



Alcohol, Smoking, and Caffeine in Relation to Fecundability, With Effect Modification by NAT2

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PURPOSE: Common polymorphisms in the *N*-acetyltransferase-2 (NAT2) metabolic enzyme determine slow or rapid acetylator phenotypes. We investigated the effects of alcohol, smoking, and caffeine on fecundability, and determined whether the effects were modified by NAT2.

METHODS: Three NAT2 polymorphisms were genotyped in 319 women office workers participating in a prospective pregnancy study (1990–1994). Women were ages 20–41 and at risk for pregnancy. Discrete-time survival analysis was used to determine the effects of alcohol, smoking, and caffeine on fecundability and evaluate effect modification by NAT2.

RESULTS: We followed 319 women (161 slow acetylators, 158 rapid) for an average of 8 menstrual cycles, resulting in 124 pregnancies. There was no effect of caffeine on fecundability. Drinking ≥ 1 alcoholic drink per day and current smoking were significantly associated with reduced fecundability, but only among slow acetylators (adjusted fecundability odds ratio [FOR] for smoking = 0.34; 95% confidence interval, 0.22–0.90; adjusted FOR for ≥ 1 drink per day = 0.20; 0.05–0.92). There was no effect among rapid acetylators.

CONCLUSIONS: NAT2 status significantly modified the effects of alcohol and smoking on fecundability, emphasizing the importance of incorporating genetic and metabolic information in studies of reproductive health. Replication of this study is warranted.

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INTRODUCTION

Subfertility and infertility are growing public health issues, largely because women are waiting until older ages to attempt pregnancy. Fecundability, a related concept, is defined as the probability of pregnancy per time unit, and is operationalized by counting the number of cycles at risk until a pregnancy occurs (time to pregnancy) (1). Smoking, alcohol, and caffeine are modifiable risk factors that are possibly related to reduced fecundability, but epidemiologic studies of these exposures have had inconsistent results (2, 3).

Caffeine was found to reduce fecundability in a prospective study by Wilcox et al. (4), where consuming > 3150 mg per month (about 1 cup of coffee per day) was associated with a 50% reduction in fecundability, which prompted research in this area. However, other prospective studies have found either no effect (5) or even increased fecundability (6).

Results for alcohol and smoking are more consistent: typically, both are associated with increased time to pregnancy (indicating reduced fecundability), although some have found no effect. Among prospective studies, Buck Louis et al. (7) found that fecundability was reduced 4% for each alcoholic beverage consumed in a 28-day period, and Jensen et al. (8) observed a dose–response between alcohol intake and increased time to pregnancy. Wilcox et al. (4) did not find an association between alcohol and fecundability. Jensen et al. (9) found reduced fecundability associated with a woman's smoking, particularly if she was also exposed to her mother's smoking *in utero*. However, Buck Louis et al. reported no effect of smoking (7).

Genetic heterogeneity is one factor that may explain the inconsistent results of previous studies. *N*-acetyltransferase-2 (NAT2) is an enzyme that catalyzes the *N*-acetylation of aromatic and heterocyclic amines (10). NAT2 metabolizes

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Selected Abbreviations and Acronyms

BMI = body mass index (kg/m^2)
 CEU = Utah residents with Northern and Western European ancestry
 CI = confidence interval
 FOR = fecundability odds ratio
 hCG = human chorionic gonadotropin
 IQR = interquartile range
 NAT2 = *N*-acetyltransferase-2 (Gene)
 NAT2 = *N*-acetyltransferase-2 (Protein)
 SNP = single nucleotide polymorphism

and detoxifies xenobiotics such as caffeine, tobacco smoke, pesticides, and prescription drugs (11, 12). Polymorphisms in the NAT2 gene determine haplotypes that correspond with slow or rapid acetylator phenotypes (13, 14).

NAT2 haplotypes may modify associations between toxicants and various outcomes. For example, slow acetylators are especially susceptible to hepatotoxicity during prescription drug treatment (15). Acetylator status also modified the association of smoking with risk of colorectal adenoma and bladder cancer, with slow acetylators being more susceptible (16, 17). No studies to date have investigated NAT2 polymorphisms as effect modifiers of exposures that affect fecundability or fertility.

