A Study of Gender-based Cytochrome P4501A2 Variability: A Possible Mechanism for the Male Excess of Bladder Cancer[1]

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Abstract

One hypothesis for the well known gender difference in bladder cancer risk is that males and females metabolize carcinogens differently. The caffeine breath test (CBT) was performed on a group of healthy men and women to determine whether there was a gender difference in P4501A2 activity. Results consistent with previous data suggesting an elevation of CBT in men were observed, although this increase was not statistically significant. Among women, however, there was a significant difference between nulliparous and parous women (P = 0.03). Parous women had CBT values similar to men, whereas the results of women who had never given birth were lower. Confirming earlier studies, women taking oral contraceptives had low CBT values. Our data suggest an effect of recent caffeine consumption, with heavy coffee drinkers having higher rates of caffeine clearance. Adjustment for other weak effects, such as age, exposure to environmental cigarette smoke, history of smoking, recent meat and cruciferous vegetable consumption, and use of alcohol or other medications, did not alter these findings.

The finding of a difference between parous and nulliparous women requires further study.

Introduction

Cytochrome P4501A2 activity may be a risk factor for a number of cancers because of its role in the activation of aromatic and heterocyclic amines and estradiol. In particular, P4501A2 activity may be important in bladder cancer risk because of enhanced ability to activate aromatic amines such as 4-aminobiphenyl. Methods to evaluate P4501A2 activity are based on measuring the rate of demethylation of caffeine through assay of metabolites in the urine or ^{13}C-labeled CO_2 in expired air, the caffeine breath test. Caffeine is also a substrate for another polymorphic enzyme, NAT-2.[3] The gene that codes for NAT-2 determines the acetylation phenotype that is highly variable in the population but fixed in an individual; the CYP1A2 phenotype also exhibits interindividual variation in human populations, and a degree of genetic control is postulated. In contrast to NAT-2, however, P4501A2 is inducible by various chemicals (1–5). The phenotype that more rapidly demethylates caffeine may be at greater risk for bladder cancer based on enhanced ability to specifically activate aromatic amine carcinogens. Earlier studies examining the distribution of caffeine metabolic phenotypes have suggested that a history of exposure to inductive toxins, ethnicity, age, and gender may contribute to the metabolic phenotype (4–6).

We have investigated the hypothesis that caffeine metabolism varies by gender. Males may have elevated levels of P4501A2 that could contribute to the marked male/female inequality in the prevalence of bladder cancer in the United States. This difference has not been attributable to the known risk factors of cigarette smoking, occupational exposures, and urinary tract infections (7).

Gender and hormonal differences have been observed in previous studies. In studies comparing the half-life of caffeine in women taking oral contraceptives, in women not taking hormone-based contraception, and in men, the clearance of caffeine was delayed in women on oral contraception and most rapid in men (8, 9). Kalow and Tang (10) found a CYP1A2 index (based on caffeine metabolite excretion) that averaged 6.36 ± 0.24 (SE) for men and 5.36 ± 0.27 for women ($P <$ 0.05) in a study of 72 women and 102 men. When 18 users of oral contraceptives (3.90 ± 0.32) were excluded, the difference was no longer statistically significant. Vistisen *et al.* (11) found a CYP1A2 index in 103 male and 90 female non-smokers to be 4.7 ± 1.6 and 4.3 ± 1.9, respectively. Healthy pubertal children exhibit a similar higher CBT values in males (4). Among healthy Japanese non-smokers, Nakajima *et al.* (12) reported a significantly higher CYP1A2 index among men than among women.

In a study of a Michigan cohort exposed to polybrominated biphenyls, compounds that induce P4501A2, the median caffeine index was higher in males than in females (7.5 and 5.4, respectively; $P < 0.01$). Polybrominated biphenyls levels were similar in the 2 groups, and 2 women taking oral contraceptives had values near the median. In this exposed population, CBT values were likewise greater in males than in females (6.6 and 4.3% over 2 h; $P = 0.05$) (5).

The primary objective of this study is to determine whether P4501A2 activity exhibits a gender difference in a healthy adult population.

