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RELATIONSHIP BETWEEN SERUM HORMONE CONCENTRATIONS, REPRODUCTIVE HISTORY, ALCOHOL CONSUMPTION AND GENETIC POLYMORPHISMS IN PRE-MENOPAUSAL WOMEN

Montserrat García-Closas[1]*, Julie Herbstman[1], Mark Schiffman[1], Andrew Glass[2] and Joanne F. Dorgan[3]

[1]*Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA*
[2]*Kaiser Permanente Health Plan, Portland, OR, USA*
[3]*Fox Chase Cancer Center, Philadelphia, PA, USA*

Reproductive characteristics, alcohol intake and polymorphisms in genes encoding sex-steroid metabolizing enzymes might influence the risk of hormone-related cancers by changing circulating concentrations of sex hormones. The relationship between these factors and serum concentrations of estradiol, progesterone, androstenedione, testosterone and DHEA was evaluated in a cross-sectional study of 218 pre-menopausal women from Kaiser Permanente Health Plan in Portland, Oregon. Risk factor information was obtained from questionnaires and hormone serum concentrations were determined by radioimmunoassays. Genotypes for *CYP11A* 5′UTR(tttta)n, *CYP17* 5′-UTR −34 T>C, *CYP19* IVS4(ttta)n, *CYP1B1* (L432V and S453N) and *COMT* (V158M) were determined from genomic DNA samples. Increasing number of full-term pregnancies was associated with a significant decrease in late-follicular progesterone levels (*p*-trend = 0.03). Increasing alcohol consumption was associated with higher estradiol levels averaged through the menstrual cycle (*p*-trend = 0.009) and higher progesterone levels during luteal phase (*p*-trend = 0.04). Androstenedione and testosterone levels were higher among light to moderate drinkers compared to non-drinkers, although we only observe a significant trend with increasing levels of alcohol consumption for androstenedione. Women heterozygous or homozygous for the *CYP1B1* L432V or the S453N polymorphisms had increased luteal estradiol levels (*p*-value = 0.04 for L432V and 0.04 for S453N). None of the other factors evaluated was significantly associated with serum concentration of hormones. In conclusion, results from this cross-sectional study of pre-menopausal women provide support for an association between light to moderate alcohol intake and elevated levels of estrogen and androgen levels. Our data suggest that circulating levels of progesterone might be related to parity and alcohol consumption, however the biological plausibility of the observed associations is unclear. We found little support for an influence of the evaluated genetic polymorphisms in the steroid synthesis and metabolism pathway on serum hormone levels, except for a possible effect of the *CYP1B1* L432V and S453N polymorphisms on serum estradiol levels.
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Key words: *sex hormones; pre-menopausal women; genetic polymorphisms; alcohol; reproductive history*

Endogenous levels of sex-steroid hormones are likely to play an important role in the etiology of female cancers, including breast and endometrial cancer.[29,63] Reproductive and lifestyle factors such as early age at menarche, late age at first full term birth, late age at menopause and alcohol consumption are thought to influence the risk of breast cancer by modifying the life-time exposure to endogenous levels of hormones.[32,52] Similarly, established risk factors for endometrial cancer such as nulliparity, early age at menarche and late age at menopause are thought to act by modulating exposure to estrogens unopposed by progestins.[31]

Sex-steroid hormones are synthesized and eliminated through complex metabolic pathways. Polymorphisms in genes coding for key enzymes involved in these pathways could modulate endogenous hormone levels and, through this mechanism, influence the risk of hormone-related cancers.[10,29] The effect of polymorphisms on circulating levels of hormones and reproductive characteristics has been evaluated only in a few studies with no consistent evidence of a substantial effect.[19,20,23–25,53,56] Breast cancer is the cancer site most studied in relation to polymorphisms in genes involved the hormone metabolism; however, the literature is generally inconclusive.[9,17,33]

In our cross-sectional study, we evaluate associations of reproductive factors, alcohol intake and genetic polymorphisms for 5 key enzymes involved in estrogen biosynthesis and metabolism, with serum concentrations of sex hormones in pre-menopausal women. The 5 genes evaluated in our study include the *CYP11A* gene coding for the cholesterol side chain cleavage enzyme that catalyzes the conversion of cholesterol to pregnenolone;[21] the *CYP17* gene coding for steroid 17-α-hydroxylase that catalyzes the conversion of pregnenolone to DHEA and progesterone to androstenedione;[21] the *CYP19* gene coding for aromatase that catalyzes the conversion of testosterone to estradiol and androstenedione to estrone;[5] the *COMT* gene coding for catechol-*O*-methyltransferase involved in the *O*-methylation of estradiol and estrone;[39] and the *CYP1B1* gene coding for a 4-hydroxylase involved in the 4-hydroxylation of estradiol and estrone[27] (Fig. 1). This is one of the few studies published to date that evaluates determinants of circulating levels of estrogen, progesterone and androgens in a relatively large cross-sectional study of pre-menopausal women.

