Research Article

Cancer Epidemiology, Biomarkers & Prevention

Caffeine, Coffee, and Tea Intake and Urinary Estrogens and Estrogen Metabolites in Premenopausal Women

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Abstract

Background: Prior studies have found weak inverse associations between breast cancer and caffeine and coffee intake, possibly mediated through their effects on sex hormones.

Methods: High-performance liquid chromatography/tandem mass spectrometry was used to quantify levels of 15 individual estrogens and estrogen metabolites (EM) among 587 premenopausal women in the Nurses' Health Study II with mid-luteal phase urine samples and caffeine, coffee, and/or tea intakes from self-reported food frequency questionnaires. Multivariate linear mixed models were used to estimate geometric means of individual EM, pathways, and ratios by intake categories, and *P* values for tests of linear trend.

Results: Compared with women in the lowest quartile of caffeine consumption, those in the top quartile had higher urinary concentrations of 16α-hydroxyestrone (28% difference; $P_{trend} = 0.01$) and 16-epiestriol (13% difference; $P_{trend} = 0.04$), and a decreased parent estrogens/2-, 4-, 16-pathway ratio ($P_{trend} = 0.03$). Coffee intake was associated with higher 2-catechols, including 2-hydroxyestradiol (57% difference, ≥4 cups/day vs. ≤6 cups/week; $P_{trend} = 0.001$) and 2-hydroxyestrone (52% difference; $P_{trend} = 0.001$), and several ratio measures. Decaffeinated coffee was not associated with 2-pathway metabolism, but women in the highest (vs. lowest) category of intake (≥2 cups/day vs. ≤1–3 cups/month) had significantly lower levels of two 16-pathway metabolites, estriol (25% difference; $P_{trend} = 0.01$) and 17-epiestriol (48% difference; $P_{trend} = 0.0004$). Tea intake was positively associated with 17-epiestriol (52% difference; $P_{trend} = 0.01$).

Conclusion: Caffeine and coffee intake were both associated with profiles of estrogen metabolism in premenopausal women.

Impact: Consumption of caffeine and coffee may alter patterns of premenopausal estrogen metabolism. *Cancer Epidemiol Biomarkers Prev; 24(8); 1174–83. ©2015 AACR.*

Introduction

Despite investigation in many large-scale epidemiologic studies, the association between coffee and breast cancer risk remains unclear. Although some evidence suggests a small inverse association between coffee and breast cancer risk (1, 2), some large prospective studies have reported null associations (3–6). Two meta-analyses reported that a 2-cup/day increase in coffee intake was associated with a nonsignificant 2% lower breast cancer risk (7, 8). Isolating the mechanism through which coffee might affect cancer risk is complicated by the chemical complexity of coffee, which comprises many potentially bioactive compounds. Coffee contains polyphenol antioxidants, which have been hypothesized to reduce breast cancer risk (9, 10), and is a major dietary source of caffeine, which has been inversely associated with breast cancer risk in some (1, 2, 7), though not all (3–6), studies. Caffeine represents a particularly interesting biologic component of coffee with respect to breast cancer, given that enzymes involved in its metabolism also play a role in estrogen metabolism (11, 12).

Parent estrogens, estrone and estradiol, are irreversibly metabolized along three different pathways, depending on the initial hydroxylation at the 2-, 4-, or 16-position of the steroid ring. Estrogen metabolites (EM) formed in each pathway are believed to have varying degrees of carcinogenic potential (13). Catechol estrogens, which have two adjacent hydroxyl groups, are formed after the initial hydroxylation of the parent estrogens at the 2- or 4-positions and may be oxidized to produce reactive quinones or inactivated by methylation of one of the adjacent their hydroxyl groups. Quinones may damage DNA directly, and also may undergo redox cycling to produce mutagenic reactive oxygen species. Laboratory studies suggest that 4-pathway catechols have more potential to induce DNA damage than 2-pathway catechols because they form covalent, depurinating adducts, and 2-methoxyestradiol may have antiestrogenic properties, inhibiting proliferation of breast cancer cells.

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doi: 10.1158/1055-9965.EPI-15-0246

Animal and laboratory evidence have demonstrated that metabolites in the 16-pathway are not only genotoxic, causing the formation of depurinating DNA adducts, but also bind tightly to the estrogen receptor, upregulate it, and increase cell proliferation (14–16). Although there is limited epidemiologic data for premenopausal women, among premenopausal women in the Nurses' Health Study II (NHSII), urinary mid-luteal levels of one 16-pathway EM and a higher ratio of 16-pathway metabolites to parent estrogens were associated with increased risk of breast cancer, while metabolites in the 2- and 4-pathways appeared inversely associated with risk (17).

The initial hydroxylation of the parent estrogens is catalyzed primarily by cytochrome P450 enzymes, which play key roles in the metabolism of caffeine. Of particular interest is the CYP1A2 isoform, which catalyzes the hydroxylation of the parent estrogens (11), and the initial demethylation of caffeine in humans (12). In prior studies, polymorphisms in the *CYP1A2* gene modified the association between coffee consumption and age at breast cancer diagnosis and estrogen receptor status in the general population (18), and modified the association between coffee intake and risk of breast cancer among *BRCA1* mutation carriers (19). Previously, in premenopausal women in the NHSII, we observed an inverse association between coffee and caffeine intake and mid-luteal plasma levels of estradiol (20). In another study of premenopausal women, coffee intake was positively associated with plasma levels of 2-hydroxyestrone, and nonsignificantly inversely associated with 16α-hydroxyestrone (21). However, it is unclear whether these associations are attributable to caffeine, or other known or unknown bioactive elements of coffee.

