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Relationship Between Caffeine Intake and Plasma Sex Hormone Concentrations in Premenopausal and Postmenopausal Women

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Abstract

BACKGROUND—Circulating estrogens and androgens are important factors in the development of various female cancers. Caffeine intake may decrease risk of breast and ovarian cancer, although the data are not entirely consistent. Whether or not caffeine affects cancer risk by altering sex hormone levels is currently unknown.

METHODS—We examined the relationship of caffeine, coffee, decaffeinated coffee, and tea with plasma concentrations of estrogens, androgens, progesterone, prolactin, and sex hormone binding globulin (SHBG) in 524 premenopausal and 713 postmenopausal women from the Nurses' Health Study (NHS) and NHSII.

RESULTS—In premenopausal women, caffeine intake was inversely associated with luteal total and free estradiol, while positively associated with luteal progesterone levels (P -trend=0.02, 0.01, 0.03, respectively). Coffee intake was significantly associated with lower luteal total and free estradiol levels, but not luteal progesterone levels (P -trend=0.007, 0.004, 0.20, respectively). Among the postmenopausal women, there was a positive association between caffeine and coffee intake and SHBG levels (P -trend=0.03 and 0.06). No significant associations were detected with the other hormones.

CONCLUSIONS—Data from this cross-sectional study suggest that caffeine may alter circulating levels of luteal estrogens and SHBG, representing possible mechanisms by which coffee or caffeine may be associated with pre- and postmenopausal malignancies, respectively. Future studies evaluating how caffeine-mediated alterations in sex hormones and binding protein levels affect the risk of female cancers are warranted.

Keywords

caffeine; sex hormones; ovarian cancer; breast cancer; cross-sectional

INTRODUCTION

Coffee is one of the most frequently consumed beverages worldwide. Although drinking habits vary between countries, coffee's widespread use has led to the evaluation of its consumption

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with various health outcomes. Coffee is a primary dietary source of caffeine, and also contains many other biologically active ingredients including minerals, polyphenols, and other phytochemicals^{1, 2}, many of which have been associated with chemopreventive and antioxidant properties. To date, the exact mechanism(s) by which coffee or caffeine may influence the development of certain conditions, particularly cancer, have not been elucidated.

Several epidemiological studies have evaluated a role of coffee and other caffeine-containing beverages in the etiology of breast and ovarian cancer, albeit, with conflicting findings³ (reviewed in⁴). Interestingly, the Nurses' Health Study (NHS) recently reported an inverse association between caffeine and risk of breast cancer among postmenopausal women⁴. Similarly, in the NHS increasing caffeine intake was associated with a decreased risk of postmenopausal but an increased risk of premenopausal ovarian cancer⁵. Due to its biochemical complexity, there are various plausible mechanisms by which coffee or caffeine may affect health. Since a substantial body of evidence has implicated sex hormones in the etiology of both breast and ovarian cancer^{6, 7}, an effect associated with coffee consumption may be due to the ability of caffeine to influence hormone metabolism⁸. Whether caffeine affects cancer risk by altering sex hormone levels is currently unclear, although sex hormones and caffeine are metabolized via similar enzymes.

Our aim was to explore the biological basis for possible associations between coffee consumption and risk of hormone-related cancers. To address this issue, we examined caffeine intake, as well as coffee, decaffeinated coffee, and tea consumption, in relation to plasma concentrations of estrogens, androgens, progesterone, prolactin, and sex hormone binding globulin (SHBG) in a cross-sectional study 524 premenopausal and 713 postmenopausal women from the NHS and NHSII.

MATERIALS AND METHODS

Study Population

The NHS was established in 1976 among 121,700 US female registered nurses, ages 30 to 55 years, and the NHSII was established in 1989 among 116,609 female registered nurses, ages 25 to 42 years. All women completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. Data have been collected on numerous ovarian and breast cancer risk factors including parity, hormone use, tubal ligation, and family history of cancer.