MATERIAL AND METHODS

Study population

Study participants were selected from women participating as control subjects in a case-control study of cervical neoplasia, nested within a prospective cohort study of the natural history of human papillomavirus (HPV) infection at the Kaiser Permanente Health Maintenance Organization in Portland, Oregon. This cohort was established in 1989–90 and included a representative sample of over 20,000 women presenting for routine cervical cytological (Pap smear) screening at Kaiser Permanente. Controls in the case-control study included 1,037 cytologically normal women in the cohort with no past history of hysterectomy or cervical neoplasia. Details on the selection of controls can be found in Liaw *et al.*[37] Briefly, controls were selected from women with normal cytological diagnosis at enrollment in the cohort study who did not develop cervical neoplasia during follow-up. Controls were matched to cervical neoplasia cases on 2 main variables: age and follow-up time. Controls completed a follow-up examination that included the collection of a cervicovaginal lavage, a blood sample

*Correspondence to: Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Blvd., EPS 7076, Rockville, MD 20852-7234.
Fax: +301-402-0916. E-mail: montse@nih.gov

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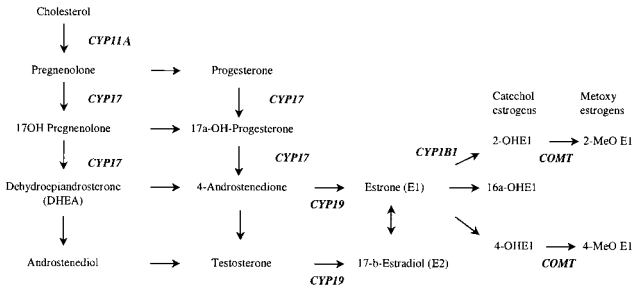

FIGURE 1 – Genes evaluated in the postulated pathway for synthesis and metabolism of steroid hormones.

and a comprehensive telephone interview of known risk factors for cervical neoplasia (tobacco use and alcohol consumption, Pap smear history, reproductive and sexual history and demographic information). Two hundred twenty-three women out of the 1,037 controls met our selection criteria aimed at selecting a representative group of pre-menopausal women with established menstrual cycles, and at reducing extraneous variation in serum levels of hormones of interest. Specifically, the selection criteria were: 1) 5 years or more since menarche and 45 years old or younger, 2) blood and cervicovaginal lavage sample collected at the follow-up visit, 3) not taking oral contraceptives, pregnant or nursing at blood collection, 4) regular menstrual cycles as reported in the medical records, 5) more than 6 months since last pregnancy, and 6) time since last menstrual period of 33 days or less. Approval for human experimentation was obtained from institutional review boards.

Hormone measurements

Blood specimens were collected using a serum separation vacuum tube and aliquots were stored at −70°C until hormone analyses were carried out. Information on date and time of day of blood collection was recorded. The day of the menstrual cycle was calculated based on self-reported information on the day of the last menstrual period at or before blood collection. All hormone measurements were carried out at Esoterix Endocrinology, Inc. (formerly known as Endocrine Sciences, Inc.; Calabasas Hills, CA) by radioimmunoassays using procedures published previously.[13] Coefficients of variation for replicate quality control samples collected from one volunteer during early follicular phase averaged 14% for estradiol, 8% for DHEA, 13% for androstenedione and 13% for testosterone; and for replicate samples collected from a second volunteer during luteal phase averaged 18% for estradiol, 17% for progesterone, 9% for DHEA, 6% for androstenedione and 9% for testosterone. The coefficient of variation for progesterone levels during early follicular phase could not be calculated because replicate samples had progesterone levels under the limit of detection for the radioimmunoassay. Serum hormone concentrations for estradiol, progesterone, testosterone, androstenedione and DHEA could not be obtained from 1, 10, 26, 8 and 12 women, respectively, because of insufficient serum sample.

Genotype determination

DNA was extracted from cervicovaginal lavage cell pellets using the Qiagen QIAamp Mini Kit according to the Blood and Body fluid protocol (Qiagen, Chatsworth, CA). PCR primer and probe sets for genotyping analyses were designed from sequences published previously: *CYP11A* 5′UTR(tttta)n,[20] *CYP17* 5′-UTR −34 T>C (*MspA1*),[6] *CYP19* IVS4(ttta)n,[57] *CYP1B1* (L432V and S453N)[2] and *COMT* (V158M).[35] About 10% repeated samples blinded to the laboratory personnel were inserted for quality control of genotype assays. The concordance for repeated samples was 100% for all genotype assays carried out. None of the genetic polymorphisms had significant departures from Hardy-Weinberg equilibrium.

