samples were analyzed for the following caffeine metabolites using an Agilent Model 1100 series HPLC (Agilent Technologies): 5-acetylamino 6-formylamino 3-methyluric acid (AFMU), 7-methylxanthine (7X), 1-methyluric acid (1U), 3-methylxanthine (3X), 1-methylxanthine (1X), 1,3-dimethyluric acid (13U), 3,7-dimethylxanthine (37X), 1,7-dimethyluric acid (17U), 1,7-dimethylxanthine (17X), 1,3-dimethylxanthine (13X), and 1,3,7-trimethylxanthine (137X, caffeine), with APAP as an internal standard. The purified standard materials were all purchased from Sigma-Aldrich with the exception of AFMU, which was purchased from Toronto Research Chemicals Inc., and 17U, which was purchased from Fluka Chemical Corp. Urine samples were batched to include all samples for one study subject in the same high performance liquid chromatography run with quality control samples included at the beginning and end of each batch. A 7-point calibration curve over a range of 0.25 to 50 mg/mL for each metabolite was analyzed with each batch of samples. Correlation coefficients for the standard curves were ≥0.99. Separation was done on a 5-μm Ultrasphere ODS C18 column 4.6 × 250 mm (Beckman) using a gradient elution. The mobile phase consisted of methanol (A), acetonitrile (B), and 0.05% acetic acid (D) with the following elution profile: 0 to 14 min 8% A, 92% D; 14 to 20 min ramp from 8% A to 19% A and 92% D to 81% D, hold for 3 min; 23 to 30 min 10% A, 8% B, 82% D; 30 to 36 min ramp from 8% B to 50% B and from 10% A to 50% A, hold for 5 min; 41 to 42 min return to starting conditions 8% A, 92% D and hold to the end of the run at 52 min. The flow rate was 1.1 mL/min throughout the entire run. Caffeine and its metabolites were identified based on retention times of the primary standards, peak purity, and spectral analysis. Inter-assay precision

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Table 1. Distribution of selected characteristics of study participants consuming controlled diets

	Women			Men		
	*CYP1A2*1F*			*CYP1A2*1F*		
	A/A	A/C	C/C	A/A	A/C	C/C
	n = 17	*n* = 15	*n* = 6	*n* = 14	*n* = 15	*n* = 6
Age, y*	29.3 ± 5.7	28.6 ± 5.7	26.6 ± 3.9	33.2 ± 6.3	28.6 ± 6.9	34.4 ± 5.1
Height, m*	1.62 ± 0.07	1.63 ± 0.09	1.63 ± 0.07	1.76 ± 0.07	1.79 ± 0.07	1.73 ± 0.06
Weight, kg*	61.6 ± 11.1	58.8 ± 8.4	61.7 ± 10.5	80.3 ± 11.3	78.2 ± 14.8	79.0 ± 11.1
Body mass index, kg/m*	23.3 ± 3.4	22.2 ± 2.2	23.2 ± 3.0	25.9 ± 3.0	24.3 ± 3.6	26.3 ± 3.5
GSTM1-null/GSTT1-null, *n* (%)	5 (29.4)	4 (26.7)	0	2 (14.0)	3 (20.0)	0
GSTM1-null/GSTT1+, *n* (%)	6 (35.3)	6 (40.0)	3 (50.0)	6 (43.0)	7 (46.7)	3 (50.0)
GSTM1+/GSTT1+, *n* (%)	6 (35.3)	5 (33.3)	1 (16.7)	6 (43.0)	5 (33.3)	3 (50.0)
GSTM1+/GSTT1-null, *n* (%)	0	0	2 (33.3)	0	0	0
Race						
Caucasian, *n* (%)	11 (64.7)	6 (40.0)	4 (66.7)	9 (64.3)	10 (66.7)	3 (50.0)
Non-Caucasian, *n* (%)	6 (35.3)	9 (60.0)	2 (33.3)	5 (35.7)	5 (33.3)	3 (50.0)

*Mean ± SD.

