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Lifestyle and nutritional correlates of cytochrome CYP1A2 activity: inverse associations with plasma lutein and alpha-tocopherol

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Cytochrome CYP1A2, a liver enzyme responsible for the metabolic activation of a number of putative human carcinogens, exhibits wide inter-individual differences in activity. In order to characterize sources of variability in CYP1A2 activity, we phenotyped (with the caffeine test) 90 subjects of various ethnic backgrounds in Hawaii. Forty-three subjects were patients with *in-situ* colorectal cancer treated by polypectomy and 47 were healthy population controls. Subjects were also administered a detailed lifestyle questionnaire, including a quantitative food frequency questionnaire, and were assessed for plasma levels of carotenoids, tocopherols, retinol, ascorbic acid, cholesterol and triglycerides. In a stepwise multiple regression, 27% of the overall variation in CYP1A2 activity was explained by seven variables. Plasma lutein explained the largest portion of the variance (7%) and was negatively associated with CYP1A2 activity ($p < 0.01$), as were use of menopausal replacement estrogens ($p = 0.04$), plasma alpha-tocopherol ($p = 0.05$) and alcohol consumption ($p = <0.01$). Acetaminophen use ($p = 0.05$), coffee consumption ($p = 0.05$) and plasma lycopene ($p = 0.06$) were positively associated with CYP1A2 activity. After adjustment for these variables, no association was found between CYP1A2 activity and sex, race, age, education, smoking, physical activity, weight, vitamin E supplements, the other plasma micronutrients measured, and dietary intakes of red meat, processed meat and cruciferous vegetables. Results were similar for colorectal cancer cases and controls. Almost two-thirds (73%) of the variability in CYP1A2 activity remained unexplained. This study confirms an enhancing effect of acetaminophen and coffee on CYP1A2 activity and suggests an inhibitory effect of estrogens, alcohol and food sources of lutein and alpha-tocopherol on this enzyme.

Keywords: CYP1A2, diet, estrogens, ethnic groups, lifestyle, nutrition.

Introduction

Cytochrome CYP1A2 is responsible for the metabolic activation of various procarcinogens such as aromatic amines and heterocyclic amines through N-oxidation (Guengerich & Shimada, 1991; Kadlubar *et al.*, 1992). CYP1A2 activity can be measured in humans by assessing the rate of demethylation of caffeine through analysis of caffeine and its metabolites in urine. Inter-individual differences in CYP1A2 activity have been associated with colorectal cancer (Lang *et al.*, 1994) and are thought to be at least partly genetically determined, although the molecular basis for a genetic polymorphism has remained elusive (Nakajima *et al.*, 1994). The enzyme is known to be inducible and previous studies have shown variation in activity level by sex, race, age, smoking status, coffee and alcohol consumption, and exposure to various combustion products and contaminants (e.g. dioxin) (Guengerach & Shimada, 1991; Kalow & Tang, 1991; Butler *et al.*, 1992; Kadlubar *et al.*, 1992; Vistisen *et al.*, 1992; Lang *et al.*, 1994; Nakajima *et al.*, 1994; Horn *et al.*, 1995). Thus, these factors can distort the assessment of the phenotype and may

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result, when evaluating disease associations, in an increase in variability and subsequent loss of statistical power, or in bias, if the distorting factors are also associated with the disease (Rothman *et al.*, 1993; Le Marchand *et al.*, 1996). Moreover, the identification of modifiable inducers and inhibitors of CYP1A2 may lead to a new means of cancer prevention if the role of this enzyme in human cancer is confirmed. We report here on a study aimed at characterizing the main lifestyle and nutritional determinants of CYP1A2 activity.

Methods

Subjects

Participants were described previously and were part of a case-control study of *in-situ* adenocarcinoma of the large bowel (Sivaraman *et al.*, 1994). Eligible patients were all those diagnosed with this condition in the main medical centers of Oahu, Hawaii, between July 1989 and October 1991 and whose treatment did not include colectomy. Controls were selected among participants in a population-based survey conducted by the Hawaii State Department of Health and were matched to each patient on sex, age (± 2.5 years) and ethnicity. Overall, 72.4% of the eligible patients and 71.6% of the eligible controls were interviewed. A total of 43 cases and 47 controls (73.4% of the eligible interviewed subjects) agreed to the biological component of the study, of whom 45 were Japanese, 15 Caucasian, 12 Filipino, 12 Hawaiian/part-Hawaiian and six Chinese. The subjects' ages ranged from 36 to 85 years, with a mean of 65.6 years. Fifty-two percent of the participants were men. All but three women were postmenopausal.