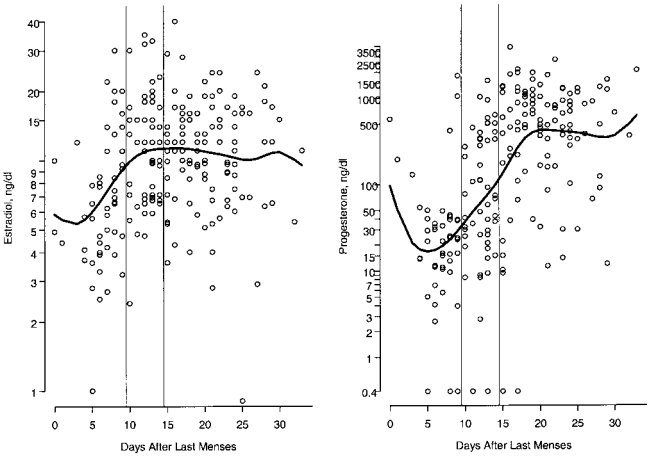

FIGURE 2 – Scatter plot smoothing of serum concentrations (ng/dl) of estradiol and progesterone by the number of days since onset of last menses.

Statistical methods

Serum hormone data were transformed to the $\log_e$ scale before analyses to improve normality. Linear regression models were used to estimate adjusted geometric mean hormone levels and to test the statistical significance of linear trends in continuous or ordered categorical covariates. A partial sum of squares F-test was used to test for heterogeneity of hormone concentrations by categorical variables in the linear models. Percentage differences in hormone levels were calculated from linear model coefficients as $(e^{\beta} - 1) \times 100$.

Adjusted geometric means for estradiol and progesterone levels stratified by variables of interest were estimated using separate models for women whose blood was collected during different phases of the menstrual cycle. We also estimated adjusted geometric means for estradiol and progesterone levels averaged across the menstrual cycles to increase the statistical power to detect associations independent of the phase of the menstrual cycle. This analysis was carried out when we found no evidence for modification of the association between hormone concentrations and the variable of interest by phase of the menstrual cycle. Phases of the menstrual cycle were defined based as: early follicular (0–9 days since onset of last menses; $N = 52$), late follicular (10–14 days since onset of last menses; $N = 54$) and luteal (15–40 days since onset of last menses; $N = 112$). Indicator variables for day of the menstrual cycle were included in each of these models to account for hormone variation within cycle phase. Data were sparse before Day 5 of the menstrual cycle because blood was collected at the time of a gynecological exam, and only a few women scheduled their appointment during menstruation. Scatter plot smoothing using kernel estimates[58] was used to graphically describe the relationship between estradiol and progesterone concentrations and day of the menstrual cycle (Fig. 2). Androgen levels do not present substantial changes during the menstrual cycle. Therefore adjusted geometric means for DHEA, androstenedione and testosterone were obtained averaged across menstrual cycle phases.

In addition to indicator variables for cycle day when blood was collected, all models for each of the hormones evaluated included a term for age and indicator variables for: time of blood collection, batch number for hormone assays, ethnic group, age at menarche, number of full-term pregnancies, and alcohol intake. A full-term pregnancy was defined as self-report of a pregnancy that ended in a live birth or stillbirth. At the time of blood collection, women were asked to report how many months since enrollment in the cohort they drank at least 1 alcoholic beverage (beer, wine or liquor), and how many alcoholic beverages they usually drank during these months. The average time since enrolled in the cohort

was 2.4 ± 1.0 (SD) years, ranging from 9 months to 4.8 years. Current average consumption of alcoholic beverage was estimated as the percentage of months when women drank at least one alcoholic beverage multiplied by the average number of consumed drinks. Scatter plots were created using S-PLUS statistical package (Seattle, WA), and all other analyses were performed using STATA statistical package (College Station, TX).

RESULTS

Population characteristics

Characteristics of the 218 pre-menopausal women included in this cross-sectional study are shown in Table I. Participants were on average 33.2 ± 6.6 (SD) years old. Over 90% of the women were Caucasian and more than 70% had at least some college education. Sixty-five women were nulliparous and 153 were parous with an average age at first full-term pregnancy of 24.7 ± 4.7 (SD) years. About 67% of the women reported not drinking or drinking an average of less than 1 alcoholic beverage/week since enrollment in the cohort. Serum concentrations of estradiol and progesterone generally approximated expected variations with the day of the menstrual cycle: estradiol rose during the follicular phase, peaked between Days 11–16 and was elevated during the luteal phase; progesterone levels were highest during the luteal phase (Fig. 2).