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[3] The abbreviations used are: NAT-2, *N*-acetyl transferase; CBT, caffeine breath test.

Materials and Methods

Study Population. Healthy adult Caucasian volunteers were recruited from an office building in Rockville, MD, among employees, their spouses, and their friends. Excluded from participation were any persons over 55 years old, those with diabetes mellitus, current smokers (any tobacco use during the last 2 years), and anyone with a medical contraindication to caffeine. Also excluded were women who were postmenopausal (surgical or natural), pregnant, or breast-feeding. These criteria were designed to maximize safety and to reduce variability.

All subjects signed a consent form and completed a screening questionnaire, which probed for medications taken in the past week, recent illness, and history of smoking and pregnancy. Subjects were instructed to fast overnight and to avoid any foods containing caffeine for 12 h before the CBT. The protocol was approved by the National Cancer Institute and Loyola University Institutional Review Board.

Clinical. Subjects (n = 113) received a letter describing the risks and benefits of the intended research. They were then contacted in person or by phone to determine their willingness to participate. Those who consented and met inclusion and exclusion criteria were scheduled for the CBT and given instructions to fast from midnight until completion of the CBT the next morning. Ten subjects failed either inclusion or exclusion criteria, nine subjects refused, and three subjects could not be scheduled. On the study date, subjects completed a questionnaire that probed for recent medication use (including over-the-counter drugs) and diet (including recent caffeine, alcohol, meat, and cruciferous vegetable consumption), provided a baseline urine sample, and were given a single dose of ^{13}C-labeled caffeine for the CBT. Samples of expired end tidal CO_2 were obtained at baseline, and then at 30 and 60 min in all subjects. For 46 subjects, samples were also collected at 90 and 120 min. Later that day, subjects voided at 4 h and collected a urine sample obtained 5 h after taking the caffeine dose.

Laboratory. The CBT consists of administering a measured oral dose of 3 mg/kg of caffeine (maximum dose, 200 mg) isotopically labeled at the 3-C position and determining the $^{13}CO_2$:$^{12}CO_2$ ratio in expired air with the use of differential gas isotope ratio mass spectrometry (5). The isotope ^{13}C is naturally occurring, nonradioactive, stable, and without harmful effect in animals (13). The CBT measures only the primary route of caffeine metabolism, 3-*N*-demethylation, which is catalyzed exclusively by P4501A2. CBT results are expressed as the percentage of administered $^{13}CO_2$ expired over 1 h.

Statistical. Analyses using univariate descriptive statistics, Student's t tests (test used unless otherwise indicated), linear correlations (Pearson and Spearman), Fisher's exact test (χ^2), and linear regression analyses were performed with the use of SAS software (SAS Institute Inc., Cary, N.C.) on a mainframe computer.

Results

Ninety-one eligible subjects, 47 men and 44 women, were given the CBT. Five women were taking oral contraceptives and were excluded from the analyses except where noted, leaving a total of 86 subjects. In this group, the mean CBT result was 1.53%, representing the percentage of administered ^{13}C exhaled during the first 1 h. The SE was 0.07, the range was 0.27–3.30, and the median was 1.43. The age range was 19–55 years, with a mean of 36.3 and a median of 37.5. There was no association of the CBT value with age (Table 1).

The CBT (mean ± SE) was 1.61 ± 0.10 for men (n = 47) and 1.43 ± 0.11 for women (n = 39). This difference was not statistically significant (P = 0.28). Women who had never borne a child (n = 20) had significantly lower CBT results, with a mean value of 1.20 ± 0.11, compared to the mean for parous women (n = 19), 1.67 ± 0.17 (P = 0.03). A similar mean was noted for women by lactation status, predominantly because most parous women had breast-fed. For women who had ever breast-fed a child for 2 weeks or longer (n = 18), the mean CBT value was 1.67 ± 0.18.

