

Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets

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Induction or inhibition of biotransformation enzymes, enzymes that activate or detoxify numerous xenobiotics, is one mechanism by which vegetables may alter cancer risk. Using a randomized crossover design, we examined the effect of various vegetable diets on cytochrome P450 (CYP) 1A2, N-acetyltransferase 2 (NAT2) and xanthine oxidase activity in humans. Men and women, non-smokers, on no medication and 20–40 years of age ate four 6-day controlled diets: basal (vegetable-free) and basal with three botanically defined vegetable groups. Enzyme activities were determined by measuring urinary caffeine metabolite ratios after a 200 mg caffeine dose on the last day of each feeding period. Mean CYP1A2 activity for 19 men and 17 women (least squares means adjusted for sex, *GSTM1* genotype, urine volume and feeding period) with basal, brassica, allium and apiaceous vegetable diets differed significantly ($P \leq 0.0005$) by diet, irrespective of the caffeine metabolite molar ratio used to describe CYP1A2 activity; brassica vegetables increased ($P < 0.04$) and apiaceous vegetables decreased ($P \leq 0.02$) activity compared with the basal and allium diets. There was no effect of diet on NAT2 and xanthine oxidase activities and none of the subjects differed by *GSTM1* genotype. These results demonstrate that while one vegetable subgroup induces human CYP1A2 activity, another subgroup inhibits it. This points to a complex association between consumption of a typical diet of various vegetables and biotransformation enzyme activities in humans, an association that may be difficult to interpret in observational studies.

Introduction

Biotransformation enzymes, such as the cytochrome P450 (CYP) isozymes, are essential for initiating conversion of lipophilic xenobiotics into more hydrophilic, water-soluble metabolites. They reduce the half-life of and duration of

Abbreviations: AFMU, 5-acetylamino-6-formylamino-3-methyluracil; CYP, cytochrome P450; FSA, Food Services of America; GST, glutathione S-transferase; NAT2, N-acetyltransferase 2; 1U, 1-methyluric acid; 17U, 1,7-dimethyluric acid; 1X, 1-methylxanthine; 17X, 1,7-dimethylxanthine (paraxanthine); 137X, 1,3,7-trimethylxanthine (caffeine).

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exposure to xenobiotics and prevent accumulation of the parent compounds. At the same time, excessive P450 activity without adequate concomitant conjugating activity may be a risk factor for cancer. The intermediate metabolites are often highly reactive compounds that can form DNA adducts or cause oxidative stress and cytotoxic effects (1). Studies in humans suggest that the prevalence of certain DNA adducts is related positively to expression and activity of the associated CYP enzymes (2,3).

CYP1A2 metabolizes various environmental procarcinogens, such as heterocyclic amines (4), nitrosamines (5) and aflatoxin B₁ (6). In humans, CYP1A2 activity, as measured by caffeine metabolite ratios, is increased by cigarette smoke (7,8), cruciferous vegetables (9,10) and well-cooked meat (11). Certain drugs and a few dietary constituents (12–14) have been associated with reduced CYP1A2 activity *in vivo*. Genetic polymorphisms in glutathione S-transferase (GST) have been shown to associate with CYP1A2 activity, perhaps by modulating circulating levels of phytochemicals that induce this P450 enzyme (15,16).

N-acetyltransferase 2 (NAT2) catalyzes acetylation of a variety of aromatic amines and hydrazines, including known carcinogens. The NAT2 gene is polymorphic; point mutations in both coding and non-coding regions can result in decreased expression, low activity or enzyme instability (17,18). Rapid and slow NAT2 phenotypes and the associated genotypes have been identified and both rapid and slow acetylation have been associated with the risk of several cancers (19). Although NAT2 is believed to be predominantly under genetic control, wide ranges exist in enzyme activity and associations have been identified between NAT2 activity and some exposures, such as use of gout medication and intake of heavily browned fish (17). To date, controlled dietary interventions have not found any effects of vegetables (9) or well-cooked meat (11) on NAT2 activity.