Age and reproductive characteristics

Age was not significantly associated with serum estradiol concentrations in any of the phases of the menstrual cycle, except for a borderline significant trend in the luteal phase (Table II). When estradiol levels were averaged across the menstrual cycle, age was significantly associated with slightly decreased levels of estradiol (−1.5% per year of age, $p = 0.03$). Increasing age was significantly associated with increasing progesterone serum concentrations during late follicular phase but not during the other phases of the menstrual cycle, p-interaction = 0.02 (Table II). When age was considered as a continuous variable, the percent difference in progesterone concentrations per year of age was −5.6% per year, $p = 0.24$ for early follicular, +23.5% per year of age, $p < 0.001$, for late follicular, −2.6% per year, $p = 0.37$ for luteal phases. Androgen concentrations (DHEA, androstenedione and testosterone) averaged across the menstrual cycle phases decreased significantly as age increased (Table II). When age was considered as a continuous variable, the percent difference in hormone concentration per year of age was −1.4% ($p = 0.009$) for DHEA, −1.9% ($p < 0.001$) for androstenedione, and −1.6% ($p < 0.001$) for testosterone.

Age at menarche was not significantly associated with estradiol levels for any of the menstrual cycles (Table II), nor when estradiol was averaged across the menstrual cycle phases (data not shown). We found no significant association between age at menarche and serum concentrations of any of the other hormones evaluated (Table II). Number of full-term pregnancies was not significantly associated with estradiol levels stratified by menstrual cycle phase (Table II) or averaged across cycle phases (data not shown). We observed a significant association between increasing number of full-term pregnancies and lower concentrations of progesterone in the late follicular phase (Table II). The p-value for the test for interaction between menstrual cycle and parity was 0.02. Among parous women, age at first full-term pregnancy or time since last pregnancy were not significantly related to serum concentration of hormones (data not shown).

TABLE I – CHARACTERISTICS OF 218 PRE-MENOPAUSAL WOMEN INCLUDED IN THE STUDY[1]

Characteristic	
Age, years	
18–25	31 (14.2)
26–30	45 (20.6)
31–35	64 (29.4)
36–45	78 (35.8)
Age at menarche, years	
<13	95 (43.6)
13	70 (32.1)
14 and older	53 (24.3)
Parity (number of full-term pregnancies)	
Nulliparous	65 (29.8)
1	43 (19.7)
2	68 (31.2)
3	42 (19.3)
Age at first full-term pregnancy (mean ± SD)[2]	24.7 ± 4.7
Race[3]	
White	201 (92.6)
Black	8 (3.7)
Other	8 (3.7)
Level of education	
High school diploma or less	56 (25.7)
Some college	97 (44.5)
College diploma or more	65 (29.8)
Current alcohol consumption	
Non-drinkers	43 (19.7)
<1 drink/week	104 (47.7)
≥1 drink/week	71 (32.6)

$N = 218$. Values are n (%) unless indicated otherwise.–[2]Among 153 women having had at least one full-term pregnancy.–[3]One woman had missing information.

Alcohol consumption

We found no significant differences in geometric mean concentrations of estradiol between non-drinkers, women drinking less than 1 drink per week and women drinking 1 or more alcoholic drinks per week, for any of the phases of the menstrual cycle (Table II). Non-drinkers, however, tended to have lower levels of estradiol than light to moderate drinkers for all menstrual cycle phases. When estradiol concentrations were averaged across the phases of the menstrual cycle, the differences reached statistical significance; women drinking less than 1 drink per week and 1 or more alcoholic drinks per week had a percent change in estradiol concentration of +36.5% ($p = 0.004$) and +36.9% ($p = 0.008$), respectively, compared to non-drinkers (p-trend = 0.009). This suggested an alcohol effect on estradiol levels independent of menstrual cycle phase (p for test for interaction between alcohol and menstrual cycle phase = 0.83). Alcohol consumption was significantly associated with higher luteal progesterone, androstenedione and testosterone levels, although we found a significant trend only for the association with luteal progesterone and androstenedione levels (Table II).