This study investigated the effects of caffeine, alcohol, and smoking on fecundability using daily exposure data and prospectively measured time to pregnancy, including subclinical pregnancies. Furthermore, this study addresses potential genetic heterogeneity by assessing whether NAT2 acetylator status modified any of these effects.

MATERIALS AND METHODS**Population**

The Study of Women Office Workers was a prospective study of fertility and menstrual function conducted from 1990 to 1994 (18, 19). A total of 4640 women completed eligibility questionnaires. Women between the ages of 18 and 40 who were sexually active in the preceding month were eligible. Exclusion criteria included the use of hormonal contraception, an intrauterine device, or current infertility (>1 year of unprotected intercourse without pregnancy). Women with a history of hysterectomy, infertility, polycystic ovaries, tubal ligations, or with partners who had vasectomies were also excluded. Of the 855 women who met these criteria, 563 (64%) agreed to participate. Fourteen of these women became newly ineligible, and 79 women did not collect any urine samples. The remaining 470 women were requested to complete daily diaries for 12 months or until pregnancy. They collected urine samples on the first 2 days of every menstrual cycle (where the first day of bleeding is considered day 1). If menses did not occur

when expected, 2 urine samples were collected 1 week after the date of expected menses, based on the usual cycle length reported in the intake interview. Urine samples were stored in the woman's freezer until a courier retrieved them. Information on year of birth, race, ethnicity, body mass index (BMI; kg/m^2), age at menarche, previous months of unprotected intercourse, desire to become pregnant, and reproductive and medical histories was recorded during an interview at the onset of the study.

Exposure Information

Women recorded daily information on caffeine, alcohol, smoking, and covariates in the diaries. Caffeine was recorded as cups of caffeinated tea, coffee, and cola. Alcohol was recorded as cans of beer, glasses of wine, and shots of liquor. Smoking was recorded as the number of cigarettes. Menstrual bleeding, intercourse (yes/no), and birth control use (e.g., barrier method, withdrawal) were indicated on a daily basis. A variable for the frequency of unprotected intercourse during the (estimated) ovulatory window was created from the intercourse and birth control variables. The 10-day window starts 7 days before and ends 2 days after the estimated day of ovulation. The day of ovulation was estimated by counting 14 days before the onset of the next menses. This method accounts for the 14-day lifespan of the corpus luteum (20) and the relatively consistent length of the luteal phase (21).

Caffeine was converted into milligrams using the following factors: 1 cup caffeinated coffee = 150 mg; 1 cup caffeinated tea = 55 mg; 1 cup caffeinated cola = 45 mg, which are within the ranges of reported values (22). Caffeine was averaged over each cycle and categorized into low (mean caffeine intake <150 mg/d), medium (150–300 mg/d) and high (>300 mg/d) groups. Alcohol was averaged over each cycle and divided into 3 categories: nondrinkers, <1 drink per day, and ≥ 1 drinks per day. Categories were created to be practically meaningful while maintaining stable sample sizes. Current smoking was divided into nonsmokers, <10 cigarettes per day and ≥ 10 cigarettes per day. These variables are all cycle specific: in other words, a woman may be categorized as a smoker for 1 cycle and a nonsmoker for the next. For cycles in which a pregnancy occurred, the mean alcohol, caffeine, and smoking values were calculated for that woman's average cycle length (e.g., 28 days) and subsequent days were excluded.

NAT2 Genotyping

DNA was extracted from the urine samples using the Qiagen MagAttract DNA Mini M48 kit in combination with the BioRobot M48 workstation (Qiagen, Valencia, CA). DNA was extracted in duplicate from 20% of the women,

to check genotyping accuracy. We genotyped 3 single nucleotide polymorphisms (SNPs) in NAT2: rs1799929 (C481T), rs1799930 (G590A), and rs1208 (A803G). SNPs were chosen based on minor allele frequencies in HapMap (>5%) and their ability to differentiate slow from rapid acetylators. Slow acetylator haplotypes were defined by having either a C→T polymorphism at rs1799929, a G→A polymorphism at rs1799930, and/or an A→G polymorphism at rs1208. Haplotypes were assigned using PHASE (version 2.1.1) using the expectation-maximization algorithm (23, 24). The *4 haplotype (wild type) is the rapid acetylator haplotype. Zero copies of this rapid haplotype determined slow acetylator status, whereas 1 or 2 determined rapid. Good concordance between genotype and acetylator phenotype has been documented (25, 26).