We sought to further elucidate the relationship between coffee and caffeine intakes and patterns of estrogen metabolism. Using a liquid chromatography/tandem mass spectrometry (LC/MS-MS) method with high sensitivity, accuracy, and reproducibility, we examined the cross-sectional relationship between intake of caffeine, coffee, tea, and decaffeinated coffee and urinary levels of 15 individual estrogens and EM (all 15 referred to as EM), total EM, and estrogen metabolism pathway measures among premenopausal women in the NHSII.

Materials and Methods

Study population

The NHSII is an ongoing prospective cohort study, established in 1989, when 116,430 registered nurses ages 25 to 42 years were enrolled. At baseline and biennially since, participants have returned mailed questionnaires with updated information about disease and exposure status. In 1996–1999, participants who were cancer-free and ages 32 to 54 years were asked to provide blood and urine samples. Of 29,611 women who provided samples, 18,521 who were premenopausal and had not been pregnant, breastfed, or used oral contraceptives in the 6 months preceding collection provided samples timed within their menstrual cycle. Women collected follicular phase blood samples during days 3 to 5 of their menstrual cycle, and blood and urine samples during the mid-luteal phase, 7 to 9 days before the anticipated start of their next cycle. Urine samples were shipped with an ice pack to our laboratory via overnight courier; 93% of samples were received within 26 hours of collection. Upon arrival at the laboratory, urine samples were aliquoted into cryotubes without preservatives and stored in liquid nitrogen freezers.

The current study population includes 110 women selected for a biomarker reproducibility study (22), and 493 controls from a nested case–control study of breast cancer (17). Of these 603 women, 587 had exposure data.

Assessment of exposure

Semi-quantitative food frequency questionnaires (FFQ) were used to assess intake of specific foods every 4 years, beginning in 1991. Possible choices for intake of coffee, caffeinated tea, and decaffeinated coffee ranged from "never or less than once per month" to "6 or more times per day." Caffeine intake was derived from self-reported intakes of coffee, soda, tea, and chocolate using their caffeine content per serving as estimated by the U.S. Department of Agriculture (USDA) food composition sources. The average amount of caffeine was estimated to be 137 mg per 8 oz serving of coffee, 47 mg per 8 oz serving of tea, 46 mg per 12 oz serving of soda, and 7 mg per 1 oz serving of chocolate.

Caffeine was adjusted for energy intake using the residual method (23), and modeled as quartiles of daily intake (mg), while intake of each specific beverage was modeled as servings per month, week, or day. Results for caffeine were unchanged when unadjusted intakes were used. We present analyses using the 1999 self-reported intakes for exposure; results using the mean of the 1995 and 1999 responses were not appreciably different.

Assessment of covariates

The questionnaire completed at urine collection included information about collection time, whether urine was first morning void, and the participant's present weight and tobacco use. To confirm menstrual cycle phase and calculate luteal day at specimen collection, 97% of participants recorded the date of their next menstrual period on a postcard returned by mail. Progesterone levels were measured in the blood sample drawn on the same day as urine collection (24, 25). We considered women with luteal progesterone levels $\geq$400 ng/dL to have donated samples in an ovulatory cycle, while participants with levels below this cutoff point were defined as having an anovulatory cycle.

Anthropometric, reproductive, and other lifestyle factors were assessed on biennial questionnaires. Information on menstrual cycle regularity and usual length was obtained in 1993. Body mass index (BMI) was calculated from weight at time of urine collection and height in 1989. Physical activity was queried every 4 years; we used the average of the 1997 and 2001 surveys to estimate physical activity in metabolic equivalent (MET) hours/week. Average alcohol intake was calculated from the 1995 and 1999 FFQs.

Laboratory methods

Details of the assay have previously been described (26, 27). Briefly, frozen 500-μL aliquots of urine were shipped to the Laboratory of Proteomics and Analytical Technologies (Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD), where levels of 15 individual EM were quantified using stable isotope dilution LC/MS-MS. Because urinary EM are generally present as glucoronide and sulfate conjugates, an initial hydrolysis step was performed using B-glucuronidase/sulfatase from *Helix pomatia*. To correct for loss of EM throughout the assay procedure and allow accurate quantification, an internal standard solution of 5 deuterated EM (17β-estradiol-d4, estriol-d3, 2-hydroxy-17β-estradiol-d5,

2-methoxy-17β-estradiol-d5, 16-epiestriol-d3) was added to each thawed aliquot. Each batch of urine samples included masked replicate quality control samples, which were used to assess intra- and interbatch assay variability. For most metabolites, coefficients of variation were below 7%, though two low concentration EM, 4-methoxyestradiol and 4-methoxyestrone, had CVs of 15% and 17%, respectively. The lower limit of quantitation for all EM was approximately 150 fmol/mL urine.

Two batches of urinary creatinine were measured at the Endocrine Core Laboratory at Emory University (Atlanta, GA); a third was measured at the laboratory of Dr. Vincent Ricchiuti at Brigham and Women's Hospital (Boston, MA). CVs were ≤9.2%. Plasma progesterone was assayed by chemiluminescent immunoassay using the Immulite Auto-Analyzer (Diagnostic Products Corp.). Overall CVs were ≤17%, while within-batch CVs were ≤4%.

Statistical analysis

EM levels were standardized by creatinine level to account for differences in urine volume and concentration. EM were assessed individually and summed as total EM; we additionally examined a number of groups (i.e., catechols) and ratios of individual EM and pathways based on shared biochemical characteristics, known metabolic pathways, and etiologic hypotheses. To improve normality, all outcome measures were log-transformed. Statistical outliers for each measure were identified using the generalized extreme Studentized deviate many-outlier approach (28), and removed from analyses [$N = 0$–16 (2-methoxyestradiol)].

