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Research Article

Alcohol Consumption in Relation to Plasma Sex Hormones, Prolactin, and Sex Hormone–Binding Globulin in Premenopausal Women

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Abstract

Background: Alcohol consumption is a consistent risk factor for breast cancer, and evidence suggests premenopausal plasma hormones are associated with breast cancer.

Methods: Plasma concentrations of estradiol, estrone, estrone sulfate, testosterone, androstenedione, progesterone, prolactin, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and sex hormone–binding globulin (SHBG) were measured in samples collected in 1996–99. Average alcohol intake was calculated from semiquantitative food frequency questionnaires collected in 1995 and 1999. We used generalized linear models to calculate geometric mean hormone concentrations across alcohol categories and the percentage difference for the highest versus lowest category.

Results: Comparing women who consumed >20 g/d with nondrinkers, levels were 25.7% higher for luteal estrone (geometric mean, 106 vs. 84.5 pg/mL; $P_{trend} = 0.001$), 27.2% higher for luteal estradiol (182 vs. 143 pg/mL; $P_{trend} = 0.006$), and 16.8% higher for SHBG (85.6 vs. 73.3 nmol/L; $P_{trend} = 0.03$); concentrations of free testosterone were 17.9% lower (0.16 vs. 0.20 ng/dL; $P_{trend} = 0.002$). Women consuming >10 g/d compared with nondrinkers had 26.5% higher concentrations of follicular estrone sulfate (950 vs. 751 pg/mL; $P_{trend} = 0.04$). We did not observe significant associations between alcohol and the other sex hormones evaluated. Significant positive associations were observed with beer intake, but not other alcohol types, for DHEA ($P_{interaction} = 0.003$) and androstenedione ($P_{interaction} = 0.006$).

Conclusion: Alcohol consumption was significantly positively associated with plasma luteal estrogen concentrations, but not with androgen levels, nor estrone or estradiol measured in the follicular phase.

Impact: Differences in premenopausal estrogen levels may contribute to the association between alcohol and breast cancer. *Cancer Epidemiol Biomarkers Prev; 23(12); 2943–53. ©2014 AACR.*

Introduction

Alcohol is associated with an increased risk of several chronic conditions, including liver disease and cancer of the oral cavity and pharynx, esophagus, larynx, and colon (1, 2). In addition, alcohol has been consistently shown to increase breast cancer risk (3–8), but the mechanisms underlying this association are unclear. A positive association between plasma sex steroid concentrations and breast cancer risk is well established among postmenopausal women (9–16) but is less consistent among premenopausal women (17–25). However, factors influencing the concentrations of these sex steroids are largely unknown. Alcohol consumption has various effects on estrogen pathways and is associated with decreased menstrual cycle variability and more frequent long cycles (26, 27). Thus, one of the suggested etiologic mechanisms to explain the role of alcohol in breast carcinogenesis is through an effect on circulating sex hormone concentrations.

Among postmenopausal women, associations between alcohol and estrogen concentrations and sex hormone–binding globulin (SHBG) have been inconsistent, while there is more evidence for associations with androgens (28–40). The measurement of hormone concentrations among premenopausal women is complicated by the variation in levels throughout the menstrual cycle. Studies among premenopausal women, with blood samples not specifically timed within the menstrual cycle, have

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suggested a positive association between alcohol consumption and estrogens (41–43) and androgens (41, 42, 44). In addition, positive associations between acute alcohol intake and estrone, estradiol, and dehydroepiandrosterone sulfate (DHEAS) were observed in a controlled feeding study among 34 premenopausal women (45). However, three small ($N < 220$) studies of alcohol and sex hormones among premenopausal women with samples timed in the menstrual cycle have been inconsistent (46–48). Furthermore, to our knowledge, no study has examined associations by type of alcohol consumed.

With the availability of samples carefully timed within the menstrual cycle, the Nurses' Health Study II (NHSII) provides a unique opportunity to assess estrogen concentrations in both the follicular and luteal phase and to evaluate associations by alcohol type in a large sample of premenopausal women. Therefore, we conducted a cross-sectional analysis of total alcohol consumption and type of alcohol consumed with plasma sex steroid concentrations, prolactin, and SHBG among 2,000 premenopausal women in the NHSII.