Although current smokers were excluded from participation, the study included 27 former smokers. They had a slightly elevated mean CBT value, 1.64 ± 0.17, compared to subjects who had never smoked (n = 59), 1.48 ± 0.07 (P = 0.32). Those who reported exposure to the cigarette smoke of others but did not smoke themselves (n = 9) had a mean CBT result of 1.76 ± 0.25, whereas the mean value of those who were not "passive smokers" (n = 77) was 1.50 ± 0.08. This difference was not statistically significant (P = 0.28).

Caffeine use was related to CBT value in this sample. In subjects who consumed four or more cups of coffee on the day before the CBT (n = 10), the mean CBT value was 1.93 ± 0.15; the mean for the remainder (n = 76) was 1.48 ± 0.08 (P = 0.05). A graded increase in CBT value was seen with increasing coffee consumption. Considering "usual intake," the mean for those who drank no caffeinated coffee (n = 34) was 1.46 ± 0.10, which rose to 1.94 ± 0.26 for subjects consuming four or more cups daily (n = 7). A similar trend was observed with caffeinated tea consumption, although not with cola. To better characterize this relationship, the upper and lower quartiles of CBT values were compared by different measures of caffeine intake. Among subjects in the lower quartile, "slow metabolizers," there was a deficit of heavy coffee drinkers; none of the 19 slow metabolizers drank 4 or more cups of coffee the day before (χ^2, Fisher's exact test; P = 0.08). Conversely, those "fast metabolizers" in the upper CBT quartile had a greater than expected frequency of heavy coffee users, with 6 of 22 reporting that level of consumption (Fisher's exact test; P = 0.01). When caffeine intake from all sources was summed and categorized into low and high levels of consumption ("low" is less than 700 mg yesterday), the same pattern was observed. For slow metabolizers, the χ^2 value was 2.46, P = 0.12; for fast metabolizers, χ^2 was 6.46, P = 0.01.

The use of alcohol in relation to the CBT was examined. For subjects who had consumed 1 or more alcoholic drinks of any kind in the 3 days before the CBT (n = 46), the mean CBT result was 1.60 ± 0.11, and for nondrinkers (n = 40) the mean was 1.44 ± 0.09. The only subgroup of alcohol drinkers with a suggestive increase in mean CBT value was beer drinkers, who consumed 3 or more beers in the 3 days preceding the CBT. For this group (n = 11), the mean value was 1.80 ± 0.27, compared to those without such exposure (n = 75), 1.49 ± 0.07 (P = 0.17). A subgroup analysis of those who drank wine or hard liquor showed no difference in mean CBT.

Meat consumption, cooking method, and doneness of meat were examined. The quantity of meat consumed 1–3 days before the CBT showed no consistent association with CBT value. If meat was prepared by grilling, barbecue, or oven broiling (methods that use high heat and may expose meat to flame or fat drippings, n = 25), the CBT was 1.66 ± 0.16; those who ate meat prepared by baking, frying, boiling, or other method had a mean CBT value of 1.44 ± 0.13 (P = 0.29). Likewise, doneness of meat shows a weak association with CBT result, with those who ate very well-done meat (n = 5) having a nonsignificantly higher mean CBT value than those