Xanthine oxidase is responsible for the metabolism of endogenous purines and pyrimidines and drugs, such as mercaptopurine and azathioprine (20). Its activity has been implicated in superoxide anion generation (21,22) and it is also involved in the formation of reactive metabolites, such as semi- and hydroquinones from estrogen quinones (23). The modulation of xanthine oxidase by macronutrients is well documented (24), however, little is known about the potential for phytochemicals to regulate this enzyme *in vivo*. Xanthine oxidase activity within individuals, as determined by caffeine metabolite ratios, appears to be very stable over time (20), but can be altered by other external factors besides diet; Vistisen *et al.* (9) observed that an intense exercise program could increase xanthine oxidase activity by 42% and allopurinol (used in the treatment of gout) inhibits the enzyme (25).

The objective of this study was to examine, under controlled dietary conditions, the effect of daily doses of commonly consumed, botanically defined vegetable groups on CYP1A2, NAT2 and xanthine oxidase activity, as measured by urinary

Table 1. Foods consumed daily on the basal vegetable-free, low phytochemical diet

| | Serving size | Amount consumed (g/day) |
|--|------------------------|-------------------------|
| Rice cereal, dry, ready-to-eat (Kellogg's) | $\frac{2}{3}$ cup | 18 |
| Corn cereal, dry, ready-to-eat (Kellogg's) | $\frac{3}{4}$ cup | 21 |
| Sugar, white, granulated | 1 packet | 6 |
| Orange flavored juice drink, ready-to-drink, regular, fortified with vitamin C | 1 × 8.45 fl. oz. box | 262 |
| Milk, skimmed | 8 fl. oz. | 245 |
| Bagel, white flour, plain | 1 medium bagel | 65 |
| Cream cheese, light, 18% fat | 0.75 oz. | 21 |
| Cracker, club (Keebler) | 8 crackers | 28 |
| Cookies, shortbread (Nabisco) | 4 cookies | 28 |
| Cheese, part-skimmed mozzarella (Kraft) | 1 × 1 oz. cheese stick | 29 |
| Sauce, white, cheese, commercial, prepared | $\frac{3}{4}$ cup | 136 |
| Noodles, macaroni, boiled, no fat added | $1\frac{1}{3}$ cup | 190 |
| Bread, rolls, French | 1 × 2 oz. roll | 57 |
| Margarine, regular, stick, salted, corn oil | 2 tsp. | 10 |
| Cookies, vanilla, cream filled (Keebler) | 4 cookies | 68 |

caffeine metabolite ratios, and to determine the effect of *GSTM1* genotype on these activities.

Materials and methods

Participants

Healthy, non-smoking men and women, aged 20–40 years, were recruited from the greater Seattle area to take part in a controlled feeding study. Participants were screened using a written survey and were ineligible if they reported any of the following: a history of gastrointestinal disorders; food allergies or intolerances; a weight change of >4.5 kg in the past year; major diet changes within the past year; exercise regimens requiring significant short-term dietary changes; oral antibiotic use within the past 3 months; body weight >150% of ideal; current drug therapy for a diagnosed disease; regular non-steroidal anti-inflammatory drug use; alcohol intake >2 drinks/day (750 ml beer, 240 ml wine or 90 ml hard liquor); job-related exposure to smoke and/or organic solvents; regular overexposure to second-hand smoke; current or planned pregnancy; no interest in participating in a controlled feeding study. Participants were requested not to take any type of medication (prescription or over-the-counter) or nutritional supplements during each feeding period and to maintain their usual physical activity. The Institutional Review Board at the Fred Hutchinson Cancer Research Center approved the study and informed consent was obtained from all participants.