Materials and Methods

Study population

The NHSII was established in 1989 among 116,430 female registered nurses, ages 25 to 42 years. Women completed a baseline questionnaire and are followed biennially to assess exposure and disease diagnoses. A total of 29,611 participants in the NHSII (ages 32–54 years) provided blood samples between 1996 and 1999. Details of the blood collection procedure are described in a prior publication (28). Premenopausal women who had neither taken oral contraceptives nor been pregnant or breastfed within 6 months completed a short questionnaire and provided blood samples in the early follicular (3–5 day) and the mid-luteal (7–9 days before expected start of their next cycle) phases of the menstrual cycle. Participants aliquoted and froze follicular plasma 8 to 24 hours after collection. Timed follicular and luteal samples were available for 18,521 of the women. A single untimed blood sample was provided for the remaining 11,090 women and all samples were shipped overnight on ice and processed by our laboratory into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored liquid nitrogen freezers since collection. The stability of sex hormones using these blood collection methods has been previously established (49).

Participants in this cross-sectional analysis served as controls in nested case–control studies of breast cancer ($n = 1{,}256$; ref. 50), ovarian cancer ($n = 44$; ref. 51), endometriosis ($n = 574$), and rheumatoid arthritis ($n = 18$; ref. 52), or as participants in a hormone reproducibility study ($n = 108$; ref. 49). A total of 2,000 premenopausal women with measured estradiol, estrone, estrone sulfate, progesterone, testosterone, androstenedione, SHBG, dehydroepiandrosterone (DHEA), DHEAS, and prolactin who provided information on alcohol consumption on either the 1995 or the 1999 questionnaire were included in this study. This study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA).

Laboratory assays

Laboratory assay methods used to quantify the concentrations of estrogens, androgens, progesterone, and prolactin have been described previously (10, 53). Briefly, estrone, estradiol, and estrone sulfate were assayed in luteal and follicular samples. Testosterone, androstenedione, and prolactin concentrations were assayed in luteal and/or follicular samples as well as untimed samples. Progesterone was measured in luteal samples, and DHEA, DHEAS, and SHBG were measured in luteal and untimed samples. Sex hormones, prolactin, and SHBG concentrations were measured in different batches at different laboratories. Assays were performed at Quest Diagnostics for three batches of estrogens, five batches of testosterone, two batches of androstenedione, and one batch of progesterone. Radioimmunoassay (RIA) following organic extraction and celite column chromatography were used to assay estrogens and testosterone. Estrone sulfate was assessed by RIA of estrone, after extraction of estrone, enzyme hydrolysis, and column chromatography. RIA was also used to assay androstenedione. Progesterone was assayed by RIA preceded by organic extraction. Four batches of estrogens and testosterone were assayed at Mayo Medical Laboratories using liquid chromatography–tandem mass spectrometry. Two batches of DHEA and androstenedione and four batches of DHEAS, SHBG, and progesterone were assayed at the Royal Marsden Hospital (London, United Kingdom). Androstenedione was assayed by RIA and DHEAS, SHBG, and progesterone were assayed by chemiluminescent enzyme immunoassay. One batch of progesterone (RIA) and three batches of SHBG (chemiluminescent enzyme immunometric assay) were assayed at Massachusetts General Hospital (Boston, MA) and one batch of SHBG and progesterone were assayed at the Children's Hospital Boston (Boston, MA). Prolactin was measured using microparticle enzyme immunoassay at the Massachusetts General Hospital, by the AxSYM Immunoassay system. C-peptide and insulin-like growth factor (IGF1) were measured from plasma samples by enzyme-linked immunosorbent assay (Diagnostic Systems Laboratory) in the laboratory of Dr. Michael Pollak (McGill University, Montréal, QC, Canada). We included 10% blinded replicates in each batch to assess laboratory precision. Within-batch coefficients of variation were between 2% and 15% for all analytes, except for a single batch of progesterone (17%).

Exposure and covariate measurement

Alcohol consumption was assessed by a semiquantitative food frequency questionnaire, administered in 1995 and 1999, and included separate items for regular beer, light beer, white wine, red wine, and liquor consumption. Alcohol consumption of the past year was assessed for wine, beer, and liquor in nine categories ranging from

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"none or <1/month" to "40+/week." Total alcohol consumption was calculated as the sum of the intake by alcohol beverage type. For this analysis, we calculated alcohol consumption as an average of the frequencies from the 1995 and 1999 questionnaires; results were similar when we restricted to 1995 consumption and are not presented here. If information on alcohol was missing on one of the questionnaires, information from the other was used. From the reported frequency, we calculated g/d of alcohol consumed and categorized it for analyses: none (reference), 0.1–10.0 g/d, 10.1–20.0 g/d, and >20 g/d. For follicular and luteal estrone sulfate, DHEA, and androstenedione, where we had a smaller sample size, we used a highest category of alcohol consumption of >10 g/d. Furthermore, we evaluated the associations between the type of alcohol consumed (beer, wine, and liquor) and sex steroid concentrations using categories of <1, 1–4, and ≥5 drinks/week. The correlations between the different alcohol types in our study population were weak to moderate, with Spearman correlation coefficients of 0.28, $P < 0.0001$ between beer and liquor, 0.41, $P < 0.0001$ between beer and wine, and 0.39, $P < 0.0001$ between wine and liquor consumption.