From 1989–1990, 32,826 NHS participants (ages 43–70 years) provided blood samples and completed a short questionnaire⁹. Briefly, women arranged to have their blood drawn and shipped with an icepack, via overnight courier, to our laboratory, where it was processed.

From 1996–1999, 29,611 NHSII participants (ages 32–54 years) provided blood samples and completed a short questionnaire¹⁰. Briefly, premenopausal women (n=18,521) who had not taken hormones, been pregnant, or lactated within 6 months provided blood samples drawn on the 3rd to 5th day of their menstrual cycle (follicular) and 7 to 9 days before the anticipated start of their next cycle (luteal, called timed samples). Other women (n=11,090) provided a single 30 mL untimed blood sample. Since collection, samples have been archived at –130 °C or colder in continuously monitored liquid nitrogen freezers. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA).

We considered a woman to be premenopausal if (1) she gave timed samples, (2) her periods had not ceased, or (3) she had a hysterectomy with at least one ovary remaining and was ≤47 years (nonsmokers) or ≤45 years (smokers). We considered a woman to be postmenopausal if

(1) her natural menstrual periods had ceased permanently, (2) she had a bilateral oophorectomy, or (3) she had a hysterectomy with at least one ovary remaining and was ≥ 56 years (nonsmokers) or ≥ 54 years (smokers)¹¹. The remaining women, most of whom had a simple hysterectomy and were 48–55 years old, were of unknown menopausal status and therefore excluded.

Participants in this study were controls from nested case-control studies of breast cancer^{10, 12} who were matched to women diagnosed with breast cancer after blood collection through June 1, 2000 (NHS postmenopausal women not using postmenopausal hormones {PMH}, n=713) or June 2003 (NHSII premenopausal women)(n=411), and a subset of premenopausal women who were included in a reproducibility study (n=113)¹³.

Laboratory Assays

Premenopausal hormone assay methods for estrogens and testosterone have been described previously¹⁴. We measured premenopausal hormone levels on the following samples: estradiol, estrone, and estrone sulfate in follicular and luteal samples; testosterone, androstenedione, prolactin, and SHBG in follicular, luteal, and untimed samples; DHEA and DHEAS in luteal and untimed samples; and progesterone in luteal samples. Follicular and luteal samples from each woman were assayed together; samples were assayed in three batches. Details regarding the methods used to assay postmenopausal hormones have been published previously^{12, 15}. The interassay coefficients of variation (CV) based on blinded replicates were 6–14%, except for progesterone (CV=17%).

Samples were assayed in a random order. When hormone values were less than the detection limit, we set the value to one half the limit (n, estrone=25, testosterone=2, androstenedione=1, DHEA=1, DHEAS=7, estrone sulfate=5). The stability of these hormones in whole blood not processed for 24–48 hours has been shown previously¹⁶.

Dietary Assessment

In both the NHS and NHSII, diet was assessed through a validated, self-administered, semi-quantitative food frequency questionnaire (FFQ)¹⁷. Caffeine consumption was calculated using US Department of Agriculture food composition sources^{18–20}. Respondents were asked the average frequency of use of caffeine-containing beverages (coffee, tea and soda) and foods (chocolate) with choices ranging from “never or almost never” to “6 or more times per day”. The estimated caffeine content used was 137mg per cup of coffee, 47 mg per cup of tea, 46mg per can or bottle of caffeinated soda, and 7mg per chocolate serving. FFQs in both cohorts asked about non-herbal tea (i.e. caffeinated) only. The frequency of consumption was modeled as cups per day for coffee and tea, and mg per day for caffeine.

We evaluated intake using two approaches. First, we looked at consumption reported on the FFQ closest to the blood collection (1990 for NHS, 1995 for NHSII). Second, we calculated the average intake from the FFQ completed four years prior to blood collection (1986 for NHS, 1991 for NHSII) and that closest to blood collection. Since the results were similar for these approaches, we only report the results of the former.