Study design

The study was conducted between October 1997 and May 1998. Genotyping for *GSTM1* and *GSTT1* was conducted on all potential study participants prior to randomization into the feeding trial (26–28). Participants were randomized, blocked on sex and *GSTM1* genotype, using a crossover study design, with each participant receiving all four experimental diets in a randomly assigned Latin Square sequence (29). Each diet was consumed for 6 days and we used a 1 day diet rotation (i.e. the same food daily) to minimize day-to-day variability. The feeding periods began with dinner on a Saturday evening and ended with biological sample collection the following Saturday morning. There was at least a 2 week wash-out period between each diet period.

Study diets

During the four feeding periods participants consumed four different diets: a basal diet devoid of fruits and vegetables (Table 1) and the basal diet supplemented with brassica (*Cruciferae*), allium (*Liliaceae*) and apiaceous (*Apiaceae* or *Umbelliferae*) vegetables. The brassica supplement comprised: 16 g ($\frac{1}{2}$ cup US household measure) fresh radish sprouts, 150 g (1 cup) frozen cauliflower [Food Services of America (FSA) Signature], 200 g (2 cups) frozen broccoli (FSA Signature) and 70 g (1 cup) fresh shredded cabbage. The allium supplement comprised: 10 g (3 tablespoons) fresh chopped chives, 100 g ($1\frac{1}{2}$ cups) fresh chopped leeks, 5 g (1 teaspoon) fresh minced garlic and 75 g ($\frac{1}{2}$ cup) fresh chopped onion. The apiaceous supplement comprised:

0.50 g (1 teaspoon) fresh dill weed, 50 g ($\frac{1}{2}$ cup) fresh celery, 5 g (3 tablespoons) fresh chopped parsley, 100 g ($1\frac{1}{4}$ cups) fresh grated parsnips and 110 g ($\frac{3}{4}$ cup) frozen carrot coins (FSA Signature). All pre-packaged foods were purchased in case lots and fresh foods were purchased from the same vendor. The nutrient content of the diets was determined using the Nutrition Data System software (Food Database v.12A, November 1996 release; Nutrient Database v.27, November 1996 release) developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN). Components of the basal diet were adjusted to accommodate the added vegetables, such that all diets provided a similar percent energy from carbohydrate, protein and fat: 60, 12 and 29%, respectively.

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

The Harris-Benedict equation was used to calculate participant energy requirements (30). All four study diets were designed to provide 2000 kcal. Participant's daily energy intakes were adjusted to maintain body weight by adding 'unit' foods. One unit consisted of a plain bagel (65 g), light cream cheese (21 g) and four shortbread cookies (28 g). This combination of foods was chosen because these foods were already included in the basal diet and the macronutrient composition was the same as that of the basal diet.

Urinary caffeine metabolites

Participants completed a 'urine caffeine test' at the end of each feeding period for the measurement of CYP1A2, NAT2 and xanthine oxidase activity. On the morning of the last day of each feeding period (i.e. the second Saturday), after a 12 h overnight fast, they arrived at the feeding center, consumed 200 mg caffeine (NoDoz; Bristol-Meyers Squibb, NY) with 240 ml water and a light breakfast (120 ml orange juice and a muffin) and remained at the feeding center for the next 5 h. Additional fluids were withheld during this period. Four hours after taking the caffeine dose, participants were asked to void and then to collect all of their urine during the fifth hour. The volume of each urine sample was measured, the pH was adjusted to 3.5 and 1 ml aliquots were stored at -70°C until analysis. Caffeine and its metabolites were extracted from the urine specimens as described previously (31), however, we used a 200 μl urine aliquot, 40 μl internal standard (4-acetamidophenol in chloroform, 120 mg/l) and a 9:1 ratio of chloroform/isopropanol (v/v) (6 ml).