Multivariate linear mixed models were used to estimate geometric means of the log-transformed metabolites, pathways, and ratios by exposure category. Tests of linear trend were conducted by modeling the median of each beverage intake category and the quartile medians of caffeine intake as continuous variables. All models were adjusted for age at urine collection, BMI at collection, height, ovulatory cycle, first-morning urine, alcohol intake, total physical activity, current tobacco use, luteal day, usual menstrual cycle length, menstrual cycle regularity, and age at first birth and parity. Additional adjustment for creatinine did not significantly change our results, and this variable was not retained in final models.

In secondary analyses, we restricted to women with ovulatory cycles, nonsmokers, or women who provided samples between luteal days 4 and 10. We also examined potential effect modification by BMI (≤25 vs. >25). Wald tests were used to assess the significance of interaction terms between dichotomous BMI and the median of the exposure category that were included in models that included all women. All P values were two-sided and tests of significance were performed at the $\alpha = 0.05$ level. All analyses were conducted using SAS v. 9.2 (SAS Institute).

Results

The mean age of the study population was 42.8 years at urine collection, and the mean BMI was 25.1 (Table 1). On the basis of plasma progesterone level, 90% of cycles were ovulatory; 86% of samples were collected 4 to 10 days prior to next menstrual period. Compared with women with the lowest caffeine intake, those with highest intake were more likely to report being current smokers, had higher alcohol intake, and were less likely to have provided a first-morning urine sample. Women with highest caffeine intake were also slightly older than those with the lowest intake and had a higher BMI.

About half of women (49.9%) reported drinking ≤6 cups of coffee/week, and 40 (6.9%) reported drinking ≥4 cups/day. Median caffeine intake was 163.1 mg/day. Consumption of tea and decaffeinated coffee was notably lower than that of coffee; 415 women (71.4%) reported drinking <1 cup of tea/week, while 36 (6.2%) reported drinking ≥2 cups/day. A total of 369 (63.5%) participants drank decaffeinated coffee <3 times/month and 44 (7.6%) drank ≥2 cups/day. No significant correlations were observed between intakes of coffee, decaffeinated coffee, and tea (all $r < 0.08$).

In multivariate analyses, caffeine intake was associated with several individual EM and metabolic ratios (Table 2). Higher caffeine intake was associated with higher urinary levels of two 16-pathway metabolites, 16α-hydroxyestrone (28% higher among women in the highest quartile of caffeine intake compared with the lowest quartile, $P_{trend} = 0.01$), and 16-epiestriol (13% higher, $P_{trend} = 0.04$). Furthermore, suggestively, though not significantly, higher 16-ketoestradiol levels were observed

Table 1. Characteristics of the premenopausal study population by quartiles of caffeine intake

	<49 mg/d	49–163 mg/d	164–366 mg/d	≥367 mg/d
N	144	144	144	145
Age at urine collection, y	42.2 (4.0)	42.9 (3.9)	43.1 (3.9)	43.4 (3.4)
Urinary creatinine, mg/L	1,146 (589)	1,209 (548)	1,077 (590)	1,094 (657)
Ovulatory cycle, %	91	90	88	90
First-morning urine sample, %	88	86	71	75
Sample collected 4–10 days before next menstrual period, %	88	87	87	81
BMI at time of urine collection, kg/m^2	24.7 (4.9)	25.0 (5.1)	25.4 (5.3)	25.6 (6.0)
Height, inches	65.1 (2.7)	65.2 (2.5)	65.1 (3.1)	65.0 (2.3)
Caucasian, %	97	96	99	95
Physical activity, MET-h/wk	20.9 (18.6)	21.3 (21.8)	22.8 (20.9)	20.7 (17.7)
Alcohol intake, g/d	2.2 (5.5)	3.5 (5.1)	4.6 (6.2)	5.3 (7.0)
Current smoker, %	2	3	6	12
Parous, %	83	81	81	82
Age at first birth, y	26.7 (4.3)	26.6 (4.6)	26.4 (4.8)	27.3 (4.9)
Regular menstrual cycles, %	96	94	94	97
Menstrual cycle length 26–31 days, %	71	66	67	63
Coffee intake, cups/day	0 (0)	0.4 (0.4)	1.6 (0.9)	3.1 (1.1)
Decaffeinated coffee intake, cups/day	0.4 (0.9)	0.3 (0.7)	0.4 (0.8)	0.2 (0.6)
Tea intake, cups/day	0.1 (0.1)	0.5 (0.7)	0.5 (1.0)	0.4 (0.8)

NOTE: Values are means (SD) or percentages.

Table 2. Multivariate-adjusted[a] geometric means of estrogen metabolism measures by quartiles of caffeine intake