The SNPs were genotyped using the Beckman Coulter GenomeLab SNPstream system (Beckman Coulter, Inc., Brea, CA) using primers designed by [Autoprimer.com](#) (27). Up to 6 ng of DNA were used for genotyping. The GenomeLab SNPstream Genotyping System Software Suite version 2.3 was used to check the quality of the genotyping results, along with visual inspection of signal intensity and clustering pattern to make individual calls where questionable.

Statistical Analyses

Time to pregnancy was measured as the number of menstrual cycles up until and including the cycle when a pregnancy was achieved. Only first pregnancies were included in the present analysis. Subclinical pregnancies were detected by measuring human chorionic gonadotropin (hCG) levels in urine samples collected on days 1 and 2 of the subsequent menstrual cycle, or 1 week after the expected date of menses, in cases where menses had not yet occurred. The hCG was analyzed and pregnancies were diagnosed as described by Small et al. (28). Briefly, 2 samples of hCG >0.25 ng/μL determined pregnancy. We used questions from the entry interview to determine an approximate number of cycles a woman had been at risk for pregnancy at study entry. In our primary analyses, we added these cycles to prospective cycles at risk, meaning that if a woman indicated she had been at risk for pregnancy for 3 cycles before enrollment, her prospective cycle 1 would instead be entered as cycle 4. As a sensitivity analysis, we instead included the prior cycles at risk in the model as a covariate.

The effects of the exposures and covariates on time to pregnancy were assessed using discrete-time survival analysis. This approach is more powerful than dichotomizing reproductive success as fertile/infertile (1). The discrete-time hazard is the probability that a woman became pregnant in a given menstrual cycle conditional on a pregnancy not occurring in prior cycles. The likelihood is equivalent to

that for binary regression models (29). We modeled the per cycle probability of conception using logistic regression and generated fecundability odds ratios representing the odds of conception in one group compared with the odds of conception in the referent group. We adjusted for variables that were significantly associated with fecundability in the multivariable model.

Interactions between caffeine, alcohol, smoking, and NAT2 were assessed by including interaction terms in the multivariable models. Three separate models were run to evaluate interactions of NAT2 acetylator status with smoking, alcohol, and caffeine. Evaluating all 3 interactions simultaneously was not feasible, given our limited sample. Models evaluating 1 interaction (e.g., smoking/NAT2) were adjusted for variables significantly associated with fecundability, in addition to being adjusted for the other exposures of interest (e.g., caffeine, alcohol). We dichotomized smoking (smoker/nonsmoker), alcohol intake (<1 vs. ≥1 drink per day) and caffeine intake (<300 vs. ≥300 mg/d) for the interaction models, to allow for stable subgroup sizes. As a sensitivity analysis, the NAT2 interaction models were restricted to non-Hispanic whites with genotype information (n = 248) to reduce the potential for confounding by population stratification.

The Emory Institutional Review Board approved the study protocol after complete de-identification of all samples, surveys and interviews.

RESULTS

Study Participants

Study participants were mostly white, non-Hispanic, married, and had some college education (Table 1). A substantial number of women smoked cigarettes (39%) and drank alcohol (89%) during follow-up. Although all participants were at risk for pregnancy according to our definition, only 23% reported that they were trying to become pregnant. The median number of cycles at risk before study entry was 1 (interquartile range [IQR] = 0, 7). Thirty-eight percent of the 470 women (n = 179) achieved pregnancy during the study.