Table 1

Group	*n*	CBT	±SE	Group	*n*	CBT	±SE
All subjects	91	1.50	0.07	Meat (eaten yesterday)			
OC[a] users excluded	86	1.53	0.07	Meat	38	1.59	0.12
Men	47	1.61	0.10	No meat	37	1.48	0.09
Women	44	1.38	0.11	Doneness			
OC users[b]	5	1.01	0.39	Rare	0		
No OC	39	1.43	0.11	Medium	25	1.37	0.11
Parous	19	1.67	0.17	Well-done	33	1.51	0.14
Nulliparous	20	1.20	0.11	Very well-done	7	1.83	0.28
Ever breastfeed	18	1.67	0.18	Cooking method			
Never breastfeed	21	1.22	0.11	Grilled, barbecued, or broiled	25	1.66	0.16
Age (in years)				Other method	24	1.44	0.13
19–29	25	1.45	0.13	Number of 4-ounce servings eaten			
30–39	27	1.67	0.12	1	29	1.60	0.13
40–55	34	1.47	0.12	2 or more	16	1.43	0.19
Smoking				Vegetables eaten yesterday (no. of servings)			
Ever smoked	27	1.64	0.17	None	57	1.60	0.09
Never smoked	59	1.48	0.07	1	20	1.31	0.17
Passive exposed	9	1.76	0.25	2	9	1.60	0.25
No passive	77	1.50	0.08	Medications in past week[b]			
Alcohol (in past 3 days)				Seldane			
Beer	28	1.53	0.14	Y	13	1.27	0.19
No beer	58	1.53	0.08	N	73	1.57	0.08
Wine	23	1.64	0.15	Sudafed			
No wine	63	1.48	0.08	Y	5	1.35	0.37
Hard Liquor	8	1.52	0.28	N	81	1.54	0.08
No liquor	78	1.53	0.08	Ibuprofen			
Drinkers	46	1.61	0.11	Y	19	1.49	0.14
Nondrinkers	40	1.44	0.09	N	67	1.53	0.09
Coffee (no. of cups yesterday)				Aspirin			
None	32	1.49	0.11	Y	17	1.46	0.17
0.5–2	32	1.45	0.13	N	69	1.55	0.08
3–4	17	1.62	0.20	Tylenol			
5+	5	2.00	0.12	Y	12	1.43	0.23
Coffee (no. of cups/usual day)				N	74	1.55	0.08
None	34	1.46	0.10	Any	40	1.50	0.11
0.5–2	23	1.48	0.14	None	46	1.56	0.09
3–4	22	1.56	0.17				
5+	7	1.94	0.26				

[a] OC, oral contraceptive.
[b] Oral contraceptive users were excluded from analysis.
[c] Users of antibiotics, exogenous sex hormones and thyroxin in past week were excluded from participation.

who ate rare, medium, or well-done meat. (1.87 ± 0.38 *versus* 1.54 ± 0.12; $P = 0.36$).

Recent consumption of cruciferous vegetables, such as broccoli, cabbage, and kale, was not associated with CBT value in this sample, nor was a vegetarian diet.

For women, the date of onset of the last menstrual period was recorded. No significant correlation between CBT and last menstrual period was observed. When the menstrual cycle was divided into three time intervals, crudely reflecting serum estrogen levels, no correlation with caffeine clearance was detected.

Individuals with a recent history of taking analgesic or antihistamine medications for a variety of minor ailments, including musculoskeletal pain, menstrual discomfort, allergies, and upper respiratory illness, exhibited slightly lower mean CBT values. These differences were not significantly different from those of individuals who did not consume these medications.

A linear regression model suggested that smoking, coffee consumption, and alcohol consumption were independently associated with CBT results. The greatest effect of these was for coffee, which appeared to increase the CBT value by 0.07 percentage points for each additional cup (up to five).

Discussion

In previous studies of induced and noninduced populations, investigators have described a small gender difference in the rate of caffeine metabolism. In this sample, we have found a similarly small difference but suggest that it is explained by a difference between parous and nulliparous women. Women who had ever given birth had a mean caffeine clearance measured by the CBT that was similar to that of men (1.67 ± 0.17 for parous women, 1.61 ± 0.10 for men). The mean CBT value for the 20 nulliparous women was significantly lower (1.20 ± 0.11), which accounted for the perceived gender difference. Whether pregnancy itself or some factor associated with it, such as lactation, is responsible for this persistent induction is unclear; we have insufficient numbers of parous women who did

not breast feed their child to distinguish between these two possibilities. The one parous woman in our sample without a history of lactation had a CBT of 1.61, near the mean for parous women.

The mechanism for this effect is not clear. Postpartum P450 isozyme changes have been observed in mice (14). In humans, it is clearly established that during pregnancy, the half-life of caffeine increases (15–16), and this is accompanied by a decline in mean caffeine use during pregnancy. Immediately after pregnancy, these changes quickly reverse (17), but to our knowledge long-term postpartum follow-up has not been reported. Our findings suggest that women rebound from the pregnancy-induced decline in caffeine clearance to a level higher than at prepregnancy. Lactation or lifestyle changes (smoking, diet, caffeine) may account for a component of this change.