The HPLC method of Butler *et al.* (8) was adjusted to optimize chromatography and reproducibility. We used a Hewlett Packard (Wilmington, DE) 1050 HPLC system consisting of quaternary pumps, a programmable auto-sampler and a diode array detector with spectral analysis software for peak purity analysis. The analytical column was Altex 5 μm Ultrasphere ODS (4.6 × 250 cm; Beckman, Fullerton, CA) with a Waters Guard-Pak Pre-column Module containing a $\mu\text{Bondapak C18}$ insert (Waters Chromatography Division, Millipore Corp., Milford, MA). The composition of the mobile phase was as follows: A, 0.5% acetic acid (pH 3.5); B, 100% methanol; C, 100% acetonitrile. The elution program was: 8% B and 0% C at 0.8 ml/min from 0 to 3.5 min, then an increase in flow rate to 1.1 ml/min from 3.5 to 4.5 min, held until 14 min; 8–19% B and 0% C, 14–20 min; 19–12% B and 0–8% C, 20–23 min; 12% B and 8% C, 23–30 min; 12–50% B and 8–0% C, 30–36 min; 50% B and 0% C, 36–41 min; 50–8% B and 0% C, 41–48 min. Finally, the flow rate was dropped to 0.2 ml/min (48–66 min). Linear gradients were used for all solvent changes. Caffeine and its metabolites were identified based on spectral analysis, retention times of standards and peak purity analysis. All samples were run in batches of 18, with two quality control samples (in-house pooled samples) within each batch. We quantified 5-acetylamin-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1U), 1-methylxanthine (1X), 1,7-dimethyluric acid (17U), 1,7-dimethylxanthine (17X) and 1,3,7-trimethylxanthine (caffeine, 137X). The standards were obtained as follows: 1U, 1X, 17X, 137X and 4-acetamidophenol (internal standard) were purchased from Sigma Chemical Co. (St Louis, MO); 17U was purchased from Fluka Chemical Corp. (Ronkonkoma, NY); AFMU was purchased from Dr B.K.Tang (University of Toronto, Toronto, Canada). The coefficients of variation for 73 consecutive batches of the quality control sample were as follows: AFMU, 3.4%; 1U, 9.0%; 1X, 5.6%; 17U, 3.9%; 17X, 5.2%; 137X, 4.4%.

Several different caffeine metabolite ratios have been used to define CYP1A2 and NAT2 activities. We defined CYP1A2 activity using four different molar ratios of caffeine metabolites: (1U+1X+AFMU)/17U (32); (1U+1X+AFMU)/17X (33); (17X+17U)/137X (8); 17X/137X (34). NAT2 activity was defined as AFMU/(1X+1U+AFMU) and xanthine oxidase activity as the molar ratio 1U/(1X+1U). According to Rostami-Hodjegan

Table II. Average daily energy and macronutrient intake of 36 individuals consuming controlled study diets

| | Basal | Brassica | Allium | Apiaceous |
|--------------------------------|------------------------|------------|------------|------------|
| Energy (kcal) | 2389 ± 82 ^a | 2327 ± 82 | 2348 ± 82 | 2380 ± 82 |
| Carbohydrate (g) ^b | 353 ± 11 | 346 ± 11 | 348 ± 11 | 357 ± 11 |
| Fat (g) ^b | 75 ± 2 | 73 ± 2 | 73 ± 2 | 74 ± 2 |
| Protein (g) ^b | 67 ± 2 | 70 ± 2 | 67 ± 2 | 65 ± 2 |
| Dietary fiber (g) ^b | 13.9 ± 0.3 | 21.3 ± 0.5 | 17.3 ± 0.4 | 21.2 ± 0.5 |

^aLeast squares means ± SE, adjusted for subject effect.

^bAnalyses performed on log-transformed data; least squares means and SE are back-transformed.

et al. (35), $AFMU/(1X+1U+AFMU)$ may best define NAT2 activity because the rate of conversion of 1X to 1U does not influence the measurement.

Statistical analysis

The CYP1A2 caffeine metabolite ratio $(AFMU+1X+1U)/17U$ and the ratio for NAT2 were approximately normally distributed, although the distribution of NAT2 indicated bimodality. The metabolite ratios for the other three determinations of CYP1A2 and for xanthine oxidase, as well as the macronutrient intake data, were skewed and were log transformed (natural log) prior to analysis.