Genetic polymorphisms

Figure 1 shows a schematic representation of the postulated pathway for synthesis and metabolism of steroid hormones and the genes evaluated in this study. The distribution of genotypes under study shown in Table III was similar to distributions reported previously in Caucasian populations.[2,6,20,35,57] Among the genetic polymorphisms evaluated in relation to hormone concentrations, we only found a significant association between the *CYP1B1* (L432V and S453N) polymorphisms and estradiol concentrations: women with the variant alleles had higher estradiol concentrations (Table IV). Although the observed association was significant only in the luteal phase, levels of estradiol were higher for all phases of the menstrual cycle and we found no significant evidence for heterogeneity of this effect by cycle phase (p-value for interaction by menstrual cycle phase = 0.11 for L432V and 0.81 for S453N). Table IV shows no difference in hormone levels for women carrying the 7 allele repeat of *CYP19* compared to non-carriers. We also failed to observed differences in hormone levels when women carrying the 7, 8 , 9 or ≥10 repeat alleles were consider separately (data not shown). We found no significant effects of potentially relevant genotype combinations on hormone levels and (data not shown). Finally, we failed to observe any significant

TABLE II – GEOMETRIC MEAN[1] SERUM CONCENTRATIONS (NG/DL) OF SEX-STEROIDS IN 218 PRE-MENOPAUSAL WOMEN STRATIFIED BY AGE, REPRODUCTIVE CHARACTERISTICS AND ALCOHOL CONSUMPTION[2]

	Estradiol[3]			Progesterone[3]			DHEA[4]	Androstenedione[4]	Testosterone[4]
	Early follicular $n = 52$	Late follicular $n = 53$	Luteal $n = 112$	Early follicular $n = 50$	Late follicular $n = 52$	Luteal $n = 106$	$n = 206$	$n = 210$	$n = 192$
Age, years									
18–25	6.0	12.3	13.9	25.1	53.6	288.2	475.8	152.6	32.3
26–30	6.7	10.4	11.8	16.6	15.5	467.7	443.5	142.5	25.0
31–35	7.2	12.7	10.0	24.5	76.7	387.2	370.1	120.7	23.7
36–45	7.1	8.8	9.7	16.5	302.4	292.1	362.3	111.9	19.6
p for heterogeneity	0.43	0.67	0.16	0.33	0.00	0.81	0.17	0.00	0.00
p for trend	0.41	0.60	0.05	0.24	0.00	0.54	0.01	0.00	0.00
Age at menarche, years									
<13	7.0	12.9	10.3	12.0	73.9	297.1	370.4	122.1	22.9
13	6.3	9.5	11.4	22.9	83.8	451.0	399.4	125.4	22.9
14 and older	7.6	10.7	10.5	43.2	52.3	323.2	436.9	132.4	25.0
p for heterogeneity	0.43	0.27	0.76	0.33	0.13	0.12	0.80	0.52	0.69
p for trend	0.19	0.71	0.85	0.13	0.05	0.17	0.65	0.91	0.65
Parity (number of full-term pregnancies)									
0	6.4	10.1	10.5	18.9	148.9	298.8	434.5	134.6	26.5
1	7.1	11.1	9.7	20.7	121.7	205.8	389.5	121.9	23.2
2	8.1	12.0	10.7	28.5	28.7	425.6	380.4	123.3	21.9
3 or more	6.1	11.8	11.9	11.4	45.8	525.1	367.4	119.9	21.6
p for heterogeneity	0.40	0.70	0.58	0.27	0.06	0.08	0.46	0.44	0.15
p for trend	0.95	0.90	0.18	0.74	0.03	0.08	0.23	0.28	0.23
Alcohol intake									
Non-drinkers	4.9	9.9	8.5	20.8	249.8	220.7	334.6	108.5	19.3
<1 drink/week	7.4	11.6	11.5	12.9	52.6	333.0	401.9	129.7	25.0
>=1 drink/week	7.6	11.4	11.1	33.2	51.2	495.0	425.9	131.1	23.8
p for heterogeneity	0.30	0.34	0.07	0.43	0.48	0.07	0.14	0.01	0.00
p for trend	0.13	0.25	0.09	0.82	0.72	0.04	0.10	0.04	0.10

[1]Adjusted for age, days since last menstrual period (0–5 days, 6–8 days, 9–10 days, 11–12 days, 13–14 days, 15–17 days, 18–20 days, 21–23 days, 24–26 days, 27–29 days and +30 days), time of blood collection (before 10:00, 10:00–11:59, 12:00–14:59 and after 15:00), batch number for hormone assays, race, age at menarche (<13, 13, ≥14 years old), number of full-term pregnancies (0, 1, 2, ≥3 pregnancies) and alcohol intake (<1 drinks per week, ≥1 drink per week).–[2]Serum hormone concentrations for estradiol, progesterone, testosterone, androstenedione and DHEA could not be obtained from 1, 10, 26, 8, 12 women, respectively, because of insufficient serum sample.–[3]Early follicular (0–9 days since onset of last menses), late follicular (10–14 days since onset of last menses) and luteal (15–40 days since onset of last menses) phases of the menstrual cycle.–[4]All cycle phases.

association between the evaluated genetic polymorphisms and age at menarche in these pre-menopausal women (data not shown).