Table 2. Average daily energy and macronutrient intake of 73 individuals consuming controlled diets

	Basal	1C	2C	1C+A
Energy (kcal)	2,403 ± 38	2,408 ± 38	2,444 ± 38	2,422 ± 38
Carbohydrate (g)	336 ± 5	339 ± 5	354 ± 6*	339 ± 6
Protein (g)	90 ± 2*	94 ± 2	99 ± 2†	95 ± 2
Fat (g)	78 ± 1	79 ± 1	77 ± 2	81 ± 2‡
Dietary fiber§ (g)	10.1 ± 0.5	19.4 ± 0.5	30.7 ± 0.5	26.8 ± 0.5

NOTE: The values are least squares means ± SE.
*The difference from each of the other three diets is statistically significant ($P < 0.001$).
†The difference from 1C and 1C+A is statistically significant ($P < 0.001$).
‡The difference from basal and 2C is statistically significant ($P < 0.05$).
§All diets are different from each other ($P < 0.0001$).

(% coefficient of variation) for the quality control samples was as follows: AFMU 6.8%, 1U 15.6%, 1X 3.7%, 3X 12.2%, 7X 6.4%, 13U 15.1%, 13X 10.7%, 17X 3.3%, 17U 4.0%, 37X 8.1%, and 137X 4.6%. Using peak heights, metabolites were quantitated based on standard curves.

Several different caffeine metabolite ratios are cited in the literature for assessment of CYP1A2 activity (5, 6, 33, 34). We used the ratio (1U + 1X + AFMU)/17U because it is the least influenced by urine flow and most sensitive to changes in CYP1A2 activity (35-37).

Additionally, participants collected their urine for 24 h starting on day 13 for assessment of urinary isothiocyanates (29, 38). Urinary isothiocyanate excretion was used as one method for confirming dietary compliance.

Statistical Analysis. Differences in CYP1A2 activity were analyzed using a linear mixed regression model (ref. 39; PROC MIXED in SAS, Release 9.0). Study participant was a random effect with the following as fixed effects in the model: diet, *CYP1A2*1F* genotype (A/A, A/C, or C/C), *GSTM1* genotype (null/non-null), *GSTT1* genotype (null/non-null), sex, period, and order. The dependent variable was the caffeine-metabolite ratio, which was log-transformed to achieve normality in distribution. Our strategy for handling missing data was to analyze the available data using SAS Proc Mixed for unbalanced data; and then to analyze the data using either random-coefficient selection models or random-coefficient pattern-mixture models depending on the drop-out mechanisms (reviewed in ref. 40). For testing of rebound in CYP1A2 activity, the dependent variable in the linear mixed regression model was the ratio of day 15/day 14 caffeine-metabolite ratio, which was log-transformed to achieve normality in distribution. All data are presented as the log-back transformed least squares means and SE of basal diet, the ratio of vegetable diets to basal diet, and the ratio of vegetable diets to each other (log-back transformed the differences between the diets). There were no statistically significant differences between analyses with and without adjustment for race; hence, data are presented without adjustment for race. All analyses were two-sided and $P < 0.05$ was considered statistically significant.

Results

Prevalence of Genotypes. Of those who met initial screening criteria and provided buccal cell samples for genotyping ($n = 468$), the overall frequency of the *CYP1A2*1F* allele in this population was 20.5%; prevalence of *CYP1A2*1F* was 10% for homozygotes and 41% for heterozygotes. This is similar to the prevalence previously reported in a population of similar ethnic background (5, 41). The prevalence of the combined *GSTM1-null/GSTT1-null* genotypes was 11%; previously reported prevalence of the combined genotypes was 7% (among Caucasians, ref. 42).

Of the 73 individuals who started the feeding study (Table 1), 4 dropped out after completing one feeding period, 5 after two periods, and 3 after three periods, resulting in 61 that completed all four feeding periods. Reasons for dropping out included changes in employment, commitment burden of the study, diet intolerance, illness, and a move out of the area.