Statistical Analysis

All analyses were stratified by menopausal status. For each analyte, we excluded women with missing values related to assay difficulties or low plasma volume. We identified and excluded values (n=0–11 for postmenopausal and 0–3 for premenopausal women) that were statistical outliers²¹. Among premenopausal women, the associations for the estrogens were assessed separately for follicular and luteal measurements. For testosterone, androstenedione, prolactin,

and SHBG, we averaged the follicular and luteal values, as levels did not vary substantially by phase^{10, 22, 23}, and included untimed samples for all hormones except estrogens²⁴.

We calculated adjusted geometric means for each log-transformed hormone by exposure category using a generalized linear model. For coffee, decaffeinated coffee and tea intakes, tests for trend were conducted by modeling the continuous variable and for caffeine modeling the quartile median intake and calculating the Wald statistic²⁵. Women with missing FFQ or hormone information were excluded for the specific analyses with missing data.

Among the premenopausal women, multivariate models were adjusted for assay batch (¹⁻³), age at blood draw (<40, 40-45, ≥45 years), fasting status (≤10, >10 hours), time of day of the blood draw(s) (1-8 a.m., 9 a.m.-noon, 1 p.m.-midnight), month of blood draw (continuous), difference between luteal draw date and date of the next menstrual period (3-7, 8-21 days, unknown/untimed), duration of past oral contraceptive use (never, <4, ≥4 years, missing), parity (yes, no), BMI at blood draw (continuous), physical activity (<5, 5-18, ≥18 MET-hr/wk), alcohol consumption (0, 0-10, >10 g/d), and smoking (never, past, current). Primary analyses were restricted to premenopausal women with ovulatory cycles at the blood draw (for timed samples); secondary analyses included all women and adjusted for ovulatory status (ovulatory [luteal progesterone ≥400ng/dl], anovulatory, untimed). Since the results were similar, we report results for the former to eliminate confounding by, and added variability due to ovulatory status.

For postmenopausal women, we adjusted for assay batch (¹⁻⁶), age at blood draw (55, 55-60, 60-65, >65 years), age at first birth/parity (nulliparous, age at first birth<25 years/1-4 children, age at first birth 25-29 years/1-4 children, age at first birth 30 years/1-4 children, age at first birth<25 years/5 children, age at first birth>25 years/5 children), and time of day of blood draw (1-8 a.m., 9 a.m.-noon, 1 p.m.-4 p.m., 5 p.m.-midnight).

We also assessed whether the associations differed by BMI, a major source of hormone production in postmenopausal women, and cholesterol, a precursor for ovarian hormone production, using multiplicative interaction terms. *P*-values were two-sided and considered statistically significant if ≤0.05.

RESULTS

There were 524 premenopausal and 713 postmenopausal women available for analysis, with a mean age at blood draw of 43 and 62 years, respectively (Table 1). Women were, on average, overweight in both groups. Among premenopausal women, 83% provided timed samples, 84% had previously used oral contraceptives, and 5% were current smokers. Among postmenopausal women, 32% had previously used oral contraceptives and 12% were current smokers. Mean parity was higher, and physical activity was lower, among the postmenopausal women. Sex hormone levels were in the expected ranges²⁴.

Cut-points for each exposure were based on the distribution in the population or on what has previously been associated with cancer risk⁵. The cut-points were ≤6 cups/week, 1 cups/day, 2-3 cups/day and ≥4 cups/day for coffee; ≤1 cups/week, 2-6 cups/week, 1 cup/day and ≥2 cups/day for tea; and ≤1-3 cups/month, 1-6 cups/week, 1 cups/day and ≥2 cups/day for decaffeinated coffee. For caffeine intake, the quartiles were based on the distribution in the premenopausal and postmenopausal women separately (see tables 3 and 5, respectively).