Questionnaire data

A detailed questionnaire on demographics, diet, and lifestyle was administered at the participants' home by trained interviewers. The dietary information consisted of a quantitative diet history (Hankin *et al.*, 1991) focussing on usual intake for the year prior to diagnosis for cases and during the year preceding the interview for controls. It also included questions on degree of browning of meat and fish (color of meat and fish surfaces) 5 years prior to interview, as well as frequency of consumption of cruciferous vegetables (cabbage, broccoli, Brussels sprouts and watercress) during the 7 days prior to the caffeine test. Alcohol, coffee and soda consumption, as well as tobacco, medication and supplement use, were ascertained for the 2 week period preceding the caffeine test. Subjects were asked to specify the time spent at various levels of physical activity during a typical workday and weekend day (Sallis *et al.*, 1985). These data were summarized over an average 24 h period and converted into metabolic equivalents (METs) using published sources. Weight was measured in light clothing.

Dosing regimen and specimen collection

Subjects were asked to refrain from consuming any foods or beverages (except water) from 10:00 pm to the time of the blood draw the following morning. After venipuncture, the subjects drank a cup of coffee made of two packets of Maxwell House instant coffee (57 mg of caffeine per packet), voided 4 h after the coffee consumption, and provided a 1 h urine specimen at the end of the fifth hour following dosing. No other source of caffeine was consumed during the 5 h period. The urine samples were acidified with ascorbate, aliquoted and stored at −70 °C until analysis.

Intra-individual variability study

Another sample of eighteen healthy, non-smoking individuals of various ethnic backgrounds participated in the intra-individual variability study. Nine male and nine female volunteers recruited among the staff of our institution were dosed with caffeine as described above once weekly for 4 weeks. No recommendation was given with regard to diet or lifestyle; however, we varied the day of the week for the caffeine test in order to optimize the daily variability due to lifestyle.

Phenotyping

Urinary ratios of [1,7-dimethyluric acid (17U) + 1,7-dimethylxanthine (17X)]/caffeine (137X) were quantified to assess CYP1A2 activity. Urine samples were quickly thawed and extracted (200 μl) according to a slight modification of the method of Butler *et al.* (1992) by adding 120 mg ammonium sulfate and 120 μl of 4-acetamidophenol (160 $\mu g\,ml^{-1}$) as an internal standard to the urine in a 15 ml centrifuge tube and mixing for 2 min. Caffeine and its metabolites were partitioned by the addition of 6 ml chloroform: isopropanol (19 : 1). The organic phase was removed after centrifugation, evaporated to dryness, then resuspended in 500 μl of the HPLC mobile phase 'A' consisting of 0.045% aqueous acetic acid containing 9% methanol. Chromatography was carried out with a Supelcosil LC-18 5 μm (4.6 mm × 250 mm) reversed phase HPLC column using a Beckman System Gold chromatograph. Samples were eluted at 1.1 $ml\,min^{-1}$ using linear gradients of mobile phase 'A' and 'B' (methanol) as follows: 0–3 min (0% B); 3–5 min (2% B); 5–6 min (2–20% B); 6–16 min (20% B); 16–21 min (60% B); 21–35 min (0% B). Identification of the analytes was done by comparison of their retention time and absorption spectra (Beckman Model 168

diode array detector) with those of authentic standards, in addition to spiking experiments.