Participants consumed ≥87% of the prescribed vegetables on each vegetable-supplemented diet; total urinary isothiocyanate concentrations (mean ± SD) further confirm adherence: 2.10 ± 2.5, 113.0 ± 61.7, 258.8 ± 170.6, and 106.0 ± 65.8 μmol/24 hours for basal, 1C, 2C, and 1C+A diets, respectively. The range of average daily intake of vegetables on the 1C diet was 319 to 662 g for men and 275 to 507 g for women. For the 2C diet, the range was 642 to 1,329 g for men and 507 to 1,011 g for women; for the 1C+A diet, it was 560 to 1,065 g and 458 to 816 g for men and women, respectively. There were some statistically significant differences among diets for intakes of individual macronutrients (Table 2); however, these were relatively small differences as all diets provided similar total energy and proportions of macronutrients as a percentage of total energy (55% carbohydrate, 15% protein, 30% fat) because we adjusted the basal-diet components to accommodate the addition of vegetables. We made no attempt to adjust for fiber differences due to the addition of vegetables. Differing views exist on which urine caffeine metabolite ratio is the least influenced by urine flow and the most sensitive to changes in CYP1A2 activity with (1U + 1X + AFMU)/17U and (17X + 17U)/137X each proposed as the most suitable ratio 4 to 5 hours after caffeine administration (34, 35, 37, 43). Using the data from the 583 urine caffeine tests completed in this study, we confirmed that (1U + 1X + AFMU)/17U was the least correlated with urine flow in our study population [Pearson correlation = 0.09 (after log transformation of data); −0.50 for the ratio (17X + 17U)/137X]. In addition, the direction of change in point estimates by diet were generally similar regardless of which ratio was used (data not shown).

Diet and Sex Differences in CYP1A2 Activity. Diet overall had statistically significant effects on CYP1A2 activity (Table 3). Compared with the basal diet, the 1C diet resulted in a 14% increase in CYP1A2 activity ($P < 0.0001$)

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and the 2C diet in a 27% increase in CYP1A2 activity. Compared with the 1C diet, the 2C diet resulted in an 11% further increase in CYP1A2 activity (P = 0.0006) and the 1C+A resulted in 13% decreased activity (P < 0.0001). The decrease in CYP1A2 activity was more pronounced when comparing the 1C+A diet with the 2C diet (22% reduction; P < 0.0001). There was no overall effect of sex, nor any differences by sex within each diet on CYP1A2 activity; however, when comparing effects of diet within sex, statistically significant diet effects were observed in one sex and not the other in some instances. Compared with the basal diet, the 1C diet induced CYP1A2 activity but men experienced only half the induction of women (Table 3). The 2C diet increased CYP1A2 activity in both men and women to the same extent compared with the basal diet, and the 1C+A diet had no effect in either sex (Table 3). Compared with the 1C diet, the 2C diet increased CYP1A2 activity further in men, but not in women, and the 1C+A diet resulted in inhibition of CYP1A2 activity in both sexes (Table 4).

***CYP1A2*1F* Genotype Effects.** There was no overall effect by *CYP1A2*1F* genotype and no genotype-by-diet interaction. However, when participants were stratified by *CYP1A2*1F* genotype, statistically significant diet effects were not always consistent across each *CYP1A2*1F* genotype. Compared with basal diet, the 1C diet resulted in 9% increased CYP1A2 activity in A/A individuals (P = 0.05), 13% increased activity in A/C (P = 0.008), and 20% increased activity in C/C (P = 0.008; Table 3). Doubling the cruciferous vegetable dose resulted in a further increase in CYP1A2 activity, but only in those individuals with at least 1 A allele (Tables 3 and 4). Addition of apiaceous vegetables negated the inducing effect of cruciferous vegetables to a similar extent for all genotypes; CYP1A2 activity on the 1C+A diet was similar to activity on the basal diet (Table 3). Compared with the 1C diet, the 1C+A diet inhibited CYP1A2 activity in A/A (13% reduction; P = 0.0018) and C/C (16% reduction; P = 0.01) but not in A/C (8%; P = 0.528; Table 4). Compared with the 2C diet, the 1C+A diet reduced CYP1A2 by 25%, 21%, and 18% in A/A, A/C, and C/C, respectively (P < 0.001 for each; Table 4).