Among premenopausal women with an ovulatory cycle at blood draw, caffeine intake was inversely associated with luteal total estradiol (mean for the highest (Q4) versus lowest (Q1) quartile=117 versus 134pg/mL; *P*-trend=0.02) and luteal free estradiol (mean, Q4 versus Q1=1.44 versus 1.70pg/mL; *P*-trend=0.01), and positively associated with luteal progesterone

levels (mean, Q4 versus Q1=1585 versus 1412ng/dL; P -trend=0.03) (Table 2). Similarly, higher coffee intake was associated with lower luteal total estradiol (P -trend=0.007) and luteal free estradiol levels (P -trend=0.004), but not with progesterone levels (P -trend=0.20) (Table 3). There were no significant associations with any other hormones. Results were similar, although somewhat weaker, when including women with anovulatory cycles (data not shown).

Decaffeinated coffee was associated with significantly lower DHEAS levels (mean, Q4 versus Q1=66.4 versus 79.6 μ g/dL; P -trend=0.04). We did not observe any other associations between decaffeinated coffee and hormone levels (data not shown). Tea intake was positively associated with follicular estradiol (mean, Q4 versus Q1=54.6 versus 45.8pg/mL; P -trend=0.05) and follicular free estradiol levels (mean, Q4 versus Q1=0.70 versus 0.57pg/mL; P -trend=0.009). There was a suggestive inverse association between tea and DHEAS (P -trend=0.08).

There were no significant interactions with BMI for any exposure (all P -interactions $\geq$ 0.11); however, the relationship of caffeine intake with DHEA and DHEAS levels differed by cholesterol intake (P -interaction=0.02 and 0.04, respectively). Caffeine intake was positively associated with DHEA and DHEAS levels among women at or above the median for cholesterol intake (P -trend=0.04 and 0.11, respectively) but not among those below the median.

Among postmenopausal women, caffeine intake was associated with higher levels of SHBG (mean, Q4 versus Q1=52 versus 47nmol/l; P -trend=0.03) (Table 4). Similarly, higher coffee intake was modestly positively associated with SHBG levels (P -trend=0.06) (Table 5). There was also a suggestion of an inverse association between caffeine and free testosterone (P -trend=0.09). Caffeine and coffee intakes were not associated with other circulating hormones. Decaffeinated coffee was modestly inversely associated with free estradiol (P -trend=0.06) and estrone sulfate (P -trend=0.07) levels, and was significantly inversely associated with DHEAS levels (mean, Q4 versus Q1=79 versus 75 μ g/dL; P -trend=0.01). We observed no significant associations between circulating sex hormones and tea intake (P -trend $\geq$ 0.22). Generally the results showed a stronger dose-response relationship with deciles of intake (data not shown).

DISCUSSION

We examined the relationships between caffeine, coffee, and tea intake with circulating sex hormones, prolactin and SHBG levels in a large cross-sectional study. Among premenopausal women, caffeine and coffee intake were inversely associated with luteal levels of total and free estradiol. Furthermore, caffeine intake was positively associated with luteal progesterone levels. We also observed a positive association between tea intake and follicular and free estradiol levels. There was a positive association of caffeine and coffee intake with SHBG levels among postmenopausal women. Decaffeinated coffee was inversely associated with DHEAS levels in both groups.

The results from three earlier studies of these associations in premenopausal women have been inconsistent^{8, 26, 27}. Lucero *et al.* reported higher early follicular estradiol levels with increasing daily caffeine and coffee consumption (n=498)⁸. Conversely, among 50 premenopausal women, green tea, but not coffee or total caffeine intake, was inversely correlated with follicular estradiol²⁷. London *et al.* reported a significant inverse correlation between caffeine intake and free estradiol among 325 perimenopausal women aged 50–60 years²⁶. In our study, premenopausal women with the highest versus lowest quartile of caffeine and coffee intake had 12–15% lower luteal total and free estradiol levels, while follicular estradiol and free estradiol levels were 16–19% higher among women in the highest versus lowest quartile of tea intake. Differences between studies may be due to small sample sizes or poor timing of blood collection during the menstrual cycle in prior studies. With our method

of blood collection, we were able to accurately calculate the date of the menstrual cycle for women with timed samples.