It has long been recognized that cigarette smoking increases caffeine clearance (18, 19), presumably from an inductive effect of an inhaled chemical. Several constituents of tobacco smoke increase the activity of hepatic cytochrome P-450 enzymes, including nicotine, 3-methylcholanthrene, and benzo(*a*)pyrene (20). Our data suggest that this induction is not fully reversible in persons with a history of smoking, although a prospective study would be necessary to establish this. The tendency for persons exposed to environmental tobacco smoke to have increased caffeine clearance is of interest, although it is based on only 9 exposed individuals. Additional investigation is required to verify the effect of passive smoking on CYP1A2 that, if confirmed, could provide a mechanistic explanation for proposed carcinogenic effects of environmental tobacco smoke, *i.e.*, through enhanced activation of carcinogens.

Recently, the role of CYP1A2 in estrogen metabolism has been shown to mediate the C2-hydroxylation of estradiol-17β. Smoking induces this metabolic pathway at the expense of C17-oxidation to ketone and C16 α-hydroxlation (21–23). The biological significance of this induction may be a reduction in the number of functional estrogen molecules and a diminished estrogen response.

Michnovicz *et al.* (21) has reported that dietary indole-3-carbinol, found in cruciferous vegetables, can alter the pathway of estrogen metabolism. The P4501A2 enzyme has multiple substrates, and compounds that induce C2-hydroxylation can be expected to increase caffeine clearance (and the CBT value) as well. A previous report identified a 12% increase in P4501A2 activity after the ingestion of 500 g of broccoli for 10 days. In our sample, eating cruciferous vegetables did not appear to increase caffeine metabolism. The most likely explanation for our failure to observe an effect is that subjects did not eat a substantial quantity of cruciferous vegetables. The day before the CBT, mean cruciferous vegetable intake was only 0.5 servings; over the previous 3 days, the mean total intake was only 1.7 servings.

This same explanation may account for our failure to find a consistent inductive effect of alcohol consumption, which has been reported previously (10). In our study population, fewer than one-half of subjects reported any alcohol consumption in the 3 days before testing, and most of the drinkers consumed less than 3 drinks over that time, an intake which may fall below a threshold for induction.

Epidemiological studies have revealed a substantial male excess of bladder cancer in the United States that persists after adjustment for known risk factors (7). We have hypothesized that increased P4501A2 expression in males could contribute to this through enhanced activation of bladder carcinogens. This would be broadly consistent with the plateau (*i.e.*, lack of further increase in risk with incremental increases in pack-year history of smoking beyond a certain level) in the bladder cancer/smoking association that contrasts to the linear dose response between lung cancer rates and increasing smoking that persists even at high smoking levels (24).

We observed an association of coffee consumption with P4501A2 activity. It is not clear whether this is due to enzyme induction by caffeine or whether the trait itself fixes the rate of metabolism and thereby the frequency of caffeine consumption by a habituated individual. Whatever the mechanism, this linkage might explain inconsistent reports of excess bladder cancer risk associated with coffee. Other effects inconsistently observed in association with coffee/caffeine consumption, such as spontaneous abortion (25), low birth weight (26), and teratogenicity (27), might also be due to enzyme activity rather than primary effects of caffeine.

We have not found a gender difference between men and all women but rather between men and a subset of women, those who have never given birth. If women develop increased P4501A2 activity after childbirth, their risk of subsequent bladder cancer would be expected to increase as well. In a study examining bladder cancer and parity, no effect of parity was observed on the cancer rate in all women. Among women who never smoked, a protective effect of prior birth was observed (odds ratio, 0.51; 95% confidence interval, 0.30–0.88) (28). If correct, this isolated effect may be the result of a quantitatively much larger effect of cigarette smoking on CYP1A2 than of parity. Alternatively, the idea that an induced CYP1A2 phenotype is an important risk factor for bladder cancer may be incorrect.

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