	Geometric mean by caffeine intake				
	<49 mg/d	**49–163 mg/d**	**164–366 mg/d**	**≥367 mg/d**	**P_{trend}**
N	144	144	144	145	
Individual and grouped EM (pmol/mg creatinine)					
Total EM	199.0	183.4	203.5	211.0	0.10
Parent estrogens	40.8	39.4	40.5	41.3	0.67
Estrone	26.8	25.4	26.4	27.4	0.54
Estradiol	13.2	12.8	12.9	13.5	0.66
Catechols	67.8	61.5	62.3	74.6	0.19
2-catechols	56.7	53.0	53.1	65.3	0.10
2-Hydroxyestrone	50.0	46.8	46.5	57.9	0.10
2-Hydroxyestradiol	5.8	5.4	5.6	6.5	0.14
4-catechols					
4-Hydroxyestrone	7.6	5.7	5.9	6.2	0.28
Methylated catechols	11.4	10.9	10.1	10.5	0.28
Methylated 2-catechols	11.1	10.6	9.7	10.1	0.25
2-Methoxyestrone	8.7	8.2	7.6	7.8	0.18
2-Methoxyestradiol	0.78	0.70	0.71	0.69	0.29
2-Hydroxyestrone-3-methyl ether	1.4	1.3	1.2	1.3	0.18
Methylated 4-catechols	0.25	0.23	0.22	0.25	0.91
4-Methoxyestrone	0.15	0.15	0.13	0.18	0.37
4-Methoxyestradiol	0.06	0.05	0.06	0.05	0.45
2-Hydroxylation pathway	70.1	64.8	64.0	76.7	0.25
4-Hydroxylation pathway	8.3	6.8	6.6	7.2	0.39
16-Hydroxylation pathway	62.3	61.0	67.3	65.3	0.33
16α-Hydroxyestrone	**9.7**	**10.1**	**11.2**	**12.4**	**0.01**
Estriol	27.5	26.4	30.6	26.2	1.00
17-Epiestriol	1.6	1.7	1.7	1.6	0.96
16-Ketoestradiol	12.2	12.6	14.2	13.7	0.07
16-Epiestriol	**5.3**	**5.4**	**5.9**	**6.0**	**0.04**
Ratios (pmol/pmol)					
2-Hydroxyestrone/16α-hydroxyestrone	5.0	4.6	4.0	4.5	0.29
4-Pathway/2-pathway	**0.11**	**0.10**	**0.10**	**0.09**	**0.01**
2-Pathway/16-pathway	1.1	1.0	0.9	1.2	0.69
2,4-Pathway/16-pathway	0.13	0.11	0.09	0.11	0.15
2-Catechols/methylated 2-catechols	**5.0**	**4.9**	**5.4**	**6.3**	**0.0002**
4-Catechols/methylated 4-catechols	31.7	25.9	27.2	26.8	0.53
Catechols/methylated catechols	**5.8**	**5.5**	**6.1**	**7.0**	**0.001**
Parent estrogens/2-, 4-, 16-pathways	**0.28**	**0.28**	**0.26**	**0.25**	**0.03**
2-Pathway/parent estrogens	1.7	1.6	1.5	1.8	0.23
4-Pathway/parent estrogens	0.20	0.17	0.16	0.17	0.20
16-Pathway/parent estrogens	1.5	1.6	1.7	1.6	0.50

[a]Adjusted for age at urine collection (continuous), BMI at collection (kg/m^2, continuous), height (continuous), ovulatory cycle (yes/no), first-morning urine (yes/no), quartiles of alcohol intake (nondrinker, ≤1.49, 1.50–4.85, >4.85 g/day), total physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET h/wk), current tobacco use (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days to next period), usual menstrual cycle length (<26, 26–31, ≥32 days), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), and age at first birth and parity (nulliparous, age at first birth <25 years/1–2 children, age at first birth 25–29 years/1–2 children, age at first birth ≥30 years/1–2 children, age at first birth <25 years/≥3 children, age at first birth ≥25 years/≥3 children). Numbers do not sum to 603 due to missing values for exposure. Significant P_{trend} shown in bold.

at higher levels of caffeine intake (12% higher, $P_{trend} = 0.07$). However, the combined 16-pathway was only nonsignificantly 5% higher at higher caffeine intake.

There were suggestive positive association between caffeine consumption and the 2-catechols, though these associations did not appear linear and were not statistically significant. Compared with women in the lowest quartile of caffeine intake, those with the highest intake had 16% higher levels of 2-hydroxyestrone ($P_{trend} = 0.10$) and 13% higher levels of 2-hydroxyestradiol ($P_{trend} = 0.14$). These suggestive associations with 2-catechols appeared to drive the positive associations observed with the 2-catechols/methylated 2-catechols ratio ($P_{trend} = 0.0002$), and the catechols/methylated catechols ratio ($P_{trend} = 0.001$), and the inverse association with the 4-pathway/2-pathway ratio ($P_{trend} = 0.01$). Finally, an inverse association between caffeine and the parent estrogens/2-, 4-, 16-pathway ratio was observed ($P_{trend} = 0.03$), largely because total EM nonsignificantly increased with caffeine intake ($P_{trend} = 0.10$).

Coffee consumption was positively associated with urinary levels of 2-catechols, but not with methylated 2- or 4-catechols (Table 3). Compared with women who reported drinking ≤6 cups/week, those who drank ≥4 cups/day had 52% higher 2-hydroxyestrone levels ($P_{trend} = 0.001$) and 57% higher 2-hydroxyestradiol levels ($P_{trend} = 0.001$). Overall, 2-catechols were 52% higher ($P_{trend} = 0.001$), catechols were 45% higher ($P_{trend} = 0.002$), and the 2-hydroxylation pathway was 41% higher ($P_{trend} = 0.01$) among participants in the top category of intake compared with the lowest.

A number of ratios comparing 2-catechol levels to other pathways were also associated with coffee consumption. Positive associations were observed for the following ratios where 2-catechols were included in the numerator: 2-pathway/16-pathway ($P_{trend} = 0.01$), 2-catechols/methylated 2-catechols

Table 3. Multivariate-adjusted[a] geometric means of estrogen metabolism measures by category of coffee intake