DISCUSSION

Among the reproductive characteristics evaluated, increasing number of full-term pregnancies was associated with a significant decrease in late follicular progesterone levels. Alcohol consumption was significantly associated with increased estradiol, androstenedione and testosterone levels averaged through the menstrual cycle, and increased luteal progesterone levels. We found little evidence for a substantial influence of genetic polymorphisms on serum hormone concentrations or age at menarche, except for a possible effect of the *CYP1B1* L432V and S453N polymorphisms on estradiol levels.

The lack of relationship between age at menarche and serum hormone concentrations is consistent with previous studies in adult pre-menopausal women of Caucasian origin.[3,15,30,48,61] Differences in circulating concentrations during adolescence and young adults are possible,[1,40] and could have been missed in our study because of the small number of women in this age range. Previous studies provide little evidence for an association between parity and long term changes in circulating levels of hormones.[4,15,30,49,61] Our data showed and association between increasing number of full-term pregnancies and significantly lower levels of late follicular progesterone. Lower progesterone levels during late follicular phase could reflect longer follicular phases of the menstrual cycle among parous women. We did not have information on cycle length, however a previous study found a tendency for shorter rather than longer cycle lengths in parous than in nulliparous women.[4]

Studies in pre-menopausal women have generally found positive associations between chronic and acute alcohol intake and

TABLE III – DISTRIBUTION OF GENOTYPES AMONG THE 218 PRE-MENOPAUSAL WOMEN INCLUDED IN THE STUDY

Genotype	*n* (%)
CYP11A 5′-UTR(tttta)n[1]	
4	180 (83.3)
>4	36 (16.7)
CYPI7 5′UTR-34 T > C	
A1/A1	81 (37.2)
A1/A2	102 (46.8)
A2/A2	35 (16.1)
CYPI9 IVS4(ttta)n[1]	
7	137 (62.8)
8	36 (16.5)
9	22 (10.1)
10	1 (0.5)
11	3 (1.4)
12	17 (7.8)
CYP1B1 L432V	
Val/Val	37 (17.0)
Val/Leu	107 (49.1)
Leu/Leu	74 (33.9)
CYP1B1 S453N[2]	
Asn/Asn	153 (70.5)
Asn/Ser	56 (25.8)
Ser/Ser	8 (3.7)
COMT V158M[1]	
H/H	47 (21.8)
H/L	120 (55.6)
L/L	49 (22.7)

[1]Two observations with missing values due to failure of amplification of PCR fragment.–[2]One observation with missing value due to failure of amplification of PCR fragment.

TABLE IV – GEOMETRIC MEAN[1] SERUM CONCENTRATIONS (NG/DL) OF SEX-STEROIDS IN 218 PRE-MENOPAUSAL WOMEN, STRATIFIED BY GENETIC POLYMORPHISMS[2]

	Estradiol[3]			Progesterone[3]			DHEA[4]	Androstenedione[4]	Testosterone[4]
	Early follicular $n = 52$	Late follicular $n = 53$	Luteal, $n = 112$	Early follicular $n = 50$	Late follicular $n = 52$	Luteal $n = 106$	$n = 206$	$n = 210$	$n = 192$
CYP11A 5′-UTR (tttta)n									
4	7.0	11.2	10.7	22.2	72.8	362.9	396.3	126.3	23.5
>4	6.6	10.7	10.1	12.6	60.2	237.8	391.6	122.6	22.4
p for heterogeneity	0.21	0.47	0.37	0.62	1.00	0.17	0.93	0.52	0.49
CYP17 5′UTR-34 T > C									
A1/A1	6.2	14.5	10.6	15.5	71.8	323.8	397.9	124.0	22.2
A1/A2	7.3	11.1	10.1	20.1	82.2	427.5	394.1	124.4	23.0
A2/2	6.7	8.0	12.2	33.2	43.2	270.4	391.5	132.3	24.9
p for heterogeneity	0.78	0.78	0.72	0.14	0.14	0.21	0.97	0.38	0.27
CYP19 IVS4(ttta)n									
7	7.1	10.4	10.9	19.1	63.4	387.8	397.5	125.2	24.0
>7	6.5	12.2	10.4	20.6	86.0	306.3	393.5	126.6	22.1
p for heterogeneity	0.83	0.50	0.33	0.26	0.45	0.22	0.80	0.66	0.19
CYP1B1 L432V									
Val/Val	7.5	10.0	9.2	37.1	75.8	273.4	402.3	125.6	21.3
Val/Leu	6.9	11.8	10.2	16.0	117.2	347.6	414.0	127.3	22.8
Leu/Leu	6.6	11.1	12.5	20.5	41.7	429.3	365.1	123.1	25.2
p for heterogeneity	0.80	0.89	0.04	0.71	0.12	0.30	0.04	0.64	0.14
CYP1B1 S453N									
Asn/Asn	6.9	10.4	10.2	17.4	58.4	341.7	384.5	124.0	22.9
Asn/Ser or Ser/Ser	6.8	12.9	11.8	26.5	108.6	367.4	422.7	129.9	24.2
p for heterogeneity	0.84	0.13	0.04	0.48	0.73	0.70	0.46	0.58	0.12
COMT V158M									
H/H	7.1	10.0	12.1	10.5	110.3	458.2	382.9	123.7	24.8
H/L	6.3	11.9	10.8	13.6	47.4	322.6	399.7	126.1	23.2
L/L	7.8	10.6	9.2	40.8	128.3	324.4	394.8	126.4	22.3
p for heterogeneity	0.75	0.45	0.41	0.08	0.16	0.69	0.67	0.43	0.95