Of the 470 women we attempted to genotype, high-quality DNA (resulting in successful genotyping) was obtained from 319 of the women. The 319 women genotyped for NAT2 were followed for an average of 8 menstrual cycles, resulting in 124 pregnancies. Of those women who became pregnant, the median number of cycles until pregnancy was achieved was 3 (IQR = 1, 7). Figure 1 shows the cumulative proportion of women who became pregnant during the study.

The 319 women who were genotyped for NAT2 were not different from the other 151 women for any of the

TABLE 1. Population characteristics of 470 women office workers and 319 women who were genotyped for NAT2

	All women (n = 470) n (%) ^a	Genotyped women (n = 319), n (%)	Chi-square p-value (2-sided) ^b
Age (yrs)			.74
19–24	41 (9)	30 (9)	
25–29	150 (32)	102 (32)	
30–34	164 (35)	113 (35)	
35–41	115 (24)	74 (23)	
Race			.21
White	376 (80)	248 (78)	
African-American or black	58 (12)	45 (14)	
Asian	13 (3)	8 (3)	
Other	23 (5)	18 (6)	
Ethnicity			.36
Hispanic	22 (5)	18 (6)	
Non-Hispanic	445 (95)	299 (94)	
Missing	3 (1)	2 (1)	
Marital status			.91
Married	309 (66)	208 (65)	
Single (never married)	127 (27)	87 (27)	
Divorced/separated/widowed	34 (7)	24 (8)	
Ever pregnant			.91
Yes	285 (61)	194 (61)	
No	185 (39)	125 (39)	
Highest education			.23
High school or technical	110 (23)	76 (24)	
Some college	156 (33)	112 (35)	
College graduate	203 (43)	131 (41)	
Missing	1 (0)	0 (0)	
BMI (kg/m ²)			.04
<18.5	23 (5)	9 (3)	
18.5–<25	297 (63)	204 (64)	
25–<30	91 (19)	62 (19)	
≥30	58 (12)	43 (13)	
Missing	1 (0)	1 (0)	
Trying to get pregnant			.39
Yes	108 (23)	73 (23)	
No	332 (71)	229 (72)	
Missing	30 (6)	17 (5)	
Months at-risk for pregnancy before study start			.22
<1	220 (47)	148 (46)	
1–3	96 (20)	60 (19)	
4–6	36 (8)	24 (8)	
>6	117 (25)	87 (27)	
Mean times unprotected intercourse in the ovulatory window during follow-up			.81
<1	293 (62)	201 (63)	
1–2	97 (21)	63 (20)	
>2	65 (14)	48 (15)	
Missing	15 (3)	7 (2)	
Mean smoking during follow-up (cigarettes/day)			.57
0	287 (61)	161 (63)	
<10	117 (25)	63 (23)	
≥10	66 (14)	38 (14)	
Mean alcohol intake during follow-up (drinks/day)			.10
0	54 (11)	34 (11)	
<1	346 (74)	230 (72)	
≥1	70 (15)	55 (17)	
Mean caffeine intake during follow-up (mg/d)			.99
≤150	256 (54)	174 (55)	
151–300	137 (29)	93 (29)	
>300	77 (16)	52 (16)	

^aPercentages may not add to 100 due to rounding.^bChi-square p-value for comparing those genotyped (n = 319) with those not genotyped (n = 151). Missings not included in chi-square calculations.