	Geometric mean by coffee intake				
	≤6 cups/wk	1 cup/day	2–3 cups/day	4+ cups/day	P_{trend}
N	293	73	181	40	
Individual and grouped EM (pmol/mg creatinine)					
Total EM	195.5	197.0	207.2	222.7	0.08
Parent estrogens	40.5	41.8	41.2	44.4	0.37
Estrone	26.1	28.0	27.6	28.5	0.29
Estradiol	13.5	12.5	13.3	14.7	0.51
Catechols	**61.2**	**62.0**	**69.4**	**88.5**	**0.002**
2-catechols	**51.9**	**54.4**	**59.7**	**79.0**	**0.001**
2-Hydroxyestrone	**45.7**	**48.4**	**52.6**	**69.6**	**0.001**
2-Hydroxyestradiol	**5.4**	**5.3**	**6.1**	**8.4**	**0.001**
4-catechols					
4-Hydroxyestrone	6.1	5.5	6.1	7.1	0.61
Methylated catechols	11.2	10.2	10.4	10.7	0.49
Methylated 2-catechols	10.8	9.8	10.0	10.4	0.46
2-Methoxyestrone	8.3	7.6	7.8	7.9	0.45
2-Methoxyestradiol	0.71	0.71	0.69	0.73	0.94
2-Hydroxyestrone-3-methyl ether	1.4	1.2	1.3	1.3	0.37
Methylated 4-catechols	0.25	0.23	0.25	0.26	0.86
4-Methoxyestrone	0.16	0.14	0.16	0.18	0.47
4-Methoxyestradiol	0.06	0.06	0.05	0.05	0.51
2-Hydroxylation pathway	**64.3**	**64.8**	**70.7**	**90.5**	**0.01**
4-Hydroxylation pathway	7.0	6.1	7.1	7.8	0.59
16-Hydroxylation pathway	65.8	65.7	65.8	61.6	0.79
16α-Hydroxyestrone	10.7	11.4	11.3	12.6	0.17
Estriol	29.4	27.9	28.3	24.6	0.30
17-Epiestriol	1.7	1.6	1.4	1.9	0.51
16-Ketoestradiol	13.1	14.0	13.7	13.6	0.45
16-Epiestriol	5.6	5.3	6.0	5.9	0.28
Ratios (pmol/pmol)					
2-Hydroxyestrone/16α-hydroxyestrone	4.3	4.1	4.5	5.0	0.32
4-Pathway/2-pathway	**0.10**	**0.10**	**0.09**	**0.09**	**0.03**
2-Pathway/16-pathway	0.94	1.0	1.1	1.4	0.01
2,4-Pathway/16-pathway	**0.10**	**0.09**	**0.11**	**0.12**	**0.53**
2-Catechols/methylated 2-catechols	**4.7**	**5.5**	**6.0**	**7.4**	**<0.0001**
4-Catechols/methylated 4-catechols	25.1	23.3	26.2	29.2	0.54
Catechols/methylated catechols	**5.4**	**6.0**	**6.7**	**8.1**	**<0.0001**
Parent estrogens/2-, 4-, 16-pathways	0.28	0.28	0.26	0.25	0.10
2-Pathway/parent estrogens	**1.5**	**1.6**	**1.7**	**2.0**	**0.003**
4-Pathway/parent estrogens	0.17	0.15	0.17	0.17	0.84
16-Pathway/parent estrogens	1.6	1.6	1.6	1.5	0.35

[a]Adjusted for age at urine collection (continuous), BMI at collection (kg/m^2, continuous), height (continuous), ovulatory cycle (yes/no), first-morning urine (yes/no), quartiles of alcohol intake (nondrinker, ≤1.49, 1.50–4.85, >4.85 g/day), total physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET h/week), current tobacco use (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days to next period), usual menstrual cycle length (<26, 26–31, ≥32 days), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), and age at first birth and parity (nulliparous, age at first birth <25 years/1–2 children, age at first birth 25–29 years/1–2 children, age at first birth ≥30 years/1–2 children, age at first birth <25 years/≥3 children, age at first birth ≥25 years/≥3 children). Numbers do not sum to 603 due to missing values for exposure. Significant P_{trend} shown in bold.

(P_{trend} < 0.0001), catechols/methylated catechols (P_{trend} < 0.0001), and 2-pathway/parent estrogens ratio (P_{trend} = 0.003). Conversely, an inverse association with coffee intake was observed for the 4-pathway/2-pathway ratio (P_{trend} = 0.03). As with caffeine, total EM nonsignificantly increased with coffee intake (P_{trend} = 0.08).

Markedly fewer associations were observed between individual EM and decaffeinated coffee and tea intakes (Tables 4 and 5). Two individual 16-pathway metabolites were inversely associated with decaffeinated coffee intake (Table 4). Compared with those who reported drinking ≤3 cups/month, participants who drank ≥2 cups/day had 25% lower estriol levels (P_{trend} = 0.01) and 48% lower 17-epiestriol levels (P_{trend} = 0.0004). Decaffeinated coffee also was suggestively but nonsignificantly inversely associated with other 16-pathway metabolites, specifically 16-ketoestradiol (top vs. bottom intake: 14% lower) and 16-epiestriol (16% lower). Overall, levels of all 16-pathway metabolites combined were 16% lower among participants in the highest category, though the test for trend was not significant (P_{trend} = 0.14).

Caffeinated tea intake was positively associated with 17-epiestriol (P_{trend} = 0.01), which was 52% higher among those consuming ≥2 cups/day compared with those who drank ≤3 cups/month (Table 5). Tea intake was also inversely associated with the 2-catechols/methylated 2-catechols (P_{trend} = 0.02) and the catechols/methylated catechols ratios (P_{trend} = 0.02).

Results from sensitivity analyses among nonsmokers (n = 560), samples 4 to 10 days before next menstrual period (n = 516), and ovulatory cycles (n = 537) did not differ appreciably from the results of main analyses. We observed some evidence of modification by BMI of the association between coffee and estrogen metabolism, with several associations appearing stronger in overweight women (BMI > 25) than in normal weight women (BMI ≤ 25). Positive associations between coffee and 2-hydroxyestrone (P-interaction = 0.02), 2-hydroxyestradiol (P-interaction = 0.06),

Table 4. Multivariate-adjusted[a] geometric means of estrogen metabolism measures by category of decaffeinated coffee intake