Plasma micronutrients

Venous blood samples were protected from light and processed within 1 h after collection. Plasma samples were stored at −70 °C and analysed in a single batch at the end of the study. Individual carotenoid levels were determined by high pressure liquid chromatography (Franke *et al.*, 1993). Briefly, plasma proteins were precipitated with ethanol containing bis-hydroxy-toluene as antioxidant and three internal standards followed by repeated (3×) hexane extraction of the lipophilic micronutrients. The combined hexane layers were dried under nitrogen and redissolved in the high pressure liquid chromatography mobile phase consisting of methanol : dichloromethane : acetonitrile (65 : 25 : 10), bis-hydroxy-toluene (0.025%) as anti-oxidant, and aqueous bis-tris-propane (2 ml l^{-1} of 0.5 M, pH7.0) as buffer to prevent analyte degradation during chromatography. Twelve carotenoids, retinol, γ- and α-tocopherol were separated on a Spherex 5-μm C_{18} column (250 × 4.6 mm) (Phenomenex, Torrance, CA) and monitored by a dual diode array detector at each individual compound's absorption maximum. Levels were determined using peak areas and calibration curves of authentic standards for the five major peaks (α-carotene, β-carotene, lycopene, lutein/zeaxanthin, and β-crytoxanthin). All 12 carotenoid fractions were used to calculated total plasma carotenoids.

Analytical accuracy and reliability were verified by participation in the National Institute of Standards and Technology 'round robin' for lipid-phase micronutrient analysis with measurements consistently within 6% of the median values reported for all laboratories and coefficients of variation for intra-assay variability not greater than 4%, with the exception of α-carotene for which the coefficient of variation was 6.8%. Plasma ascorbic acid was measured with the dichlorophenolindophenol method (Vanderjagt *et al.*, 1986). Total plasma cholesterol and plasma triglycerides were measured enzymatically using diagnostic kits NO. 352-50 and NO. 339-50, respectively, from Sigma Chemical Co. (St Louis, MO).

Data analysis

The objective of the analysis was to study the variability of CYP1A2 activity, as assessed by the caffeine test. The metabolic ratio was log transformed, as $\log(x + 1)$, in order for its distribution to approximate normality. This was confirmed by the Wilk-Shapiro test ($p = 0.53$) and by low values for skewness (0.44) and kurtosis (0.09). In the intra-individual variability study, the coefficient of variation (CV), averaged across individuals, was taken as a measure of variability (Snedcor & Cochran, 1967). The CV was also used to measure variability in laboratory measurement.

For the inter-individual variability study, multiple analysis of covariance was used to examine how much variability was accounted for by the covariates. A stepwise procedure was executed to determine the importance of each variable after other covariates were controlled for. Ninety-five percent confidence intervals for each regression parameter were computed as the estimate ± 1.96 times the SE. The variables selected for the regression were those shown in the past to both affect levels of CYP1A2 or other xenobiotic metabolizing enzymes and/or to be associated with cancer risk. These variables have the potential not only to increase the background variability in CYP1A2 activity but also to act as confounders of an association between N-oxidation and cancer. Pearson's correlation coefficients were used to investigate the linear relationships between the log-transformed variables.

Results

Intra-individual and laboratory variability

Among the eighteen subjects dosed with caffeine once a week for 4 weeks, the mean coefficient of variation for the urinary metabolic ratio [(17U + 17X)/137X] was 23.2% (range: 10.8%–39.0%). Analyses of duplicate samples in our laboratory resulted in good reproducibility of the measurement of the metabolic ratio, with intra- and inter-assay coefficients of variation of 6.5% and 7.7%, respectively. These intra-individual and laboratory variations are comparable to published data (Butler *et al.*, 1992; Nakajima *et al.*, 1994; McQuilkin *et al.*, 1995).

CYP1A2 activity

The metabolic ratio ranged from 0.2 to 42.7, with a median of 4.5. The distribution of the ratio appeared log-normal but the relatively small size and mixed ethnic composition of the sample precluded any formal search for a mixture of distributions evocative of a polymorphism in the gene regulating the enzyme. There was no statistically significant difference in the geometric mean ratio for cases and controls ($p = 0.95$). The race-adjusted odds ratios (OR) (and 95% confidence intervals [CI]) for *in-situ* colorectal cancer for the second and third tertiles of the metabolic ratio, compared to the first, were 0.7 (0.3–2.1) and 1.0 (0.2–2.8), respectively. However, there was a statistically significant difference ($p = 0.004$) in the mean

Table 1. CYP1A2 activity by sex, age, diet and lifestyle among *in-situ* colorectal cancer cases and population controls