Because of the similar associations of coffee and caffeine with luteal estrogen levels, it is probable that caffeine is the component influencing estrogen metabolism. Since there was suggestive evidence for higher testosterone levels with higher intakes of caffeine and coffee, caffeine may be inhibiting *CYP19*, or aromatase, the key enzyme mediating the conversion of androgens to estrogens^{24, 28}. Nevertheless, it is unclear why this would not also influence follicular estrogen levels or sex hormone levels in postmenopausal women where aromatase plays a more critical role in dictating estrogen and testosterone levels. We observed a significant trend for higher circulating progesterone with higher caffeine but not coffee intake. To our knowledge, there are no prior reports of caffeine and progesterone levels in humans; however, one study in male rats showed increased plasma progesterone with intraperitoneal injections of caffeine²⁹.

Since we did not observe any effect of coffee or caffeine on follicular estrogen levels, the positive association with tea could be attributed to another component rather than caffeine. Our results require further exploration and should be interpreted with caution given that there are no prior assessments of this association and the number of women with daily tea consumption was small (24% consumed ≥ 1 cup/day). Nagata *et al.* reported an inverse correlation between follicular estradiol levels and green but not black tea among premenopausal women²⁷. Another study reported that tea intake was not associated with estradiol and SHBG levels⁸. We were not able to evaluate the effect of green tea since our FFQ asked about non-herbal tea intake, which consists primarily of black tea in the United States.

Although androgens and estrogens are well-established risk factors for postmenopausal breast cancer³⁰, the role of these hormones in premenopausal breast cancer is not fully understood. Our findings of lower luteal estrogen levels with higher caffeine intake represents one potential protective mechanism for premenopausal hormonally-dependent cancers. Of the two studies examining estrogens and breast cancer risk in premenopausal women, one reported no association³¹, and the other reported an increased risk of premenopausal breast cancer only with follicular, but not luteal, estradiol levels¹⁴. The elevated levels of follicular estrogens with tea intake suggests that tea may increase the risk of hormone-related cancers, although to our knowledge no epidemiologic data have reported an association between tea intake and premenopausal breast cancer risk. The higher progesterone levels with caffeine intake requires further exploration particularly given the inconsistency in the data regarding a role for this hormone in breast cancer development^{14, 31} and the possible inverse relationship with ovarian cancer⁶. Furthermore, our findings do not explain the increased risk of premenopausal ovarian cancer with caffeine intake we and others have reported^{5, 32, 33}.

Among postmenopausal, but not premenopausal, women, high intakes of caffeine or caffeinated coffee were associated with a 13% higher SHBG levels versus low intakes. Four previous studies have reported a similar positive association with caffeine and/or coffee intake and levels of SHBG^{8, 26, 27, 34}, however, only one study was limited to postmenopausal women³⁴. Since SHBG is the major carrier of estrogen and testosterone, we expected to see a concomitant inverse association with free levels of these two hormones. We did not observe a strong effect; however, there was suggestive evidence for decreasing free testosterone levels with increasing caffeine intake; testosterone preferentially binds SHBG versus estradiol³⁵. Similarly, Ferrini *et al.* reported an inverse association between caffeine intake and bioavailable testosterone levels, but a positive association with estrone levels³⁴. The lack of an association with estrogen levels may be attributed to the sensitivity of our assays and consequently, inability to detect small changes in circulating levels particularly given the low estrogen, and to a lesser extent androgen, production among postmenopausal women.