We evaluated age, smoking, body mass index (BMI), height, age at menarche, usual menstrual cycle length and pattern, parity, age at first birth, breastfeeding history, oral contraceptive use, family history of breast cancer, benign breast disease diagnosis, and physical activity as potential confounders of the association between alcohol consumption and sex steroid concentrations. Information on current weight, age, and blood collection date, time, and fasting status were reported on the questionnaire at blood collection. Age at menarche, height, and family history of breast cancer were reported on the baseline questionnaire in 1989. Usual menstrual cycle length and pattern were ascertained in 1993. Information on breastfeeding history, duration of oral contraceptive use, age at first birth, parity, benign breast disease history, smoking status, and physical activity were reported on the 1997 questionnaire. BMI was calculated from weight in kilograms from the questionnaire at blood collection divided by height in meters squared from the baseline questionnaire in 1989.

Statistical analyses

Because sex steroid hormone levels vary throughout the menstrual cycle, we evaluated the estrogens in the follicular and luteal phases separately, and progesterone in the luteal phase. We used the average of the follicular and luteal blood sample values, when available, for testosterone, free testosterone, androstenedione, and prolactin as concentrations did not vary substantially by menstrual phase and the average concentration more accurately represents long-term levels (49, 54). Statistical outliers in hormone concentrations were identified using the generalized extreme Studentized deviate (ESD) many-outlier detection approach (55); 0 (estrone sulfate, DHEA, and DHEAS) to 13 (prolactin) extreme values were excluded from the analyses. We noticed batch-to-batch variation in our quality control samples and adjusted for batch according to the methods described by Rosner and colleagues (56), as we have used previously (57).

We used generalized linear models to calculate geometric mean hormone concentrations across alcohol consumption categories standardized to the marginal distribution of the covariates and estimated the percentage difference and 95% confidence intervals (CI) contrasting the means for the highest versus lowest category of alcohol consumption. Contrast tests were used to assess statistical differences in associations by alcohol type (beer, wine, and liquor; ref. 58). We modeled continuous alcohol consumption using medians of the categories and examined linear trends with the Wald test. Potential confounders were included in the model based on our prior knowledge of factors associated with alcohol intake and sex steroid concentrations. The final model included age at blood draw, BMI, smoking status, race, age at menarche, parity, and physical activity as categorized in the footnote of Table 3. We additionally included the date and time of day of blood collection, and fasting status in the model to reduce extraneous variation. We evaluated usual menstrual cycle length and pattern, height, biopsy-confirmed benign breast disease, family history of breast cancer, breastfeeding history, and BMI at the age of 18 years as potential covariates, but their inclusion in the model did not change our results, so they were not retained. Models that included luteal or average of timed samples were also adjusted for the difference between luteal blood draw date and date of next menstrual period.

We assessed whether associations of alcohol with hormone concentrations varied by BMI at blood draw (<25 vs. ≥25 kg/m^2), age (<45 vs. ≥45 years), and by menstrual cycle phase (follicular vs. luteal) for the estrogen models. The Wald test was used to test for modification by including interaction terms between BMI, age, or menstrual cycle phase and a continuous alcohol intake variable weighted by the median of alcohol use in each category. We conducted *a priori* sensitivity analyses among nonsmokers only and among women whose timed samples were collected during an ovulatory cycle (defined by luteal progesterone ≥400 ng/dL). As SHBG is strongly inversely correlated with BMI (42), we explored the possibility that alcohol may affect SHBG through insulin sensitivity by conducting secondary analyses restricted to women with these measures (c-peptide, $n = 665$; IGF1, $n = 667$) and adjusting for c-peptide and IGF1 along with batch in the full model. All statistical tests were two-sided, and were considered statistically significant at $P < 0.05$. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc.).