		Geometric mean (17U + 17X/137X)[a]		
		Cases	*Controls*	*All*
		(n = *43*)	(n = *47*)	(n = *90*)
Sex	Male	5.29	6.23	6.30
	Female	3.91	4.29	4.42
		$p = 0.28$	$p = 0.17$	$p = 0.06$
Age (years)	36–62	5.86	4.47	5.47
	63–71	3.08	5.54	4.95
	72–85	3.45	5.79	5.19
		$p = 0.91$	$p = 0.16$	$p = 0.49$
Years of schooling	1–12	5.00	4.60	5.28
	13–19	3.75	5.66	5.17
		$p = 0.30$	$p = 0.49$	$p = 0.91$
Smoking (cigarettes per day)	0	4.56	5.25	5.36
	1–40	3.01	5.21	4.26
		$p = 0.42$	$p = 0.99$	$p = 0.46$
Alcohol (drinks per day)	0	5.09	5.02	5.31
	1–40	3.94	6.06	5.13
		$p = 0.36$	$p = 0.52$	$p = 0.86$
Coffee (cups per day)	<1	3.96	3.46	3.94
	1–10	5.27	6.38	6.26
		$p = 0.33$	$p = 0.02$	$p = 0.01$
Red meat (g per day)	0–39	4.07	5.64	5.14
	40–95	4.00	4.00	4.42
	96+	5.46	6.60	6.64
		$p = 0.60$	$p = 0.35$	$p = 0.21$
Chicken and fish (g per day)	0–30	4.08	5.61	5.08
	31–90	5.28	5.66	5.98
	91+	4.03	4.18	4.49
		$p = 0.63$	$p = 0.60$	$p = 0.42$
Cruciferous vegetables (times in last 7 days)	0	3.45	5.76	5.21
	1+	6.49	3.65	5.27
		$p = 0.03$	$p = 0.14$	$p = 0.96$
Color of meat surface	Did not eat	–	2.07	2.45
	Lightly brown	5.07	3.08	4.19
	Moderately brown	4.49	5.43	5.46
	Heavily brown	4.29	5.99	5.54
		$p = 0.91$	$p = 0.34$	$p = 0.60$
Color of fish surface	Did not eat	–	4.54	5.57
	Lightly brown	3.61	2.65	3.28
	Moderately brown	6.00	6.82	6.78
	Heavily brown	4.27	5.64	4.98
		$p = 0.36$	$p = 0.15$	$p = 0.07$
Plasma lutein ($\mu g\,l^{-1}$)	⩽188.0	5.08	6.10	6.02
	188.1–271.7	3.79	5.03	4.99
	271.8+	4.04	4.61	4.69
		$p = 0.65$	$p = 0.68$	$p = 0.51$

Table 1. (Continued)

		Geometric mean (17U + 17X/137X)[a]		
		Cases	*Controls*	*All*
		(n = *43*)	(n = *47*)	(n = *90*)
Plasma β-cryptoxanthin ($\mu g\,l^{-1}$)	⩽117.7	4.87	6.12	6.08
	117.8–357.3	4.54	5.79	5.43
	357.4+	3.56	3.56	3.89
		$p = 0.64$	$p = 0.18$	$p = 0.13$
Plasma lycopene ($\mu g\,l^{-1}$)	⩽122.4	4.41	4.83	5.15
	122.5–181.5	3.32	6.24	5.29
	181.6+	5.60	4.75	5.24
		$p = 0.31$	$p = 0.65$	$p = 0.99$
Plasma α-carotene ($\mu g\,l^{-1}$)	⩽38.7	5.35	6.50	6.55
	38.8–77.7	4.26	5.11	5.10
	77.8+	4.23	3.59	4.16
		$p = 0.74$	$p = 0.19$	$p = 0.13$
Plasma β-carotene ($\mu g\,l^{-1}$)	⩽133.1	5.65	5.24	6.05
	133.2–328.9	4.58	6.32	5.85
	329.0+	3.59	3.88	3.74
		$p = 0.06$	$p = 0.40$	$p = 0.31$
Total plasma carotenoids	⩽99.2	5.93	6.00	6.83
	99.3–173.4	4.23	6.09	5.20
	173.5+	3.79	3.43	3.83
		$p = 0.36$	$p = 0.12$	$p = 0.03$
Plasma ascorbic acid ($mg\,dl^{-1}$)	⩽1.05	5.88	4.44	5.83
	1.06–1.5	5.20	6.54	4.76
	1.6+	3.01	4.50	5.10
		$p = 0.13$	$p = 0.36$	$p = 0.10$
Plasma α-tocopherol ($\mu g\,dl^{-1}$)	⩽101.6	4.86	6.19	6.23
	101.7–143.3	3.24	5.52	4.65
	143.4+	4.96	3.68	4.69
		$p = 0.37$	$p = 0.25$	$p = 0.33$
Plasma γ-tocopherol ($\mu g\,dl^{-1}$)	⩽130.9	4.38	5.41	5.09
	131.0–215.6	4.32	5.02	5.14
	215.7+	4.79	5.01	5.51
		$p = 0.94$	$p = 0.96$	$p = 0.93$
Replacement estrogen	Non-users	4.26	5.02	4.94
	Users	3.23	3.86	3.68
		$p = 0.47$	$p = 0.51$	$p = 0.25$
Acetaminophen	Users	4.47	4.91	5.06
	Non-users	4.34	9.46	9.25
		$p = 0.97$	$p = 0.13$	$p = 0.10$