Higher SHBG levels have been associated with a lower risk of postmenopausal breast cancer among both users and non- users of PMH^{7, 12, 36}. Only one study has evaluated the relationship between SHBG concentrations and ovarian cancer risk, observing an inverse association among women diagnosed before age 55³⁷. In general, the protective effect of caffeine and/or coffee on breast and ovarian cancer appears to be strongest among postmenopausal women^{4, 5}. Hormonal changes in postmenopausal women include a substantial decrease in estradiol and estrone levels, but only a small change in androgen synthesis by the ovaries and adrenal glands²⁴. This suggests that the inverse association between caffeine intake and risk of postmenopausal ovarian and breast cancer may be mediated by its effect on hepatic production of SHBG and subsequent reduction in free testosterone. Indeed, high endogenous testosterone levels have been clearly implicated in the etiology of postmenopausal breast cancer^{7,12}, although a positive association with risk of ovarian cancer is less clear³⁸.

Since adipose tissue is an important source of sex hormones in postmenopausal women, we also evaluated whether BMI modified any of these associations. The inverse association between caffeine and SHBG appeared strongest among overweight/obese women. Although not statistically significant, this possible interaction warrants further evaluation since adiposity has consistently been associated with lower SHBG levels.

We did not observe associations between caffeine, coffee, or tea with levels of androgens, estrogens, or prolactin in the postmenopausal women. The null association with prolactin is of interest given that this hormone has been associated with an increased risk of premenopausal¹⁰ and postmenopausal breast cancer¹⁵. However, in other studies of postmenopausal women, black tea has been associated with higher plasma levels of estrone and prolactin^{39, 40}.

The inverse association between decaffeinated coffee and DHEAS requires further confirmation given the small percentage of women that regularly consumed decaffeinated coffee in our cohort (≥ 2 cups/day: 12% for premenopausal, 31% for postmenopausal women) and more importantly, the biological effect of decaffeinated coffee on adrenal androgens levels has never been explored.

This is the largest study to examine the relationship between endogenous androgens, estrogens, prolactin and SHBG with caffeine, coffee and tea intake. We were able to obtain timed blood samples from a large number of premenopausal women to accurately assess hormone concentrations during both the luteal and follicular phases and we limited our analysis of postmenopausal women to those not using PMH. The major limitation of this study is the inability to establish a temporal relationship between the exposure (i.e. caffeine) and hormones levels, although it is unlikely that endogenous sex hormones would influence coffee consumption. Also, validation studies have shown a high correlation between self-reported coffee intake on the FFQ compared to that from a 28-day diet record ($\rho=0.75$)⁴¹.

In summary, our data suggest that caffeine-mediated changes in circulating levels of luteal estrogens and SHBG represent possible mechanisms by which coffee or caffeine may be associated with pre- and postmenopausal hormonally-related malignancies, respectively. Since hormones are clearly implicated in the etiology of many female cancers, further evaluation of how caffeine-mediated alterations in sex hormones and binding protein levels affect the risk of breast or ovarian cancer are warranted.

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TABLE 1

Characteristics of Premenopausal and Postmenopausal Women in the Nurses' Health Study and Nurses' Health Study II, Respectively