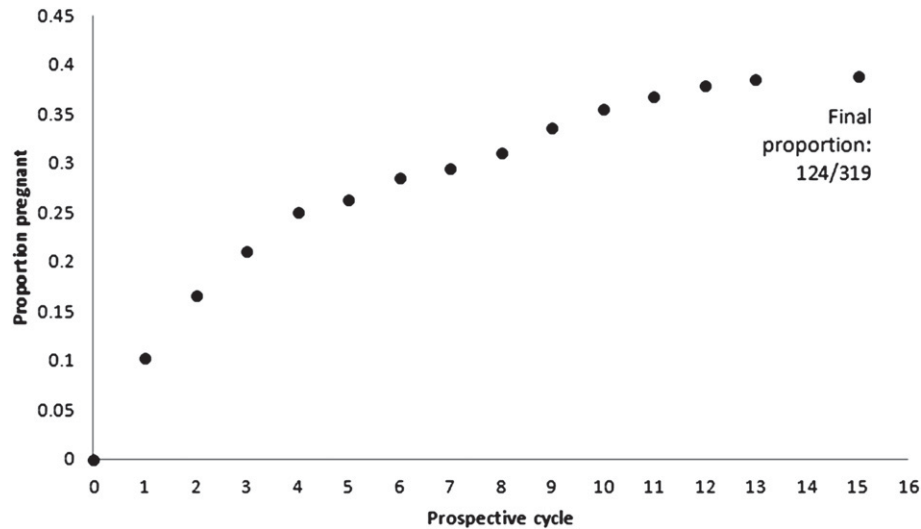


FIGURE 1. Cumulative proportion of genotyped women achieving pregnancy during the study.

characteristics shown in Table 1, with the exception of BMI, where there were fewer women with a BMI < 18.5 who were successfully genotyped than expected. The *p*-values in Table 1 were obtained by comparing the distribution of characteristics of those who were genotyped (*n* = 319) with those who were not genotyped (*n* = 151).

The median intakes of the exposures of interest were 2 alcoholic drinks per week (IQR = 0, 5), 2 caffeinated beverages per day (IQR = 1, 3), and 0 cigarettes per day (IQR = 0, 1). The maximum reported values for any cycle were 68 alcoholic drinks per week, 17 caffeinated beverages per day, and 56 cigarettes per day; each maximum value came from a different participant.

NAT2 Genotyping Results

The minor allele frequencies of the NAT2 SNPs among non-Hispanic whites were similar to those in the CEU population of HapMap (Utah residents of Northern and Western European ancestry): 0.39 for rs1799929, 0.30 for rs1799930, and 0.40 for rs1208 in the study sample, compared with 0.42, 0.30, and 0.39 in HapMap (30, 31). The concordance of genotype duplicates was 100% for all 3 SNPs. The frequency of the rapid acetylator haplotype in the sample was 0.28, and thus the frequency of the slow acetylator haplotype was 0.72. Of the 319 women, 161 (50%) carried 2 copies of a slow haplotype and were assigned “slow” acetylator status. The other individuals carried ≥1 copy of the rapid acetylator haplotype and were assigned “rapid” acetylator status. Of the non-Hispanic whites, 54% were slow acetylators; this is consistent with other estimates (59%, 62%) in other Caucasian populations (26). African Americans had a higher proportion of rapid acetylators than whites (65% vs. 46%).

Effects of Smoking, Alcohol, and Caffeine on Time to Pregnancy

The multivariable model shown in Table 2 includes the 3 exposures of interest along with covariates that were significantly associated with fecundability. Age at menarche, marital status, ethnicity, black race, other race, partner's age, and gravidity (0, >0) were not associated with fecundability when included as covariates, and did not meaningfully alter the associations of caffeine, alcohol, or smoking with fecundability (results not shown). Older age and obesity (BMI > 30) were significantly associated with reduced fecundability (fecundability odds ratio [FOR] < 1). A higher frequency of unprotected intercourse during the ovulatory window and the intention to become pregnant were associated with a higher fecundability (FOR > 1).

Alcohol intake was significantly associated with reduced fecundability. A dose-response effect was apparent, with those who drink <1 drink per day experiencing a 30% reduction, and those who drink ≥1 drinks per day experiencing a 50% reduction in the FOR when compared with nondrinkers (Table 2; FOR, 0.50; 95% confidence interval [CI], 0.28–0.89). Smoking and caffeine were not associated with fecundability in this model whether modeled categorically (as shown) or continuously as cigarettes per day or milligrams of caffeine per day (results not shown). Including prior cycles at risk as a covariate in the analysis (as opposed to prospectively measured cycles) did not alter the results.