Results

The mean age at blood draw for our study population was 42.7 years, and the mean BMI was 26.1 kg/m^2. Reported alcohol consumption ranged from 0 to 67.9 g/d with an average among drinkers of 5.6 g/d

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Table 1. Characteristics at blood draw of 2,000 premenopausal women in the NHSII

	Average alcohol consumption, g/d			
	None ($n = 618$)	0.1–10.0 ($n = 1,162$)	10.1–20.0 ($n = 157$)	>20.0 ($n = 63$)
Age in years (mean, SD)	42.7 (4.0)	42.7 (4.0)	43.1 (4.0)	43.7 (4.1)
BMI, kg/m^2 (mean, SD)	26.8 (7.0)	25.9 (7.1)	24.4 (5.9)	25.0 (5.8)
BMI at age 18, kg/m^2 (mean, SD)	21.4 (3.3)	21.1 (3.1)	20.4 (2.2)	20.8 (2.4)
Height in inches (mean, SD)	64.9 (2.8)	64.9 (2.5)	65.5 (2.7)	65.3 (2.5)
Physical activity in MET-hours/week (mean, SD)	14.9 (15.5)	18.8 (18.0)	21.9 (18.6)	24.4 (22.1)
Parous, %	84.6	80.3	72.0	76.2
Parity[a] (mean, SD)	2.4 (1.0)	2.3 (0.9)	2.3 (1.0)	2.2 (0.9)
Age at first birth[a] (mean, SD)	26.4 (4.4)	26.8 (4.4)	26.7 (4.7)	27.3 (4.1)
Past oral contraceptive use, %	82.4	85.9	87.9	92.1
Past breast feeding history, %	68.9	68.1	61.2	68.3
Current smoker, %	5.7	7.4	17.2	17.5
Family history of breast cancer, %	7.8	10.2	11.5	9.5
Benign breast disease history, %	16.2	17.3	17.8	17.5
Caucasian, %	94.7	93.9	95.5	95.2
Age at menarche between 12–13 y, %	61.7	58.1	61.2	60.3
Usual menstrual cycle pattern regular, %	89.3	93.1	93.3	93.6

[a]Among parous women.

(~2–3 drinks/week). Compared with nondrinkers, women who consumed the highest quantities of alcohol were more likely to be current smokers, physically active, have past oral contraceptive use, and report a regular menstrual cycle pattern (Table 1). Alcohol drinkers were also slightly older at first birth and were less likely to be parous as compared with nondrinkers. The distributions of the plasma sex hormones, prolactin, and SHBG concentrations are displayed in Table 2. The Spearman rank correlation coefficients between the hormone concentrations ranged from 0.001 for DHEAS and luteal estradiol to 0.90 for follicular free estradiol and follicular total estradiol, with a median correlation coefficient of 0.12 (Supplementary Table S1).

Table 2. Plasma hormone concentrations among 2,000 premenopausal women in the NHSII

Plasma hormone	*N*	Median (10th–90th percentile)
Follicular estradiol, pg/mL	1,405	46.7 (22.1–101)
Luteal estradiol, pg/mL	1,533	134 (72.4–237)
Follicular free estradiol, pg/mL	1,369	0.58 (0.30–1.18)
Luteal free estradiol, pg/mL	1,517	1.69 (0.93–2.86)
Follicular estrone, pg/mL	1,425	40.6 (25.0–67.7)
Luteal estrone, pg/mL	1,580	84.3 (51.1–142)
Follicular estrone sulfate, pg/mL	444	661 (299–1,517)
Luteal estrone sulfate, pg/mL	449	1,454 (573–3,326)
Luteal progesterone, ng/dL	1,596	1,397 (250–2,695)
DHEA, ng/dL[a]	476	614 (346–1,127)
DHEAS, µg/dL[a]	1,244	86.9 (39.5–163)
Testosterone, ng/dL[b]	1,967	23.5 (14.2–36.8)
Free testosterone, ng/dL[b]	1,908	0.20 (0.10–0.37)
Androstenedione, ng/dL[b]	627	99.6 (60.1–164)
Prolactin, ng/dL[b]	1,303	14.5 (8.3–28.7)
SHBG, nmol/L[b]	1,926	64.6 (32.5–116)

[a]Luteal/untimed.
[b]Average of follicular and luteal measures, or untimed.