Characteristic	Premenopausal (n=524) ¹	Postmenopausal (n=713)
Age at blood draw, y (mean, SD)	43.4 (3.8)	61.6 (4.7)
Age at menarche, y (mean, SD)	12.5 (1.4)	12.5 (2.0)
Parity (mean, SD) ²	2.31 (0.9)	3.6 (1.7)
BMI at blood draw, kg/m ² (mean, SD)	25.1 (5.9)	26.2 (4.8)
Ever OC use (n, %)	440 (84%)	229 (32%)
Timed sample (n, %)	437 (83%)	n/a
Age at menopause, y (mean, SD)	n/a ³	49.0 (5.1)
Alcohol consumption, g/day (mean, SD)	3.7 (6.6)	5.4 (10.3)
Total physical activity (MET-h/week) (mean, SD)	21.0 (27.9)	15.9 (19.5)
Current smoker (n, %)	29 (5%)	89 (12%)
Mean plasma hormone levels	Median (10 th – 90 th Percentile)	Median (10 th – 90 th Percentile)
Estrone (pg/mL)		25 (14–43)
Follicular ⁴	41 (26–63)	
Luteal ⁵	79 (50–124)	
Estradiol (pg/mL)		7 (4–14)
Follicular	46 (22–98)	
Luteal	124 (80–198)	
Free estradiol (pg/mL)		0.10 (0.04–0.25)
Follicular	0.59 (0.31–1.11)	
Luteal	1.61 (0.97–2.53)	
Estrone Sulfate (pg/mL)		197 (85–527)
Follicular	674 (306–1508)	
Luteal	1477 (561–3235)	
Progesterone (ng/dL)	1481 (740–2571)	n/a
Testosterone (ng/dL)	23.5 (14.5–36.5)	22 (12–40)
Free Testosterone (ng/dL)	0.2 (0.1–0.3)	0.2 (0.1–0.4)
Androstenedione (ng/dL)	106 (63.3–172)	57 (29–109)
DHEA (ng/dL)	638 (346–1130)	217 (88–431)
DHEAS (μg/dL)	79.3 (39.9–140)	85 (32–175)
Prolactin (ng/mL)	14.8 (8.6–26.4)	8.30 (5.12–15.9)
SHBG (nmol/L)	64.3 (32.7–112)	50.8 (23.3–90.8)

¹ Among ovulatory premenopausal women.

² Among parous women only.

³ Not applicable.

^{4,5} Follicular and luteal levels only apply to premenopausal women.

Adjusted Geometric Mean Levels^a of Estrogens, Androgens, Progesterone, Prolactin and SHBG by Quartile of Caffeine Intake in Premenopausal Women

TABLE 2

Hormone	n	Caffeine quartiles (mg/day)				P – trend ^b
		≤ 70	> 70 – ≤ 190	> 190 – ≤ 371	> 371	
Sample size range		102–133	94–126	99–131	79–120	
Estrone (pg/mL)	389	40	40	42	40	0.89
Follicular	417	81	78	80	76	0.32
Luteal						
Estradiol (pg/mL)	388	46	46	49	49	0.35
Follicular	383	134	126	123	117	0.02
Luteal						
Free estradiol (pg/mL)	366	0.57	0.57	0.64	0.59	0.50
Follicular	374	1.70	1.60	1.64	1.44	0.01
Luteal						
Estrone Sulfate (pg/mL)	377	684	690	693	661	0.72
Follicular	378	1486	1446	1497	1250	0.16
Luteal						
Progesterone (ng/dL)	422	1412	1333	1495	1585	0.03
Testosterone (ng/dL)	477	23	23	24	24	0.14
Free Testosterone (ng/dL)	473	0.18	0.19	0.20	0.19	0.48
Androstenedione (ng/dL)	488	102	101	109	105	0.27
DHEA (ng/dL)	409	616	614	654	654	0.23
DHEAS (µg/dL)	409	74	76	80	77	0.58
Prolactin (ng/mL)	493	15	14	15	15	0.95
SHBG (nmol/L)	501	63	60	59	66	0.37

^a Adjusted for assay batch, age at blood draw, fasting status (follicular and luteal phase), time of day of blood draw (follicular and luteal), month of blood draw, difference between luteal blood draw date and date of the next menstrual period, duration of oral contraceptive use, parity, BMI at blood draw, physical activity level, alcohol consumption, and smoking status.

^b Trend across quartile medians for caffeine, using the Wald test.

Adjusted Geometric Mean Levels^a of Estrogens, Androgens, Progesterone, Prolactin and SHBG by Quartiles of Coffee Intake in Premenopausal Women