***GSTM1/GSTT1* Genotype Effects.** There was no overall effect of the *GSTM1/GSTT1* genotype, and there were no differences by genotype within each diet. However, there were two instances where the effect of diet was not consistent across all *GSTM1/GSTT1* genotypes. Compared with the basal diet, the 1C diet modestly induced CYP1A2 activity by 10%, 12%, and 7% in *GSTM1+/GSTT1+*, *GSTM1-null/GSTT1+*, and *GSTM1-null/GSTT1-null* individuals, respectively, but reached statistical significance only for *GSTM1+/GSTT1+* and *GSTM1-null/GSTT1+* ($P \leq 0.02$ for both; Table 3). Conversely, CYP1A2 induction by the 2C diet was statistically significant for all three genotypes: compared with the basal diet, the 2C diet resulted in a 25%, 24%, and 35% increase in CYP1A2 activity in *GSTM1+/GSTT1+*, *GSTM1-null/GSTT1+*, and *GSTM1-null/GSTT1-null*, respectively (P < 0.0001 for all three). Inhibition of CYP1A2 activity by the 1C+A diet compared with the 1C diet reached statistical significance in *GSTM1-null/GSTT1+* and *GSTM1-null/GSTT1-null* only, but was statistically significant for all three genotypes when compared with the 2C diet (Table 4). The inhibition of CYP1A2 by the 1C+A diet relative to the 2C diet was the only instance where there was a statistically significant difference in response by genotype: inhibition was greater in *GSTM1-null/GSTT1-null* individuals than in *GSTM1+/GSTT1+* individuals (respectively, 33% reduction versus 18%; P = 0.02).

Rebound in CYP1A2 Activity. The ratio of day 15 CYP1A2 activity to day 14 CYP1A2 activity was compared between the 1C diet and the 1C+A diet for 36 of the 73 participants. The ratio was higher for the 1C+A diet compared with the 1C diet (1.05 ±.01 and 1.01 ±.01, respectively; P = 0.025). The *CYP1A2*1F* genotype did not modify the effect (P for interaction = 0.37).

Discussion

This is the first investigation to our knowledge of the effects of combined intake of two botanical families with opposing effects on CYP1A2 activity. This is also the first comparison of effects on CYP1A2 activity from different doses of cruciferous vegetables. In a randomized, crossover, controlled feeding study, we observed that combined intake of cruciferous and apiaceous vegetables reduced CYP1A2 activity relative to cruciferous intake alone. We also report a dose-response induction of CYP1A2 activity by cruciferous vegetable consumption in men but not in women. There were statistically

Table 3. Effects of diet on CYP1A2 activity: the ratio between responses to basal and vegetable-supplemented diets