[a] Adjusted by covariance analysis for race, and case-control status when appropriate.

metabolic ratio between Caucasians (7.6; Butler *et al.*, 1992; Vistisin *et al.*, 1992) and Japanese (4.6; Vistisen *et al.*, 1992; Nakajima *et al.*, 1994).

Table 1 presents the relationship of sex, age, education, smoking, alcohol, coffee, red meat, chicken and fish, and cruciferous vegetable intakes, color of meat and fish surfaces, plasma carotenoids, tocopherols and ascorbic acid levels, and use of postmenopausal replacement estrogens and acetaminophen with CYP1A2 activity. The results were reasonably similar

Table 2. Stepwise regression of CYP1A2 activity[a] on diet, plasma micronutrients, medication use and other covariates[b]

Variables[c]	*Regression coefficient (95% CI)*[d]	p	R^{2e}
Plasma lutein ($\mu g\,l^{-1}$)	−0.0017 (−0.0029, −0.0005)	0.006	0.07
Use of replacement estrogen	−0.3060 (−0.5890, −0.0230)	0.04	0.11
Use of acetaminophen	0.5576 (0.0128, 1.1024)	0.05	0.13
Plasma lycopene ($\mu g\,l^{-1}$)	0.0015 (−0.0001, 0.0030)	0.06	0.17
Plasma α-tocopherol ($\mu g\,dl^{-1}$)	−0.3163 (−0.6263, −0.0062)	0.05	0.19
Alcohol (drinks per day)	−0.1430 (−0.2438, −0.0422)	0.007	0.23
Coffee (cups per day)	0.0923 (0.0007, 0.1838)	0.05	0.27

[a] Log[(17U + 17X)/137X + 1].
[b] Potential covariates included: sex; age; race; case-control status; years of schooling; daily intakes of red meat, chicken and fish, processed meat, and cruciferous vegetables; color of meat surface; color of fish surface; cigarettes per day; alcoholic drinks per day; cans of soda per day; physical activity (METs); use of vitamin A, vitamin E and beta-carotene supplements; use of aspirin, menopausal estrogens, acetaminophen, and non-steroidal anti-inflammatory agents; family history of colorectal cancer; personal history of diabetes; weight; and plasma levels of lutein, beta-cryptoxanthin, lycopene, alpha-carotene, beta-carotene, total carotenoids, retinol, gamma-tocopherol, alpha-tocopherol, ascorbic acid, cholesterol, and triglycerides.
[c] Variables listed in order of entry into the stepwise regression.
[d] 95% confidence interval.
[e] Cumulative R^2.

for cases and controls for all variables except cruciferous vegetable which was associated with CYP1A2 activity in cases only. Weak associations with CYP1A2 activity were also suggested for sex, coffee, brownness of fish, total plasma carotenoids, plasma vitamin C level and acetaminophen use. In this univariate analysis, men had a higher metabolic ratio than women, and coffee consumption and brownness of fish were positively associated with CYP1A2 activity. There was also a negative association between the metabolic ratio and total plasma carotenoid levels, plasma vitamin C levels and acetaminophen use. There was no association of the metabolic ratio with the remaining variables in Table 1, or with weight, physical activity, family history of colorectal cancer, personal history of diabetes, plasma levels of retinol, cholesterol and triglycerides, or the use of beta-carotene, alpha-tocopherol and vitamin A supplements, aspirin, or non-steroidal anti-inflammatory agents (data not shown).