TABLE 3

Hormone	n	Coffee quartiles				P – trend ^b
		6 cups/week	1 cup/day	2–3 cups/day	≥ 4 cups/day	
Sample size range		176–230	30–39	118–167	39–56	
Estrone (pg/mL)	379	40	42	41	39	0.79
Follicular	380	81	76	79	74	0.27
Luteal						
Estradiol (pg/mL)	390	46	50	47	52	0.42
Follicular	385	132	126	118	116	0.007
Luteal						
Free estradiol (pg/mL)	368	0.57	0.66	0.60	0.63	0.78
Follicular	376	1.69	1.57	1.53	1.43	0.004
Luteal						
Estrone Sulfate (pg/mL)	391	666	796	697	641	0.87
Follicular	419	1446	1579	1425	1251	0.27
Luteal						
Progesterone (ng/dL)	424	1345	1728	1509	1510	0.20
Testosterone (ng/dL)	479	23	24	24	25	0.15
Free Testosterone (ng/dL)	475	0.18	0.20	0.20	0.19	0.53
Androstenedione (ng/dL)	490	101	108	108	105	0.47
DHEA (ng/dL)	410	612	704	635	685	0.46
DHEAS (µg/dL)	410	72	85	81	79	0.26
Prolactin (ng/mL)	495	15	15	15	15	0.60
SHBG (nmol/L)	503	61	60	62	68	0.32

^a Adjusted for covariates listed in Table 2.

^b Trend across continuous coffee intake, using the Wald test.

Adjusted Geometric Mean Levels^a of Estrogens, Androgens, Prolactin, and SHBG by Quartiles of Caffeine Intake in Postmenopausal Women Not Taking PMH

TABLE 4

Hormone	n	Caffeine quartiles (mg/day)					p – trend ^b
		< 99	99 – < 219	219 – < 378	> 378		
Sample size range		139–175	131–170	131–169	129–175		
Estrone (pg/mL)	528	25	24	25	25	0.81	
Estradiol (pg/mL)	664	7.3	6.4	7.1	6.8	0.68	
Free estradiol (pg/mL)	633	0.10	0.09	0.10	0.09	0.33	
Estrone Sulfate (pg/mL)	659	200	208	213	208	0.58	
Testosterone (ng/dL)	666	22	21	21	22	0.68	
Free Testosterone (ng/dL)	650	0.22	0.21	0.20	0.20	0.09	
Androstenedione (ng/dL)	527	56	53	55	58	0.40	
DHEA (ng/dL)	511	189	198	208	216	0.11	
DHEAS (µg/dL)	534	77	76	77	84	0.31	
Prolactin (ng/mL)	661	9.3	8.2	8.7	8.6	0.44	
SHBG (nmol/L)	669	47	46	48	53	0.03	

^a Adjusted for assay batch, we adjusted for assay batch, age at first birth/parity, fasting status, and time of day of blood draw, BMI at blood draw, physical activity level, alcohol consumption, and smoking status.

^b Trend across quartile medians for caffeine, using the Wald test.

Adjusted Geometric Mean Levels^a of Estrogens, Androgens, Prolactin, and SHBG by Quartiles of Coffee Intake in Postmenopausal Women Not Taking PMH

Hormone	n	6 cups/week	Coffee quartiles				p – trend ^b
			1 cup/day	2–3 cups/day	> 4 cups/day		
Sample size range		193–255	42–50	177–229	85–114		
Estrone (pg/mL)	533	25	22	25	24	0.94	
Estradiol (pg/mL)	671	7.0	6.3	6.9	6.7	0.83	
Free estradiol (pg/mL)	640	0.10	0.09	0.10	0.09	0.47	
Estrone Sulfate (pg/mL)	668	213	190	208	200	0.99	
Testosterone (ng/dL)	673	22	20	21	22	0.88	
Free Testosterone (ng/dL)	657	0.22	0.20	0.20	0.20	0.30	
Androstenedione (ng/dL)	532	56	49	54	61	0.37	
DHEA (ng/dL)	516	196	197	199	231	0.14	
DHEAS (µg/dL)	539	80	69	77	86	0.26	
Prolactin (ng/mL)	668	8.9	8.9	8.4	8.9	0.61	
SHBG (nmol/L)	676	48	48	48	54	0.06	

^a Adjusted for covariates listed in Table 4.

^b Trend across continuous coffee intake, using the Wald test.