	Basal CYP1A2 activity*	1C/Basal fold change†	2C/Basal fold change†	1C+A/Basal fold change†
Total (n = 73)	2.91 ± 0.09	1.14 ± 0.04‡	1.27 ± 0.04‡	0.99 ± 0.03
Men (n = 35)	3.06 ± 0.13	1.09 ± 0.05	1.27 ± 0.06‡	0.95 ± 0.04
Women (n = 38)	2.77 ± 0.12	1.19 ± 0.05‡	1.27 ± 0.06‡	1.04 ± 0.05
*CYP1A2*1F*				
A/A (n = 31)	3.12 ± 0.13	1.09 ± 0.05	1.26 ± 0.06‡	0.95 ± 0.04
A/C (n = 30)	2.87 ± 0.13	1.13 ± 0.05‡	1.31 ± 0.06‡	1.03 ± 0.05
C/C (n = 12)	2.76 ± 0.19	1.20 ± 0.08‡	1.23 ± 0.09‡	1.00 ± 0.07
GSTM1/GSTT1				
M1+/T1+ (n = 26)	2.91 ± 0.13	1.10 ± 0.05‡	1.25 ± 0.05‡	1.02 ± 0.04
M1-null/T1+ (n = 31)	3.07 ± 0.12	1.12 ± 0.04‡	1.24 ± 0.05‡	0.95 ± 0.04
M1-null/T1-null (n = 14)	2.95 ± 0.19	1.07 ± 0.07	1.35 ± 0.10‡	0.90 ± 0.07

*Least squares means ± SE of caffeine metabolite ratio (measure of CYP1A2 activity).
†Least squares means ± SE of fold change in caffeine metabolite ratio; analyses were done on log-transformed data and adjusted for baseline CYP1A2 activity, *CYP1A2*1F* genotype, *GSTM1* genotype, *GSTT1* genotype, sex, period, and order; the results presented are after back-transformation.
‡Significant comparison between vegetable and basal diets (P < 0.05).

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Table 4. Fold change in CYP1A2 activity: the ratio between responses to the vegetable diets

	2C/1C*	1C+A/1C*	1C+A/2C*
Total (*n* = 73)	1.11 ± 0.03†	0.87 ± 0.03†	0.79 ± 0.03†
Men (*n* = 35)	1.16 ± 0.05†	0.87 ± 0.04†	0.75 ± 0.03†
Women (*n* = 38)	1.06 ± 0.05	0.87 ± 0.04†	0.82 ± 0.04†
*CYP1A2*1F*			
A/A (*n* = 31)	1.15 ± 0.05†	0.87 ± 0.04†	0.75 ± 0.03†
A/C (*n* = 30)	1.16 ± 0.05†	0.92 ± 0.04	0.79 ± 0.04†
C/C (*n* = 12)	1.03 ± 0.07	0.84 ± 0.06†	0.82 ± 0.06†
GSTM1/GSTT1			
M1+/T1+ (*n* = 26)	1.13 ± 0.05†	0.93 ± 0.04	0.82 ± 0.03†
M1-null/T1+ (*n* = 31)	1.12 ± 0.04†	0.85 ± 0.03†	0.76 ± 0.03†
M1-null/T1-null (*n* = 14)	1.26 ± 0.09†	0.84 ± 0.06†	0.67 ± 0.05†

*Least squares means ± SE of fold change in caffeine metabolite ratio, adjusted for baseline CYP1A2 activity, *CYP1A2*1F* genotype, *GSTM1* genotype, *GSTT1* genotype, sex, period, and order; analyses were done on log-transformed data; the results presented are after back-transformation.
†Significant comparison between the two vegetable diets ($P < 0.05$).

significant diet differences by *CYP1A2*1F* and *GSTM1/GSTT1* genotypes. Although CYP1A2 activity had not returned to baseline within 24 hours after the removal of vegetables on the 1C+A diet, there was a statistically significant change indicating activity was recovering.

The ability of apiaceous vegetables to achieve a net inhibitory effect on CYP1A2 despite concurrent consumption of cruciferous vegetables implies a potential chemopreventive mechanism explained as follows: in addition to inducing CYP1A2, constituents of cruciferous vegetables also induce GST and other conjugating enzymes (44) which can detoxify many carcinogens, including reactive intermediates formed by CYP1A2. Because apiaceous vegetables do not induce GSTs (44) but inhibit CYP1A2, the combined intake of both botanical families may modulate biotransformation towards greater detoxification and less activation of procarcinogens. This combined effect warrants further investigation.