We analyzed the data using a linear mixed regression model (36) (PROC MIXED in SAS, Release 6.12) and included the following effects in the model: study participant, *GSTM1* genotype, sex, sex by genotype, period, period by sex, period by genotype, period by sex by genotype, urine volume, diet, diet by urine volume, diet by genotype, diet by sex, carryover, sex by carryover, genotype by carryover. Study participant was a random effect and all other effects were fixed. We present least squares means and standard errors for each diet, adjusted for other effects in the model. The plotted, smoothed probability density functions of enzyme activities were estimated using a Gaussian kernel (37).

Results

Twenty-one women and 19 men completed all four feeding periods, however, four women were unable to provide urine samples during the fifth hour on at least one occasion and had low urine volumes (<20 ml) on other occasions. Because delayed urine collection or low urine flow can alter caffeine metabolite ratios (35), these individuals were excluded from the analysis. To minimize any potential effect of urine flow on caffeine metabolite ratios, we also adjusted for urine volume in the model, even though there was no difference in urine volume by diet ($P = 0.3$). We present data on 17 women (4 *GSTM1*+ and 13 *GSTM1* null) and 19 men (10 *GSTM1*+ and 9 *GSTM1* null). Only two of these individuals (a *GSTM1* null woman and a *GSTM1*+ man) were *GSTT1* null. Within sex, measures of height, weight, body mass index and ethnicity (~70% Caucasian) did not differ by *GSTM1* genotype.

Table II outlines participants' mean energy and macronutrient intakes on the four controlled study diets. Components of the basal diet were adjusted to maintain a similar carbohydrate, protein and fat distribution on the diets when vegetables were added, however, no attempts were made to adjust for the additional dietary fiber in the vegetables.

Figure 1 displays the smoothed probability density functions of CYP1A2 activity on the four controlled diets. We found no overall evidence of a bimodal distribution of CYP1A2 activities for our study participants. Least squares means (± SE) of CYP1A2 activity (adjusted for sex, *GSTM1* genotype, urine volume, feeding period and carryover effects) on the control, brassica, allium and apiaceous diets are presented in Table III. Overall, the effect of diet was significant for all four caffeine metabolite ratios used to define CYP1A2 activity ($P \leq 0.0005$). Similarly, compared with the control diet, the brassica diet