Interactions With NAT2

The 3 interaction models revealed significant interactions between NAT2 status and smoking and alcohol, but not caffeine (Table 3). Models adjusted for age only are presented, as well as fully adjusted models which include the

TABLE 2. Associations with time to pregnancy in a multivariable model^a

Variable	Fecundability odds ratio	95% confidence interval	Score test <i>P</i> -value (2-sided)
Age			.04
Per year	0.96	0.92–1.00	
Unprotected intercourse during the ovulatory window			<.0001
Times per window	1.36	1.25–1.48	
Trying to get pregnant			.002
No	Ref		
Yes	1.80	1.23–2.60	
Body mass index (kg/m ²)			.02
<18.5	0.69	0.27–1.75	
18.5–<25	Ref		
25–<30	0.83	0.54–1.30	
≥30	0.40	0.21–0.73	
Average caffeine intake (mg/d)			.83
<150	Ref		
150–300	1.02	0.67–1.56	
≥300	0.89	0.58–1.38	
Average alcohol intake (drinks/day)			.05
0	Ref		
<1	0.70	0.47–1.04	
≥1	0.50	0.28–0.89	
Smoking (cigarettes/day)			.80
0	Ref		
<10	1.04	0.65–1.67	
≥10	1.19	0.71–2.00	

^aAll variables listed are in the model simultaneously.

important covariates shown in Table 2. The minimally adjusted models suggested possible interactions between alcohol and smoking with NAT2 status. The fully adjusted models revealed statistically significant interactions. In the NAT2/smoking interaction model, the FOR for smoking among slow acetylators was 0.34 (95% CI, 0.22–0.90) and among rapid acetylators was 1.65 (95% CI, 0.93–2.94; 2-sided $p_{\text{interaction}} = .002$). These values can be calculated from the values shown in Table 3 (e.g., the effect of smoking among slow acetylators = $0.35/1.05 = 0.34$). In the NAT2/alcohol interaction model, the FOR for alcohol (≥1 vs. <1 drink per day) was 0.20 (95% CI, 0.05–0.92) among slow acetylators and 1.16 (95% CI, 0.61–2.22) among rapid acetylators ($p_{\text{interaction}} = .03$). There was no interaction between NAT2 and caffeine intake (≥300 vs. <300 mg/d): among slow acetylators, FOR = 0.81 (95% CI, 0.36–1.81); among rapid, FOR = 0.96 (95% CI, 0.50–1.85).

In each of these models, NAT2 acetylator status alone was not associated with fecundability, although there was some suggestion that rapid acetylators have a higher fecundability. When acetylator status was added to the no-interaction model in Table 2, the fecundability odds ratio comparing rapid versus slow acetylators was 1.44 (95% CI, 0.89–2.35).

When the 3 interaction models shown in Table 3 were restricted to the non-Hispanic whites ($n = 247$), the smoking/NAT2 interaction was still significant ($p_{\text{interaction}}$

= .03) and the significance of the alcohol/NAT2 interaction was not changed ($p_{\text{interaction}} = .03$). The effect estimates for the interaction models were similar (data not shown).

Factors Associated With Retention in the Study

To investigate whether differential drop out from the study may have biased our results, we compared characteristics of those who remained in the study for >6 cycles with those who voluntarily left the study during or before the 6th cycle. We excluded women who became pregnant during or before the 6th cycle from this analysis since they did not have an opportunity to remain in the study past 6 cycles. Women who remained in the study were not different from women who dropped out with respect to age, race, ethnicity, body mass index, current smoking, alcohol consumption, caffeine consumption, or NAT2 acetylator status (Chi-square $p > .10$ for each variable; results not shown). However, women who remained in the study had higher levels of education ($p = .05$).

DISCUSSION

In this study, we estimated the effects of caffeine, alcohol, and smoking on fecundability, and assessed whether these effects were modified by NAT2 acetylator activity. No effect