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Table 3. Adjusted geometric mean concentration[a] of hormones by categories of average alcohol consumption among 2,000 premenopausal women in the NHSII

		Total alcohol intake, g/d					
Hormone	***N***	**0**	**0.1–10.0**	**10.1–20.0[e]**	**>20**	**P_{trend}**	**Percentage difference[b] and 95% CI**
Range, *N*		(130–618)	(251–1,162)	(55–157)	(38–63)		
Follicular estradiol, pg/mL	1,405	69.7	66.8	63.0	113	0.07	62.1% (6.4%–147%)
Luteal estradiol, pg/mL	1,533	143	156	166	182	0.006	27.2% (5.4%–53.5%)
Follicular free estradiol, pg/mL	1,369	0.74	0.71	0.70	0.71	0.66	−4.0% (−29.7%–30.9%)
Luteal free estradiol, pg/mL	1,517	1.81	1.89	2.01	2.01	0.10	11.5% (−6.4%–32.8%)
Follicular estrone, pg/mL	1,425	46.9	46.3	44.8	53.8	0.25	14.9% (−2.5%–35.3%)
Luteal estrone, pg/mL	1,580	84.5	91.7	94.8	106	0.001	25.7% (9.7%–44.0%)
Follicular estrone sulfate, pg/mL	444	751	801	950	—	0.04	26.5% (1.8%–57.4%)
Luteal estrone sulfate, pg/mL	449	1,513	1,605	1,900	—	0.10	25.6% (−4.4%–65.0%)
Luteal progesterone, ng/dL	1,596	1,220	1,252	1,193	1,334	0.64	9.4% (−8.5%–30.8%)
DHEA, ng/dL[c]	476	919	845	826	—	0.35	−10.1% (−24.1%–6.5%)
DHEAS, μg/dL[c]	1,244	131	125	121	152	0.30	16.4% (−4.5%–42.0%)
Testosterone, ng/dL[d]	1,967	23.9	23.9	23.0	23.7	0.52	−0.7% (−11.5%–11.5%)
Free testosterone, ng/dL[d]	1,908	0.20	0.19	0.18	0.16	0.002	−17.9% (−28.4% to −5.9%)
Androstenedione, ng/dL[d]	627	131	128	128	—	0.81	−2.1% (−12.2%–9.1%)
Prolactin, ng/dL[d]	1,303	21.9	21.4	24.0	23.2	0.11	5.9% (−10.0%–24.7%)
SHBG, nmol/L[d]	1,926	73.3	75.5	74.4	85.6	0.03	16.8% (4.5%–30.5%)

[a]All geometric mean concentrations are adjusted for age at blood collection (continuous), BMI at blood collection (continuous), smoking (never smoker, past smoker with ≥5 years since quitting, past smoker with <5 years since quitting, current smoker of <15 cigarettes per day, current smoker of 15+ cigarettes per day), duration of past oral contraceptive use (never, <4 years, 4+ years), age at first birth/parity (nulliparous, 1–2 children and age at first birth <25 years, 1–2 children and age at first birth ≥25 years, ≥3 children and age at first birth <25 years, ≥3 children and age at first birth ≥25 years), age at menarche (<12, 12–13, >13 years), physical activity in MET-hours/week (continuous), date of blood collection (month/year, ≤1/97, 2/97–1/98, 2/98–1/99, ≥2/99), time of day of blood collection (1–8 a.m., 9 a.m. to noon, 1–4 p.m., 5 p.m. to midnight), and fasting status (<10, ≥10 hours). Models that included luteal or average of timed samples also were adjusted for the difference between luteal blood draw date and date of next menstrual period (3–7, 8–12, 13–17, 18–21 days, unknown/untimed).

[b]Percentage difference for highest category of alcohol use vs. no alcohol use.

[c]Luteal/untimed.

[d]Average of follicular and luteal measures, or untimed.

[e]Geometric mean concentration of follicular estrone sulfate, luteal estrone sulfate, DHEA, and androstenedione reflect alcohol intake (g/d) of 10+ g/d in highest category of alcohol use.