[1]Geometric means are adjusted for age, days since last menstrual period (0–5 days, 6–8 days, 9–10 days, 11–12 days, 13–14 days, 15–17 days, 18–20 days, 21–23 days, 24–26 days, 27–29 days and +30 days), time of blood collection (before 10:00, 10:00–11:59, 12:00–14:59 and after 15:00), batch number for hormone assays, race, age at menarche (<13, 13, ≥14 years old), number of full-term pregnancies (0, 1, 2, ≥3 pregnancies) and alcohol intake (<1 drinks per week, ≥1 drink per week).–[2]Serum hormone concentrations for estradiol, progesterone, testosterone, androstenedione and DHEA could not be obtained from 1, 10, 26, 8, 12 women, respectively, because of insufficient serum sample.–[3]Early follicular (0–9 days since onset of last menses), late follicular (10–14 days since onset of last menses) and luteal (15–40 days since onset of last menses) phases of the menstrual cycle.–[4]All cycle phases.

circulating levels of estrogens.[14,22,42,44–46,51,55,61] Our findings are consistent with these observations, although the limited number of women in each phase of the menstrual cycle, large variations in estradiol levels and low level of alcohol consumption in this study population made the characterization of this relationship difficult. Alcohol consumption was also associated with elevated androgen levels, particularly androstenedione and testosterone. Light to moderate levels of alcohol consumption have been found to be associated with androgen levels in pre-menopausal women in some but not all studies. For instance, cross-sectional studies of pre-menopausal women have reported associations between low/moderate levels of alcohol consumption and higher levels of androstenedione and testosterone.[7,14,41] A recent prospective study among 611 pre-menopausal women, however, failed to find an association between low/moderate alcohol consumption and testosterone levels.[59] Alcohol at low levels has been shown to stimulate the adrenals, which could account for elevated androgen production.[8] Alcohol could influence ovarian androgen production through effects on gonadotrophin production[60] and other regulatory factors. Alcohol could also alter androgen metabolism and excretion, however, little is known about the effects of low to moderate alcohol ingestion on androgen metabolism in women. Our study also showed an association between light/moderate alcohol intake and surprisingly higher luteal progesterone levels. This finding is not supported by previous studies[55] and there is no clear biological explanation for the magnitude or direction of the observed association. Thus, more information is needed to make conclusions on the effect of moderate alcohol consumption on progesterone levels.

Only a few studies have evaluated the relationship between genetic polymorphisms in hormone metabolizing enzymes and serum hormone concentrations. In our study population, carriers of the variant alleles for the *CYP1B1* L432V and S453N polymorphisms had higher concentrations of luteal estradiol than women homozygous for the wild-type allele. This finding is consistent with a recent report of modestly higher levels of estradiol among post-menopausal women with the variant alleles for these polymorphisms.[11] The *CYP1B1* gene encodes an enzyme involved in the conversion of estradiol to catechol estrogen metabolites, believed to be mammary carcinogens.[38] The *CYP1B1* variant alleles have been found to have higher catalytic efficiency than the enzyme encoded by the wild-type allele.[26,36] Thus, we would expect variant allele carriers to possibly have lower rather than higher levels of estradiol. As pointed out by De Vivo *et al.*,[11] circulating levels of estrogen metabolites produced by CYP1B1 would be a more direct measure of CYP1B1 enzymatic activity than estradiol levels. As assays for these metabolites become available, future studies will be able to better evaluate the influence of CYP1B1 polymorphisms on circulating levels of hormones.