The Pearson correlation coefficients for the correlation of the metabolic ratio with the various plasma micronutrients were: −0.24 for lutein ($p = 0.02$), −0.27 for β-cryptoxanthin ($p = 0.01$), −0.26 for α-carotene ($p = 0.01$), −0.26 for β-carotene ($p = 0.01$), 0.08 for lycopene (0.48), −0.31 for total carotenoids ($p = 0.004$), −0.20 for α-tocopherol ($p = 0.06$), 0.03 for γ-tocopherol ($p = 0.75$), −0.07 for ascorbic acid ($p = 0.50$), −0.14 for retinol ($p = 0.19$), −0.06 for cholesterol ($p = 0.60$) and −0.05 for triglycerides ($p = 0.65$).

Predictors of CYP1A2 activity

The results of the stepwise regression are presented in Table 2. Among the covariates competing for inclusion in the multivariate model, only seven were selected. Plasma lutein was selected first as it explained the largest portion (7%) of the variance in the metabolic ratio. Acetaminophen use, coffee consumption and plasma lycopene were directly associated, whereas plasma lutein, use of replacement estrogens, plasma α-tocopherol and alcohol consumption were inversely associated with CYP1A2 activity. The association with plasma lycopene did not reach statistical significance ($p = 0.06$). Together, these variables explained 27% of the variation in the metabolic ratio. Sex, race, brownness of fish, plasma vitamin C levels and cruciferous vegetable intake which all showed some association with the metabolic ratio in univariate analysis, were not selected for inclusion in the multivariate model.

Discussion

This cross-sectional study conducted in a multiethnic population showed that plasma lutein, lycopene and α-tocopherol levels, coffee and alcohol intake, and replacement estrogen and acetaminophen use explained a sizeable portion (27%) of the variance in CYP1A2 activity. As a result, it appears judicious to collect information on these factors when assessing relationships of CYP1A2 to cancer risk since they may not only increase variability but, if also associated with

disease, act as confounders either spuriously inflating or attenuating the association.

Despite the use of a detailed lifestyle questionnaire, 73% of the variability in CYP1A2 activity remained unexplained in our data. This is consistent with the proposed existence of a genetic polymorphism that would explain most of the inter-individual variation in CYP1A2 activity. Such a polymorphism has been suspected based on the multimodal distribution of the metabolic ratio observed in most (Kalow & Tang, 1991; Butler *et al.*, 1992; Vistisen *et al.*, 1992; Lang *et al.*, 1994; Nakajima *et al.*, 1994) but not all (Kalow & Tang, 1991; Vistisen *et al.*, 1992) populations. However, sequencing of the *CYP1A2* gene in a Japanese study has not shown any differences of nucleotide sequence between the slow and the fast metabolizer phenotypes (Nakajima *et al.*, 1994). The search is now focussing on regulatory elements outside the gene.

In the present study, smoking was not associated with CYP1A2 activity. This is somewhat surprising in light of the inducing effect for smoking found in most past studies (Kalow & Tang, 1991; Butler *et al.*, 1992; Vistisen *et al.*, 1992; Lang *et al.*, 1994; Nakajima *et al.*, 1994; Horn *et al.*, 1995). However, the effect of smoking has been found to be variable among individuals (Vistisen *et al.*, 1992) and absent in some groups of African Americans (Nakajima *et al.*, 1992) and Chinese (Butler *et al.*, 1992). A lower amount of smoking by these populations has been proposed as an explanation for this discrepancy. The same may apply to our sample, since smokers in this study were few ($n = 8$) and averaged only 16 cigarettes per day.

Coffee consumption has been consistently found to be associated with an increased metabolic ratio in past studies (Kalow & Tang, 1991; Vistisen *et al.*, 1992; Horn *et al.*, 1995), as in this study. Much less information is available on the effect of alcohol intake on CYP1A2 in humans. The short-term consumption of four drinks (48 mg of ethanol) was found to elevate the metabolic ratio by 12% in the study by Kalow & Tang (1991). In constrast, usual alcohol consumption during the 2 weeks preceding the caffeine test was found to be inversely associated with CYP1A2 activity in the present study.