TABLE 3. Models examining interactions with NAT2 acetylator status

Interaction model ^a	NAT2 status/exposure	Adjusted for age (n = 319 women; 2413 cycles)				Adjusted for variables shown in Table 2 ^a (n = 295 women; 2327 cycles)			
		No. of cycles ^b	Fecundability odds ratio	95% CI	Score test p-value for interaction (2-sided)	No. of cycles	Fecundability odds ratio	95% CI	Score test p-value for interaction (2-sided)
NAT2/caffeine (mg/d)	Rapid/<300	859	(ref)		.35	822	(ref)		.21
	Slow/<300	871	0.75	0.49–1.15		839	0.72	0.45–1.14	
	Rapid/≥300	292	0.93	0.50–1.73		292	0.96	0.50–1.85	
	Slow/≥300	387	0.62	0.34–1.14		374	0.58	0.30–1.12	
NAT2/alcohol (drinks/day)	Rapid/<1	881	(ref)		.07	844	(ref)		.03
	Slow/<1	1086	0.80	0.54–1.19		1040	0.83	0.54–1.28	
	Rapid/≥1	270	0.92	0.50–1.69		270	1.16	0.61–2.22	
	Slow/≥1	176	0.18	0.04–0.73		173	0.17	0.04–0.72	
NAT2/smoking	Rapid/nonsmoker	751	(ref)		.03	714	(ref)		.002
	Slow/nonsmoker	871	0.91	0.59–1.41		842	1.05	0.64–1.70	
	Rapid/smoker	400	1.06	0.63–1.78		400	1.65	0.93–2.94	
	Slow/smoker	388	0.34	0.16–0.74		372	0.35	0.15–0.82	

^aFully adjusted models include age, BMI, frequency of unprotected intercourse during the ovulatory window, and pregnancy intention, as in Table 2. The model examining smoking interaction was also adjusted for caffeine and alcohol, in the categories shown in Table 2; the model examining alcohol interaction was adjusted for caffeine and smoking; and likewise for caffeine.

^bThe number of cycles for the fully adjusted model is shown. A woman may be in one exposure category (e.g., slow/smoker) for one cycle and a different category (e.g., slow/nonsmoker) for the next. Each woman contributed an average of 8 cycles to the study.

of caffeine on fecundability was found, regardless of NAT2 acetylator status. Smoking and alcohol significantly reduced fecundability, but only among women who were slow acetylators. These associations and interactions were adjusted for potential confounders including age, frequency of unprotected intercourse, intention to become pregnant, and BMI, and persisted when the population was restricted to non-Hispanic whites.

Although several studies have observed a detrimental effect of smoking on fecundability (9, 32–34) others have not (6, 7, 35). Without stratifying on NAT2, no effect of smoking was observed in this study. Thus, it is possible that genetic heterogeneity obscured the effect of smoking on fecundability in previous studies.

There are several mechanisms by which smoking may affect fecundability, which are discussed in a recent review (3). Smoking has been related to measures of ovarian aging, including increased levels of follicle-stimulating hormone and decreased levels of antimüllerian hormone (36–38). Smoking may also cause alterations in estrogen synthesis and metabolism (39), irregular menstrual cycles (39, 40), and chromosomal errors (41). Each of these effects could theoretically be mediated by a combination of toxicants present in cigarette smoke; however, the observed interaction between smoking and NAT2 on fecundability suggests that any effects of smoking on fecundability are mediated by the aromatic and heterocyclic amine carcinogens found in cigarette smoke, which are metabolized by NAT2 (12).

A significant effect of alcohol on fecundability was observed in this population without accounting for NAT2.

However, a more detailed examination revealed that the effect was limited to the slow acetylators. Although there is presently no evidence that NAT2 could directly metabolize ethanol, other studies have also reported an interaction between NAT2 and alcohol use with respect to various outcomes. In 1 study, alcohol intake was associated with increased risk of oral squamous cell cancer, but only among rapid or intermediate acetylators (42). In a case-control study of bladder cancer, alcohol had a much stronger effect among slow acetylators (43). One explanation is that alcohol intake may be correlated with intake of other toxicants present in alcoholic beverages (e.g., ethyl carbamates, tetra-beta carbolines, or food additives) or other substances (e.g., cooked meat), which contain toxicants that are metabolized by NAT2 (44, 45). Thus, the effect modification observed with alcohol could be a reflection of 1) the effect modification of the substances correlated with alcohol intake, 2) the alcohol adding to total body burden of toxicants, so that NAT2 and other enzymes cannot metabolize all toxicants as efficiently, or 3) interactions between alcohol and smoking, which we were not able to accommodate in our analysis because of the limited sample size.