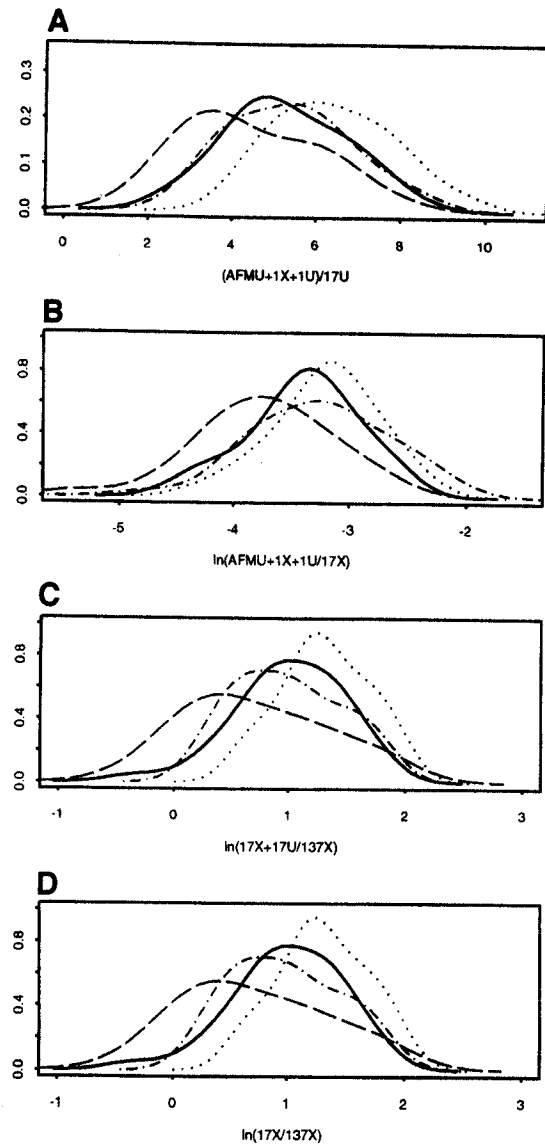


Fig. 1. Smoothed probability density functions of CYP1A2 activities on four controlled diets (—, basal; ····, brassica; - - -, allium; - · - ·, apiaceous) using the following caffeine metabolite ratios: (A) $(AFMU+1X+1U)/17U$; (B) $(AFMU+1X+1U)/17X$; (C) $(17U+17X)/137X$; (D) $17X/137X$.

increased significantly ($P < 0.04$) and the apiaceous diet reduced ($P \leq 0.02$) CYP1A2 activity.

Figure 2A and B displays the smoothed probability density functions of NAT2 and xanthine oxidase activities. Our results indicate that the vegetable diets had no effect on NAT2 ($P = 0.4$) and xanthine oxidase activities as measured by caffeine metabolite ratios ($P = 0.2$). Least squares means of NAT2 and xanthine oxidase activities were similar on all diets (Table III).

We also examined these enzyme activities in relation to *GSTM1* genotype, NAT2 phenotype and the genotype/phenotype by diet interactions. *GSTM1* genotype had no overall effect on CYP1A2 activity ($P = 0.5$) and the *GSTM1* genotype by diet interaction was not statistically significant (P values ranged from 0.06 to 0.9 depending on the caffeine metabolite ratio used). There was no effect of *GSTM1* genotype on NAT2 or xanthine oxidase activities either. Acetylator status (slow or rapid) had no effect on NAT2 activity in response to the

Table III. Urine volumes and CYP1A2, NAT2 and xanthine oxidase activities in 36 participants consuming controlled vegetable-free (basal) and vegetable-containing diets

| | Basal | Brassica | Allium | Apiaceous |
|-------------------------------|--------------------------|---------------|---------------|---------------|
| Urine volume (ml) | 58 ± 6 ^a | 61 ± 6 | 53 ± 6 | 67 ± 6 |
| CYP1A2 | | | | |
| (AFMU+1X+1U)/17U ^b | 5.50 ± 0.27 ^a | 6.47 ± 0.27 | 5.54 ± 0.28 | 4.76 ± 0.27 |
| (AFMU+1X+1U)/17X ^c | 0.035 ± 0.003 | 0.041 ± 0.004 | 0.037 ± 0.003 | 0.026 ± 0.002 |
| (17U+17X)/137X ^d | 2.68 ± 0.25 | 3.68 ± 0.34 | 2.75 ± 0.27 | 2.19 ± 0.20 |
| 17X/137X ^d | 2.66 ± 0.25 | 3.65 ± 0.33 | 2.73 ± 0.27 | 2.18 ± 0.20 |
| NAT2 ^e | 0.21 ± 0.03 | 0.21 ± 0.03 | 0.21 ± 0.03 | 0.21 ± 0.03 |
| Xanthine oxidase ^f | 0.43 ± 0.01 | 0.43 ± 0.01 | 0.43 ± 0.01 | 0.44 ± 0.01 |

^aLeast squares means ± SE, adjusted for sex, *GSTM1* genotype, feeding period and carryover effects. In addition, enzyme activities are also adjusted for urine volume.

^bActivity on the brassica and apiaceous diets differed significantly from basal; $P = 0.0001$ and $P = 0.002$, respectively.

^cActivity on the brassica and apiaceous diets differed significantly from basal; $P = 0.035$ and $P = 0.0001$, respectively.

^dActivity on the brassica and apiaceous diets differed significantly from basal; $P = 0.0006$ and $P = 0.02$, respectively.