	Geometric mean by decaffeinated coffee intake				
	≤1–3 cups/month	1–6 cups/week	1 cup/day	2+ cups/day	P_{trend}
N	369	132	36	44	
Individual and grouped EM (pmol/mg creatinine)					
Total EM	208.1	212.0	175.8	205.2	0.60
Parent estrogens	42.5	45.2	33.5	39.6	0.28
Estrone	28.1	28.7	21.5	26.8	0.42
Estradiol	13.8	14.8	10.8	12.2	0.07
Catechols	65.9	74.8	63.6	76.6	0.33
2-catechols	56.7	64.3	54.8	65.2	0.35
2-Hydroxyestrone	50.0	56.8	48.7	57.2	0.39
2-Hydroxyestradiol	5.8	6.4	5.3	7.0	0.19
4-catechols					
4-Hydroxyestrone	6.4	6.8	4.3	6.8	0.73
Methylated catechols	10.9	11.9	8.7	11.1	0.86
Methylated 2-catechols	10.5	11.5	8.4	10.7	0.93
2-Methoxyestrone	8.2	9.0	6.3	8.4	0.86
2-Methoxyestradiol	0.71	0.73	0.61	0.72	0.69
2-Hydroxyestrone-3-methyl ether	1.3	1.4	1.2	1.5	0.27
Methylated 4-catechols	0.26	0.26	0.20	0.27	0.96
4-Methoxyestrone	0.17	0.16	0.14	0.16	0.64
4-Methoxyestradiol	0.06	0.06	0.05	0.07	0.38
2-Hydroxylation pathway	68.6	77.2	65.8	76.5	0.46
4-Hydroxylation pathway	7.3	7.5	5.4	7.8	0.84
16-Hydroxylation pathway	67.9	65.6	61.2	57.3	0.14
16α-Hydroxyestrone	11.3	11.3	11.4	10.7	0.99
Estriol	**29.7**	**30.3**	**23.5**	**22.3**	**0.01**
17-Epiestriol	**1.8**	**1.5**	**1.7**	**0.92**	**0.0004**
16-Ketoestradiol	14.0	13.1	13.0	12.0	0.31
16-Epiestriol	6.0	5.7	5.0	5.1	0.11
Ratios (pmol/pmol)					
2-Hydroxyestrone/16α-hydroxyestrone	4.3	4.8	4.1	5.3	0.42
4-Pathway/2-pathway	0.10	0.10	0.09	0.09	0.56
2-Pathway/16-pathway	1.02	1.15	1.0	1.2	0.28
2,4-Pathway/16-pathway	0.10	0.11	0.10	0.13	0.31
2-Catechols/methylated 2-catechols	5.4	5.6	5.7	6.1	0.27
4-Catechols/methylated 4-catechols	25.2	27.7	24.1	27.7	0.91
Catechols/methylated catechols	6.1	6.3	6.3	6.9	0.25
Parent estrogens/2-, 4-, 16-pathways	0.27	0.28	0.22	0.28	0.84
2-Pathway/parent estrogens	1.6	1.7	1.8	1.8	0.22
4-Pathway/parent estrogens	0.17	0.17	0.18	0.19	0.39
16-Pathway/parent estrogens	1.6	1.5	1.9	1.4	0.62

[a]Adjusted for age at urine collection (continuous), BMI at collection (kg/m^2, continuous), height (continuous), ovulatory cycle (yes/no), first-morning urine (yes/no), quartiles of alcohol intake (nondrinker, ≤1.49, 1.50–4.85, >4.85 g/day), total physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET h/week), current tobacco use (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days to next period), usual menstrual cycle length (<26, 26–31, ≥32 days), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), and age at first birth and parity (nulliparous, age at first birth <25 years/1–2 children, age at first birth 25–29 years/1–2 children, age at first birth ≥30 years/1–2 children, age at first birth <25 years/ ≥3 children, age at first birth ≥25 years/≥3 children). Numbers do not sum to 603 due to missing values for exposure. Significant P_{trend} shown in bold.

and 4-hydroxyestrone (P-interaction = 0.11) were observed only among overweight women. Positive associations between coffee intake and 2-catechols ($P_{interaction}$ = 0.02), catechols ($P_{interaction}$ = 0.02), 2-hydroxylation pathway ($P_{interaction}$ = 0.04), 4-hydroxylation pathway ($P_{interaction}$ = 0.13), and total EM ($P_{interaction}$ = 0.05) were limited to, or stronger among, overweight women. Consequently, the 2-catechols/methylated 2 catechols ratio ($P_{interaction}$ = 0.01), and catechols/methylated catechols ratio ($P_{interaction}$ = 0.01) were also limited to, or stronger among, overweight women. For other exposures, results stratified by BMI generally appeared similar.

When we summed regular and decaffeinated coffee, we observed associations that were similar to those seen for each exposure individually, though the magnitude of the associations appeared somewhat attenuated (data not shown). Positive associations were observed between total coffee intake and 2-hydroxyestrone (33% higher, ≥4 cups/day vs. ≤6 cups/week; P_{trend} = 0.001) and 2-hydroxyestradiol (35% higher; P_{trend} = 0.0001), and inverse associations were seen for estriol (17% lower; P_{trend} = 0.03) and 17-epiestriol (33% lower; P_{trend} = 0.02). Adjustment for caffeine did not materially change these results, though associations with 16-pathway metabolites appeared somewhat stronger (i.e., 17-epiestriol: 47% lower; P_{trend} = 0.0003). Similarly, results for tea were generally unchanged when adjusted for caffeine.