The finding that men experienced only half the induction of women of CYP1A2 on the 1C diet and that only men experienced further induction with the 2C diet compared with the 1C diet, suggests that men may require more cruciferous vegetables than women to induce CYP1A2 activity, given that activity on the basal diet was similar. All participants received vegetables based on the same dose per kilogram of body weight, to allow more effective investigation of sex differences. Limited data suggest that estrogen may induce expression of CYP1A2 (45); however, the current evidence from a review of pharmacokinetic studies using various CYP1A2 substrates suggests that, although not conclusive, men have higher CYP1A2 activity than women but more well-designed studies are needed that account for CYP1A2 inducers such as diet and smoking (46).

Although other studies have found that *CYP1A2*1F* has no effect on inducibility (47, 48), *CYP1A2*1F* has primarily been reported to alter inducibility, with C/Cs having lower inducibility than A/As (5, 49). We thus hypothesized that there would be no difference in CYP1A2 activity between genotypes on the basal diet, but those homozygous for *CYP1A2*1F* (C/C) would then have the lowest percent change in CYP1A2 activity in response to the inducing effect of cruciferous vegetables. However, our results were in the opposite direction for the 1C diet compared with basal (i.e., the greatest increase in activity was among those with the C/C genotype, the least among the A/A). Response to the 2C diet compared with that to 1C was as expected; only those with at least one A allele responded to the increased dose of cruciferous vegetables. Surprisingly, heterozygotes did not respond to the 1C+A diet compared to the 1C diet although both homozygote groups (A/A and C/C) experienced a 13% and 16% reduction in activity, respectively, following the inclusion of apiaceous vegetables. It is unclear whether A/C could be resistant to the inhibiting effect of apiaceous vegetables or if this unexpected observation is due to noise in the data. Common polymorphisms in *CYP1A2* have been found to be in linkage disequilibrium, thus polymorphisms other than *CYP1A2*1F* may influence results (50). Additionally, although a 2-fold variation in CYP1A2 induction has been reported with the *CYP1A2*1F* genotype (5, 49), the variance in constitutive expression of CYP1A2 is reported in excess of 60-fold with recent evidence suggesting *trans*-acting factors may have a more significant role in the regulation of CYP1A2 expression and warrant further investigation (51). Another unexpected result was that, compared with the basal diet, CYP1A2 activity was statistically significantly increased in *GSTM1-null/GSTT1-null* individuals when they consumed the 2C diet but not the 1C diet. We hypothesized that having the *GSTM1-null/GSTT1-null* genotype would result in the largest induction of activity by both the 1C and 2C diets due to decreased metabolism of inducing agents (i.e., indoles and isothiocyanates). The reason for no significant effect on CYP1A2 activity in *GSTM1-null/GSTT1-nulls* on the 1C diet is unclear, but one possible explanation could be alternative metabolic pathways for indoles and isothiocyanates (such as other GSTs; ref. 52) that can compensate for the lack of GSTM1 and GSTT1 at lower levels of the compounds (1C diet) but not higher levels (2C diet). Moreover, other polymorphisms may exist in other biotransformation pathways that influence the effect of indoles and isothiocyanates, which may vary in prevalence between Asian and Caucasian populations and partially explain the inconsistent association among cruciferous vegetables, cancer, and GST status reported in the epidemiologic literature. Moyer et al. (53) reported two nonsynonymous single nucleotide polymorphisms in *GSTM1* with varying prevalence in 100 Caucasian-Americans compared with 100 Han Chinese-Americans. The frequency of the $Asn^{85}Ser$ polymorphism was 6.8% in the Caucasian-American sample yet not present in the Han-Chinese American sample; the frequency of the $Lys^{173}Asn$ (rs1065411) polymorphism was 25% in the Caucasian-American group but 61% in

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the Han-Chinese American sample. Further investigation of the impact of other biotransformation polymorphisms on phytochemical disposition is warranted.