^eNAT2 activity was calculated using the molar ratio AFMU/(1X+1U+AFMU); effect of diet was not significant ($P = 0.4$).

^fXanthine oxidase activity was calculated using the molar ratio of 1U/(1X+1U); effect of diet was not significant ($P = 0.2$).

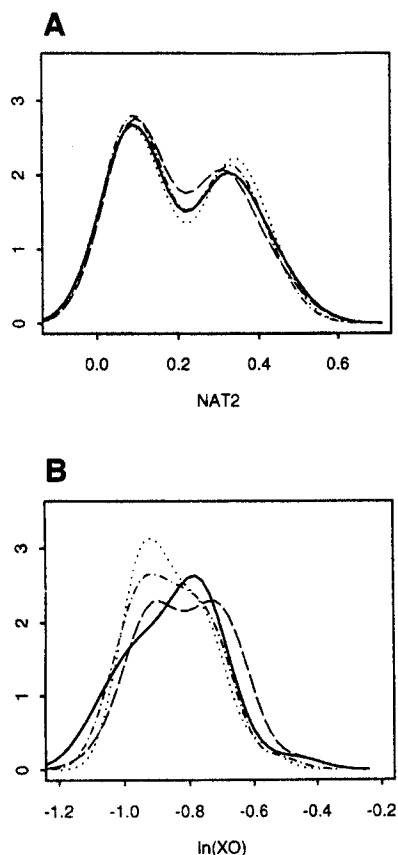


Fig. 2. Smoothed probability density functions of (A) NAT2 and (B) xanthine oxidase activities, as measured by caffeine metabolite ratios, on the four controlled diets: basal (—); brassica (.....); allium (- · -); apiaceous (- -).

vegetable diets ($P = 0.4$). Acetylator status was clearly differentiated on all diets, with no overlap in activities between slow and rapid acetylators (data not shown; Figure 2A data are smoothed); 17 participants were rapid acetylators (47%) and 19 were slow acetylators (53%).

None of the enzyme activities differed by sex and there were no sex by diet interactions. The women in our study

were pre-menopausal and were not using oral contraceptives or other hormones; 76% were nulliparous and reported regular menstrual cycles of 25–35 days in length. Excluding the four women who were parous and comparing only the 13 nulliparous women and 19 men did not change the null result.

Discussion

We have demonstrated that under controlled dietary conditions, at moderate levels of intake, brassica vegetables increased, apiaceous vegetables decreased and allium vegetables did not change CYP1A2 activity when compared with a basal, vegetable-free diet. Depending on the caffeine metabolite ratio used, mean CYP1A2 activity was 18–37% higher with consumption of 428 g brassica vegetables compared with the basal, vegetable-free diet. Other smaller feeding studies in humans have reported the capacity of similar amounts of particular brassica vegetables, specifically broccoli (9,10) and cabbage and Brussels sprouts (38), to increase CYP1A2 activity by ~10–20%. Crucifers are a rich source of glucosinolates; 25 of the more than 100 known glucosinolates have been detected at varying levels in cruciferous vegetables (39). In rats, ingestion of glucobrassicin, a major glucosinolate in broccoli, has been shown to increase CYP1A2 activity against 7-methoxyresorufin, a CYP1A2 substrate (39).

Addition of selected apiaceous vegetables (namely carrots, parsnips, celery, dill and parsley) to the basal diet reduced mean CYP1A2 activity by ~13–25%, depending on the caffeine metabolite ratio used. Certain apiaceous vegetables, including celery, parsnips and parsley, are significant sources of furanocoumarins (40), compounds which have been shown to inhibit several human P450s (41). Some *Rutaceae* (e.g. citrus fruits) also contain furanocoumarins and inhibition of CYP3A4 and CYP1A2 activity by grapefruit juice has been attributed in part to the presence of furanocoumarins (12,42,43). Direct supplementation of a specific furanocoumarin, 5-methoxypsoralen (1.2 mg/kg), in humans also resulted in a CYP1A2-dependent inhibition of caffeine *N*-demethylation (44), further supporting the inhibitory effects of this class of compounds.