There was no discernable effect of caffeine on time to pregnancy in this population. This is the first study to report findings from daily, prospective information on caffeine intake and prospectively ascertained pregnancies, including subclinical pregnancies, while controlling for frequency of intercourse, smoking, and alcohol. Although our findings are consistent with many null or equivocal studies of caffeine intake and reproductive outcomes (2),

misclassification of caffeine intake could have biased any effect estimates toward the null. In a recent validity study, self-reported caffeine use in a prospective daily diary was moderately correlated with salivary caffeine concentrations ($r = 0.68$) (46). In addition, our study assumed that each cup of coffee (or tea or soda) contained the same amount of caffeine, but in reality this varies, causing further misclassification. In addition, we did not have measurements from other caffeine sources such as chocolate or medications.

A limitation of this study was the small numbers in some of the risk groups, resulting in wide confidence intervals for some measures. Only a subset of the women could be genotyped, which affected the final sample size. However, there were no differences in characteristics between the studied eligible women ($n = 319$) and those with insufficient DNA, reducing the likelihood that our subsampling resulted in selection bias. In addition, NAT2 SNP frequencies were virtually identical to those found in the CEU population of HapMap, further indicating that this subset was a representative sample.

Another limitation of this study is that we did not have information on the partner's behaviors, such as smoking. These behaviors might have altered the fecundability of some women, although it should not meaningfully change the interaction between the women's acetylator status and her exposures on fecundability.

There was some misclassification of the measurement of unprotected intercourse, made evident by the fact that 12 women became pregnant even though they did not report having unprotected intercourse. Women may have misreported their unprotected intercourse, or birth control may not have been effective. For this reason, we included women in the analysis whether or not they reported having unprotected intercourse. Our inclusion of some women who may not have truly been at risk for pregnancy may have reduced the overall apparent fecundability of this population. A sensitivity analysis restricted to cycles in which unprotected intercourse was reported did not have meaningfully different effect estimates, although p -values were attenuated for some variables due to reduced sample size and power (data not shown).

The lower apparent fecundability of this population compared with populations in other prospective studies may be a result of several additional factors. First, we did not exclude subfecund women (women who were at risk for pregnancy 1–12 months before the study). These women may also have different susceptibilities to factors that affect pregnancy rates, such as smoking or alcohol intake. Second, only 23% of the women in this sample reported that they were consciously trying to become pregnant. Of those who reported trying ($n = 73$), the fecundability was higher (58% became pregnant during the study). Finally, women office workers are known to be less fertile than the general

population, because of the concept of the “infertile worker effect”: In essence, by limiting the sample to office workers, we have excluded fertile women who may be at home already taking care of children (47).

Because this population consisted of a mixture of women with varying pregnancy intentions, it is more representative of the general population than other studies that restrict to women who are all attempting pregnancy. During the years of this study approximately 50% of all pregnancies in the United States were unintended (48). However, our study is subject to possible confounding by factors associated with pregnancy intention. We attempted to control for such unmeasured confounders by adjusting for stated pregnancy intention and frequency of unprotected intercourse during the ovulatory window. Women who were attempting pregnancy were not different from the remaining women by age, smoking, alcohol intake, or caffeine intake (results not shown).

The apparent effects of alcohol and smoking on time to pregnancy could be a result of very early pregnancy losses (before the expected start of the next menstrual cycle), as opposed to a delay in conception. In our study, we tested hCG at the start of every bleeding segment (or 1 week after expected menses) and included all subclinical pregnancies in the analysis. However, losses occurring close to implantation may have been missed in our analysis.

In summary, we observed strong and significant effects of alcohol and smoking on time to pregnancy among women who were slow NAT2 acetylators. When slow and rapid acetylators were combined, the effects of alcohol were attenuated and the effects of smoking were no longer detectable. This research emphasizes the need to incorporate genetic information about metabolic enzymes to better interpret and understand the effects of xenobiotics on human health.

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