None of the other polymorphisms evaluated presented significant associations with circulating levels of hormones. The lack of association between the *CYP11A* polymorphism and serum hormone concentrations is consistent with a recent study of hirsutism and polycystic ovary syndrome patients, and normal pre-menopausal women in Spain.[56] This and our study do not support 2 earlier reports of increased serum androgen levels among polycystic ovary syndrome patients without the 4 allele repeat of the *CYP11A* gene from the United Kingdom[20] and Greece.[12] Feigelson *et al.*[19] reported higher serum levels of estradiol and progesterone measured around Day 11 of the menstrual cycle among women homozygous for the *CYP17* A2 allele compared to heterozygous or homozygous for the *CYP17* A1 allele, in a sample of young

nulliparous women in a cohort of Asian, African-American and Latino women.[19] This is not supported by data from our population of pre-menopausal women of predominantly white origin, even when young nulliparous women in the study were considered separately (data not shown). Haiman *et al*.[24] initially found post-menopausal women with the *CYP17 A2/A2* genotype to have elevated levels of estrone, estradiol, testosterone, androstenedione, and dehydroepiandrosterone sulfate compared to women with the *A1/A1* genotype.[24] An updated analysis that included a larger number of women than in the first report, however, showed only a weak borderline-significant effect on estrogens, and no positive associations with androgens.[23] Thus, to date there is little data to support a substantial effect of the *CYP17* 5′-UTR −34 T>C polymorphism on serum levels of hormones in pre- or post-menopausal women. Age at menarche has been found to be associated with the *CYP17* 5′-UTR −34 T>C polymorphism in some[18,28,47] but not all studies,[16,24,34,62] although we failed to observe such association. Haiman *et al*.[25] reported decreased levels of estrogens and estrone to androstenedione ratio among women with the 7 allele repeat of *CYP19* compared to non-carriers, and increased levels of estrogens and estrone to androstenedione ratio (measure of aromatase activity) among post-menopausal women with the 8 repeat allele of *CYP19* compared to non-carriers. A study of post-menopausal women enrolled in a multiethnic cohort study found no evidence for an association between the *CYP19* pentanucleotide polymorphism and plasma estrone to androstenedione ratio.[53] Our study of pre-menopausal women also failed to observe an association between the *CYP19* pentanucleotide polymorphism and plasma hormone levels. Particular combinations of allelic variants in the steroid synthesis and metabolism pathway rather than single variants might be required to observe substantial effects on circulating levels of hormones. We failed to observe any significant effect of allelic combinations in the 5 genes studied on hormone levels. The relevance of these combined analyses in this study population is limited, however, because of the small number of women with potentially important combinations of allelic variants. Our study was limited to polymorphisms in estrogen metabolizing genes that had been described previously. A more comprehensive evaluation of polymorphisms in these genes involving an exhaustive search for polymorphisms in these genes and their possible haplotypes is needed to better understand the influence of genetic variation on circulating levels of hormones.

Serum hormone levels were determined from a single blood sample per women. Although a single measurement might be adequate to characterize hormone levels of androgens, this might not be as informative for hormones with large fluctuations during the cycle such estradiol and progesterone.[50] Other sources of random error in hormone measurements in this study include a relatively large inter-assay variation commonly seen in hormone assays, and misclassification of the day of menstrual cycle (due to errors in self-reported date of onset of last menses and lack of information on the date of the next menses). These sources of random error could have limited our ability to detect true associations between the factors evaluated and hormone concentrations, especially for estradiol and progesterone. Although these sources of error would not explain the observed associations, the relatively large number of comparisons being made in this study requires being conservative in the interpretation of the results. Despite the potential for error in hormone and day of the cycle measures, the pattern of estradiol and progesterone changes across the menstrual cycle was reasonably close to expected, and we were able to detect the expected decline in androgen levels with increasing age, providing support for the quality of the data.

In conclusion, results from this population-based cross-sectional study of pre-menopausal women support the association reported previously between light to moderate alcohol intake and elevated levels of estrogens, and provides evidence for a further association with elevated androgen levels. Our data suggest that circulating levels of progesterone might be related to parity and alcohol consumption, however the biological plausibility of the observed associations is unclear. We found little support for an influence of the evaluated genetic polymorphisms in the steroid synthesis and metabolism pathway on serum hormone levels, except for a possible effect of the *CYP1B1* L432V and S453N polymorphisms on serum estradiol levels. Understanding the potential influence of genetic polymorphisms in the estrogen metabolism pathways on circulating levels of hormones will require studies of larger populations of women with accurate hormone measurements, and a more exhaustive evaluation of allelic variants and their possible haplotypes in key genes involved this pathway.

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