The gain in CYP1A2 activity one day after vegetables were removed from the 1C+A diet compared with the 1C diet was not as pronounced as we hypothesized based on the rapid metabolism of furanocoumarins (27, 28). However, furanocoumarins in apiaceous vegetables may not merely inhibit CYP1A2; several furanocoumarins are mechanism-based inactivators of other cytochrome P-450s (54-56). With mechanism-based inactivation, the enzyme produces a reactive intermediate from the parent compound which then irreversibly binds to the enzyme, permanently inactivating it. The only way to overcome the loss of enzyme activity *in vivo* is synthesis of new enzyme. The net inhibitory effect of combined exposure to CYP1A2 inducers and inhibitors, along with the modest recovery in activity one day after phytochemical sources were removed, suggests that the rapidly metabolized furanocoumarins may be irreversibly inactivating the enzyme.

There are several strengths to our approach. First, participants were recruited based on their genotypes; this ensured adequate statistical power to conduct analyses by genotype. The crossover design provides considerable reduction in interindividual variation because each participant experiences all treatments or interventions; thus, although the genotype for all other genes is unknown, there is no genetic variance between intervention groups. This enables a clearer investigation of the genes, specific genetic variances, and dietary components of interest. The detailed inclusion and exclusion criteria further minimized variability, allowing more direct investigation of the effect of diet by decreasing known nondietary factors that influence CYP1A2 activity. Additionally, participants ate controlled diets, with most of the study vegetables served on site at dinner to enhance adherence. Because men typically weigh more than women, study vegetables were provided based on body weight to achieve uniform dosing and allow investigation of sex effects. Lastly, using phenotype measurements (urinary caffeine metabolism) as the end point overcomes issues related to making inferences from measurements of RNA or protein levels to the broader physiologic state.

Nonetheless, there are a few limitations to this study. First, participants were free living and could have consumed nonstudy foods that influence CYP1A2 activity without our knowledge. They also could have refrained from eating some of the study foods. However, participants were encouraged to honestly report any deviations from the tightly prescribed diets either verbally or on the daily food checklists, and staff developed good rapport with participants to facilitate such disclosures. Second, it is possible, despite uniform dosing of vegetables, that exposure to the bioactive compounds during the four-year study was not uniform due to natural variation in phytochemical content in plant foods. Our efforts to address this included purchasing frozen items in large lots from the same vendor, purchasing fresh items from the same vendor, and uniform storage and food preparation conditions. Moreover, variation in final exposure can still occur even if equal amounts of phytochemicals are consumed. This is due to interindividual differences in the metabolic disposition of the compounds. For example, interindividual variation in phytochemical-metabolizing gut microbiota can lead to variation in bioavailability (57). Although the crossover design can minimize the impact of this interindividual variation, it cannot influence the effect of potential changes in gut microbiota over time within the same individual. It should also be noted that, although we used a relatively quantitative and robust measure of CYP1A2 phenotype, the involvement of other metabolic and transport processes involved in both caffeine and phytochemical disposition may contribute additional variability to the data. Lastly, our small sample size limited power to undertake formal genotype-diet interaction estimates.

In summary, we report that combined consumption of cruciferous and apiaceous vegetables results in net CYP1A2 inhibition; the inhibitory effect of apiaceous vegetables is sufficient to overcome the inductive effect of crucifers. Increasing the dose of cruciferous vegetables leads to further increases in CYP1A2 activity. Differences between controlled diets were influenced by sex, *CYP1A2*1F* genotype, and *GSTM1/GSTT1* genotype. Removal of apiaceous vegetables from the diet resulted in modest and incomplete, though statistically significant, recovery of CYP1A2 activity after 24 hours. However, some of our *a priori* hypotheses, which were based on existing epidemiologic data, are not supported by our results. The interindividual variation related to sex, genetics, dietary patterns, etc., underscores the complexity and challenge in elucidating the relationship of dietary modulation of biotransformation and disease susceptibility in observational studies. More controlled feeding studies are warranted to further elucidate potential mechanisms for diet-disease associations detected in observational studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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