Cruciferous vegetables, when eaten in large amounts, have been shown to induce CYP1A2. For example, Vistisen *et al.* (1992) showed that a daily diet of 500 g of broccoli for 10 days increases CYP1A2 activity by 12%. However, in agreement with our data, no effect was detected for these vegetables assessed as part of the usual diet in two past studies (Vistisen *et al.*, 1992; Horn *et al.*, 1995). Thus, it is likely that induction does not occur with the dietary amounts commonly consumed in the US population.

Intake of high-temperature cooked meat containing large amounts of heterocyclic amines has been shown to increase CYP1A2 activity (Sinha *et al.*, 1994). In the present study, we failed to find an association of the metabolic ratio with intake of red meat or with an index of brownness of meat or fish after adjustment for other covariates. This may be due to our lack of information on recent intake since these questions referred to the past year for red meat or the previous 5 years for brownness.

In past studies, CYP1A2 activity was found to be lower in women than men (Kalow & Tang, 1991; Vistisen *et al.*, 1992; Nakajima *et al.*, 1994; Horn *et al.*, 1995). In some instances, this sex difference was explained by an effect of parity or oral contraceptive use (Kalow & Tang, 1991; Vistisen *et al.*, 1992; Horn *et al.*, 1995). We also found that women had lower metabolic ratios than men and that this difference was no longer significant after adjusting for use of postmenopausal estrogens, consistent with the known impairing effect of estrogen on caffeine clearance (Reitveld *et al.*, 1984; Abernethy & Todd, 1985).

CYP1A2 has also been shown to be involved in the bioactivation of paracetamol to its hepatotoxic metabolite (Raucy *et al.*, 1989). Consistent with an induction effect, use of acetaminophen was found to be positively associated with CYP1A2 activity in our data. The clinical significance of this association for fast metabolizers with liver disease needs to be explored.

We know of only one study that has examined the effect of physical activity on CYP1A2 activity (Vistisen *et al.*, 1992). Although vigorous exercise for 30 days was found to increase CYP1A2 activity; usual moderate exercise in that study, as in ours, was not associated with caffeine metabolism.

The effects of vitamins, especially vitamin deficiencies, on P450 activity have been extensively studied in experimental animals, but not in humans. Whereas a general effect of severe vitamin deficiencies has been shown to be the decreased metabolic function and lowered levels of P450-dependent activities, a mild deficiency in a certain nutrient has often been found to enhance P450-dependent activities (Yang *et al.*, 1992). None of our subjects had vitamin deficiencies, and our results are pertinent to differences with the 'normal' range of vitamin levels. We know of no other studies in humans examining the relationship between CYP1A2 activity and plasma micronutrient levels. Our finding of a negative association with plasma lutein may reflect the inhibitory effect of certain constituents

in green leafy vegetables, for which plasma lutein level is a good marker of intake (Le Marchand *et al.*, 1994). It may also reflect the effects of components in other foods correlated with lutein intake.

In vivo and/or *in vitro* studies have suggested that severe vitamin E deficiency can increase, and ascorbic acid supplementation decrease, P450 dependent activities (Chen & Ding, 1987; Kiyohara *et al.*, 1991; Williams *et al.*, 1992). Similarly, serum alpha-tocopherol and vitamin C levels have recently been shown to be inversely correlated with CYP1A1-dependent PAH-DNA adducts levels in smokers (Grinberg-Funes *et al.*, 1994). Consistent with these results, we found an inverse association of plasma alpha-tocopherol levels with CYP1A2 activity. However, we found no association between plasma vitamin C and CYP1A2 activity, after adjustment for other covariates.

Our use of cancer patients in this study is unlikely to have distorted the variation in CYP1A2 activity since they all had an intact colon at time of study, and since similar associations were found in Table 1 for cases and controls. In conclusion, this cross-sectional study confirms an enhancing effect of acetaminophen and coffee on CYP1A2 activity and suggests an inhibitory effect for estrogens, alcohol and food sources of lutein and alpha-tocopherol on this enzyme. Since CYP1A2 may play an important role in the etiology of several common cancers, these associations need to be further explored, preferably with a prospective study design.

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