Discussion

In this population of premenopausal women, we observed several associations between coffee and caffeine intakes and luteal urinary levels of individual EM, metabolic pathways, and ratio measures. Interestingly, there were relatively few individual EM that were associated with more than one exposure. Coffee intake was positively associated with levels of both 2-catechol EM, 2-hydroxyestrone and 2-hydroxyestradiol, and several ratio and

Table 5. Multivariate-adjusted[a] geometric means of estrogen metabolism measures by category of tea intake

	Geometric mean by tea intake				
	≤1–3 cups/month	**1–6 cups/week**	**1 cup/day**	**2+ cups/day**	**P_{trend}**
N	415	80	50	36	
Individual and grouped EM (pmol/mg creatinine)					
Total EM	210.7	194.2	189.6	215.5	0.76
Parent estrogens	42.3	40.3	39.4	44.5	0.79
Estrone	27.9	26.6	25.5	27.7	0.66
Estradiol	13.6	13.2	13.2	15.4	0.17
Catechols	69.7	70.0	62.6	61.5	0.35
2-catechols	59.9	60.7	54.0	52.5	0.38
2-Hydroxyestrone	52.8	53.8	47.9	45.9	0.39
2-Hydroxyestradiol	6.1	6.0	5.6	5.5	0.29
4-catechols					
4-Hydroxyestrone	6.4	6.6	5.7	6.4	0.79
Methylated catechols	10.9	10.7	11.7	12.2	0.34
Methylated 2-catechols	10.5	10.4	11.3	11.8	0.32
2-Methoxyestrone	8.1	8.2	8.8	8.7	0.63
2-Methoxyestradiol	0.72	0.72	0.66	0.75	0.80
2-Hydroxyestrone-3-methyl ether	1.4	1.2	1.4	1.4	0.80
Methylated 4-catechols	0.26	0.23	0.24	0.27	0.76
4-Methoxyestrone	0.17	0.16	0.15	0.18	0.82
4-Methoxyestradiol	0.06	0.05	0.06	0.05	0.66
2-Hydroxylation pathway	71.9	72.6	66.4	66.4	0.59
4-Hydroxylation pathway	7.4	7.0	6.8	7.3	0.88
16-Hydroxylation pathway	67.4	58.6	60.2	74.0	0.29
16α-Hydroxyestrone	11.6	9.4	10.2	10.7	0.83
Estriol	28.9	26.7	25.5	34.7	0.13
17-Epiestriol	**1.6**	**1.7**	**1.6**	**2.4**	**0.01**
16-Ketoestradiol	14.0	12.4	11.5	15.5	0.48
16-Epiestriol	5.9	5.2	5.5	6.3	0.44
Ratios (pmol/pmol)					
2-Hydroxyestrone/16α-hydroxyestrone	4.4	5.6	4.9	4.1	0.76
4-Pathway/2-pathway	0.10	0.10	0.09	0.10	0.62
2-Pathway/16-pathway	1.05	1.1	1.1	0.86	0.20
2,4-Pathway/16-pathway	0.10	0.12	0.12	0.08	0.26
2-Catechols/methylated 2-catechols	**5.7**	**5.5**	**4.8**	**4.4**	**0.02**
4-Catechols/methylated 4-catechols	26.4	26.8	24.8	21.4	0.32
Catechols/methylated catechols	**6.3**	**6.2**	**5.4**	**5.0**	**0.02**
Parent estrogens/2-, 4-, 16-pathways	0.27	0.27	0.28	0.28	0.85
2-Pathway/parent estrogens	1.7	1.7	1.7	1.5	0.34
4-Pathway/parent estrogens	0.17	0.17	0.17	0.15	0.36
16-Pathway/parent estrogens	1.6	1.5	1.5	1.7	0.39

[a]Adjusted for age at urine collection (continuous), BMI at collection (kg/m^2, continuous), height (continuous), ovulatory cycle (yes/no), first-morning urine (yes/no), quartiles of alcohol intake (nondrinker, ≤1.49, 1.50–4.85, >4.85 g/day), total physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET h/week), current tobacco use (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days to next period), usual menstrual cycle length (<26, 26–31, ≥32 days), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), and age at first birth and parity (nulliparous, age at first birth <25 years/1–2 children, age at first birth 25–29 years/1–2 children, age at first birth ≥30 years/1–2 children, age at first birth <25 years/≥3 children, age at first birth ≥25 years/≥3 children). Numbers do not sum to 603 due to missing values for exposure. Significant P_{trend} shown in bold.

group measures involving these metabolites. In contrast, caffeine, caffeinated tea, and decaffeinated coffee were each associated with individual 16-pathway EM.

Prior to the development of the high-throughput LC/MS-MS method, measuring multiple EM was largely infeasible and inaccurate (26). Consequently, prior epidemiologic studies of caffeine, coffee, and EM focused largely on 2-hydroxyestrone and 16α-hydroxyestrone. Early evidence suggested that among premenopausal women not using oral contraceptives, daily coffee consumption was positively associated with plasma levels of 2-hydroxyestrone and 2-hydroxyestrone/16α-hydroxyestrone ratio, and nonsignificantly inversely associated with 16α-hydroxyestrone level (21). Bradlow and colleagues (29) also reported that coffee intake was positively associated with premenopausal 2-hydroxyestrone/16α-hydroxyestrone ratio in plasma, but not urine. However, in a large, multiethnic group of premenopausal women, coffee intake was not related to urinary 2-hydroxyestrone levels, though a positive association was observed between this metabolite and caffeine from non-coffee sources (30).

In addition to supporting previous findings of a positive association between coffee intake and 2-hydroxyestrone, our study provides evidence that coffee may be associated with 2-hydroxyestradiol, another 2-catechol. The strong association between coffee and 2-catechols in our study contributed to several associations with ratio measures, indicating that coffee intake may increase metabolism in the 2-pathway, relative to the 4- and 16-pathways. Coffee has many constituents that may influence estrogen metabolism, including caffeine. Metabolism of caffeine is catalyzed primarily by CYP1A2 enzymes, and laboratory and human studies have suggested that caffeine is as an inducer of CYP1A2 activity (12). This hepatic enzyme also plays a key role in 2-, 4-, and 16-hydroxylation of parent estrogens, though it is believed to be more active in hydroxylation at the C-2 position of the parent estrogens than at the C-4 or C-16α positions (11).