Given the human data available to date, the inhibition of CYP1A2 by vegetables appears to be specific to *Apiaceae*. The allium vegetables had no effect on CYP1A2 activity and

Vistisen *et al.* (9) also found no effect of a 500 g mixture of vegetables (green beans, peas and lettuce) under controlled dietary conditions. In a population-based study, Le Marchand *et al.* (13) reported an inverse association between CYP1A2 activity and several nutritional factors, including plasma lutein, plasma α -tocopherol and alcohol consumption. Lutein itself has not been shown to alter CYP1A2 activity (45,46), thus the association with plasma lutein probably reflects effects of food components associated with lutein intake or intake of some high-lutein foods that also contain CYP1A2-inhibiting phytochemicals (47). Plausible candidates include celery, parsley and other non-cruciferous, green leaf vegetables.

In previous reports, CYP1A2 activity was ~23% higher in individuals who expressed the *GSTM1* null allele compared with those who expressed at least one *GSTM1* allele ($P = 0.1$) (48) and, among frequent consumers of broccoli, *GSTM1* null individuals had a 21% higher geometric mean level of CYP1A2 activity relative to *GSTM1*+ individuals ($P = 0.01$) (15). MacLeod *et al.* (48) proposed that individuals who lack *GSTM1* activity may retain higher levels of a compound that is normally conjugated by *GSTM1* and that also, in its unconjugated form, affects CYP1A2 activity. On the basis of those prior observations, we postulated that on the controlled brassica diet *GSTM1* null individuals would have higher CYP1A2 activity. Among our participants, *GSTM1* genotype did not appear to modulate CYP1A2 activity and least squares mean CYP1A2 activity on all four diets was the same in *GSTM1* null and *GSTM1*+ individuals. In addition, the *GSTM1* genotype by diet interaction was not statistically significant. This lack of a significant effect of *GSTM1* genotype may be due to the small sample size or a glucosinolate dose in the brassica diet that was too low to differentially induce CYP1A2 activity, although the latter seems unlikely given the overall CYP1A2 response. Other polymorphisms may have a greater effect on dietary response; for example, *GSTT1* genotype has also been implicated in isothiocyanate metabolism (16).

Our study, like that of Vistisen *et al.* (9), showed that NAT2 and xanthine oxidase activity did not change with addition of vegetables to the basal diet. In epidemiological studies, few environmental factors have been reported to alter NAT2 activity (as estimated by caffeine metabolite ratios), consistent with the assumption that NAT2 is largely under genetic control and is not readily inducible (17). Xanthine oxidase indices also have been shown to be very stable (20), even in free-living individuals on their habitual diets.

We observed no overall sex difference in enzyme activities and no sex by diet interaction. This is one of few studies that have compared caffeine metabolism in men and women under controlled dietary conditions. Observational studies report small or no sex differences in CYP1A2, but report effects of oral contraceptives and parity (reviewed in ref. 49). In our study, the lack of even a trend toward different CYP1A2 activities in similarly aged, non-smoking men and women under controlled dietary conditions suggests that the small differences reported in some observational studies may be due more to environmental factors, rather than inherent sex differences. Xanthine oxidase activity was reported to be ~20% higher in liver tissue from men than from women (50). However, in our study and others (9,20) this difference has not been detectable in caffeine metabolite ratios.

In summary, we observed an increase in CYP1A2 activity when individuals consumed a controlled diet containing a selection of brassicaceous vegetables and a decrease in activity

when their diets contained a selection of apiaceous vegetables. This is the first randomized, controlled intervention study to report the capacity of apiaceous vegetables to suppress CYP1A2 activity in humans. The differential CYP1A2 response to the vegetable diets also clearly demonstrates the potential challenges of trying to isolate effects of specific foods on enzyme activities in population-based, observational studies.

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