Interestingly, we observed no significant association between caffeine and urinary levels of either 2-catechol EM, though some evidence of a nonlinear positive association was observed with 2-hydroxyestrone and 2-hydroxyestradiol. Independent of caffeine, coffee may also act as an inducer of CYP1A2 (31, 32), potentially by polycyclic aromatic hydrocarbons (PAH) produced by high-temperature brewing (33). Laboratory evidence also suggests that caffeic acid and chlorogenic acid, two polyphenols found in coffee, inhibit 2-catechol and 4-catechol methylation (34), which may explain the finding of a positive association between coffee and 2-catechols, though no associations with 4-catechols were observed. The associations between total (regular plus decaffeinated) coffee and 2-catechols persisted after adjustment for caffeine, and inverse associations with 16-pathway EM appeared stronger, suggesting that non-caffeine elements of coffee may be important in estrogen metabolism. However, given that coffee accounted for 72% of caffeine consumption among NHSII participants in 1999, it is difficult to disentangle the roles of caffeine versus other bioactive components of coffee in our population.

In contrast to regular coffee, decaffeinated coffee was not associated with 2-pathway EM, but rather, lower levels of two 16-pathway metabolites, estriol and 17-epiestriol. Decaffeinated and regular coffee differs in their caffeine content, and potentially in their antioxidant composition. Chemical analyses have detected lower levels of polyphenols in decaffeinated compared with regular coffee, possibly because of the decaffeination process (35, 36). In addition, it is possible that the relatively low range of decaffeinated coffee consumption in our study limited our ability to detect significant associations.

Laboratory evidence suggests that compared with 4- and 16-pathway EM, metabolites in the 2-pathway are hypothesized to have less genotoxic and estrogenic potential (13, 14, 37–39). Thus, our findings that coffee appears associated with higher 2-pathway activity may suggest a potential biologic mechanism through which coffee may reduce breast cancer risk, by shifting metabolism toward a profile in which 2-pathway metabolism is enhanced. However, associations between coffee and 2-pathway EM were limited to the 2-catechols, not the methylated 2-catechols that have been shown to inhibit growth of breast cancer cells *in vitro* (40). In addition, the associations between comprehensive profiles of estrogen metabolism and breast cancer risk have not been well studied, with the exception of our previous investigation in the NHSII (17). In that analysis, we did observed only suggestive, though not significant, associations between urinary levels of 2-and 4-pathway EM and risk. We also observed a positive association with a single 16-pathway EM, 17-epiestriol, as well as inverse associations with both parent estrogens. Thus, further work is needed to clarify relationships between EM profiles and breast cancer in humans. Finally, the relevance of urinary EM to breast cancer risk is as yet unclear, because these measures quantify excreted EM, which may be less indicative of estrogenic activity in breast tissue than plasma measurements.

The strengths of our study include well-timed luteal urine samples and precise and accurate quantification of 15 individual EM using a high-throughput approach. However, the cross-sectional nature of our study limits our ability to conclusively establish temporality. Furthermore, we only collected a single, luteal phase urine sample. Though luteal phase urinary EM have relatively high reproducibility over a 3-year period (22), we cannot extrapolate the associations with coffee, caffeine, and tea intake over other phases of the menstrual cycle. Although unmeasured confounding is always a possibility in an observational study, we carefully controlled for several factors associated with caffeine intake and urinary EM. For example, smoking was associated with caffeine intake in our population, and has been shown to be associated with patterns of estrogen metabolism (41); however, we adjusted for smoking, and results were unchanged when restricted to nonsmokers. Our assessment of exposure relied on self-reported data. However, results of a recent study in premenopausal women suggest that measurements of caffeine from FFQ and 24-hour recall are highly correlated ($r = 0.73$; $P < 0.01$), though FFQ measurements tend to be higher (42). Though we were not able to distinguish between specific types of coffees and teas, which may vary in caffeine content and chemical composition, associations between coffee and caffeine intakes derived from FFQ data and other outcomes in the Nurses' Health Studies suggest that self-reported intake is relatively well measured (43, 44). Finally, the large number of multiple comparisons performed could lead to the finding of statistically significant associations by chance. Because of correlations between metabolites, a Bonferroni-correction may be overly conservative. Nonetheless, at a corrected *P*-value of 0.003 (0.05/15 individual EM), some results remained statistically significant, including associations of 2-hydroxyestrone and 2-hydroxyestradiol with coffee.

In conclusion, our results suggest a relation of coffee and caffeine consumption with patterns of estrogen metabolism in premenopausal women. Our results support previous findings of a relationship between coffee and 2-hydroxyestrone, and possibly with the other 2-catechol, 2-hydroxyestradiol. In addition, we found associations between caffeine and 16-pathway metabolites, and suggestive associations with methylated 2-catechols. Further studies are needed to confirm our results.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Ziegler, A.H. Eliassen
Development of methodology: R. Ziegler
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.E. Hankinson, X. Xu, R. Ziegler, A.H. Eliassen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.S. Sisti, S.E. Hankinson, R.M. Tamimi, B. Rosner, R. Ziegler
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.H. Eliassen
Study supervision: A.H. Eliassen

Grant Support

This study was supported by Infrastructure Grant UM1 CA176726 and Research Grants CA67262 (to S.E. Hankinson) and CA50385 from the National Cancer Institute and the Intramural Research Program of the Division of Cancer Epidemiology and Genetics of the National Cancer Institute, and with federal funds of the National Cancer Institute awarded under Contract HHSN261200800001E to SAIC-Frederick. J.S. Sisti was funded by R25 CA098566 and T32 CA900137.

Received March 10, 2015; revised May 8, 2015; accepted May 22, 2015; published OnlineFirst June 10, 2015.

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