The geometric mean concentrations of plasma sex steroids, prolactin, and SHBG across alcohol consumption categories are shown in Table 3. Alcohol consumption was positively associated with concentrations of luteal estradiol ($P_{trend} = 0.006$), luteal estrone ($P_{trend} = 0.001$), and SHBG ($P_{trend} = 0.03$) and was inversely associated with concentrations of free testosterone ($P_{trend} = 0.002$). Compared with nondrinkers, hormone levels for women who consumed >20 g/d were 27.2% higher for luteal estradiol (182 vs. 143 pg/mL), 25.7% higher for luteal estrone (geometric mean, 106 vs. 84.5 pg/mL), and 16.8% higher for SHBG (85.6 vs. 73.3 nmol/L); concentrations of free testosterone were 17.9% lower (0.16 vs. 0.20 ng/dL). Compared with nondrinkers, women consuming >10 g/d had 26.5% higher concentrations of follicular estrone sulfate (950 vs. 751 pg/mL; $P_{trend} = 0.04$). Although trends were not statistically significant ($P_{trend} = 0.07$–0.25), hormone concentrations for women consuming >20 g/d were 62.1% higher for follicular estradiol, 14.9% higher for follicular estrone, and 25.6% higher for luteal estrone sulfate compared with nondrinkers. Interaction terms between alcohol and menstrual cycle phase were not significant for estradiol, estrone, and estrone sulfate ($P = 0.26$–0.88). We did not observe significant associations between alcohol and testosterone, androstenedione, progesterone, DHEA, or DHEAS.

The observed associations did not differ substantially by age, BMI, or when restricted to samples collected during ovulatory cycles or among nonsmokers only (data not shown). Although we observed a significant interaction between age and alcohol for the association with progesterone levels, this interaction was no longer significant when we restricted the sample to ovulatory women ($P = 0.68$). To ensure that the results were robust after

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eliminating potential outliers with heavier alcohol consumption, we conducted a sensitivity analysis, excluding 9 women who reported >3 drinks/day. Results from this analysis were consistent with our overall findings, suggesting that the observed associations are not being driven by the heavy alcohol drinkers in our study population. In secondary analysis restricted to women with plasma c-peptide and IGF1 measures, the association between alcohol and SHBG was only slightly attenuated with adjustment for these biomarkers.

The associations between wine consumption and plasma sex hormone concentrations were similar to the overall results (Table 4). Compared with nondrinkers of any alcohol, women who consumed ≥5 glasses of wine per week had 17.3% higher concentrations of luteal estradiol ($P_{trend} = 0.05$), and 9.1% lower concentrations of free testosterone ($P_{trend} = 0.02$). Women who consumed ≥5 glasses of beer per week had 5.1% higher concentrations of DHEA ($P_{trend} = 0.04$), 12.3% higher concentrations of androstenedione ($P_{trend} = 0.01$) as compared with nondrinkers. We did not observe any significant trends with liquor consumption after adjusting for other alcohol types; however, we had few liquor drinkers. Positive trends for wine consumption remained significant after adjustment for beer and liquor consumption across all hormones. For beer drinkers, significant positive trends with luteal estradiol, luteal free estradiol, free testosterone, and SHBG were no longer significant, while positive trends for DHEA and androstenedione became significant, after adjustment for other beverage types. Positive trends for liquor consumption and luteal estradiol, luteal estrone, and SHBG were no longer evident after adjusting for wine and beer intake. Significant differences in associations by alcohol type were observed only for DHEA ($P = 0.003$) and androstenedione ($P = 0.006$).

We observed significant differences between red and white wine for androstenedione ($P = 0.02$), and suggestive differences between regular and light beer for follicular estradiol ($P = 0.05$; data not shown). Women who reported ≥5 glasses of white wine per week had 6.3% lower concentrations of androstenedione compared with nondrinkers ($P_{trend} = 0.06$), with no suggestive trends among red wine drinkers. Follicular estradiol levels were 36.1% higher for light beer drinkers who consumed ≥5 drinks/week as compared with nondrinkers ($P_{trend} = 0.06$) and a positive trend was not significant for regular beer drinkers ($P_{trend} = 0.12$).

Discussion

In this large cross-sectional study among premenopausal women, we observed strong positive associations between alcohol consumption and circulating concentrations of luteal estrogens and SHBG and an inverse association between alcohol and free testosterone levels. Although there were few heavy drinkers, the associations appeared linear and significant changes in hormone levels were observed even among women with modest alcohol consumption. Of note, 60.1% of women in our cohort drank wine, and the associations with wine consumption were similar to the total alcohol results. Our results suggest potential differences in associations with sex steroid concentrations for beer and liquor, although these findings are based on smaller numbers.

Previous cross-sectional studies of premenopausal women (range, $n = 205$–2,719), including a large collaborative article with 471 of the 2,000 NHSII women in the current analysis, have observed suggestive or significant positive associations between alcohol and circulating estrogens (41, 42, 46, 48). However, in cross-sectional studies with samples timed in the menstrual cycle, consistent associations with luteal estrogens have not been observed. In two small studies ($n = 205$, 218), suggestive positive associations were observed between alcohol and plasma luteal estradiol (46, 48), with a significant linear trend in one of the studies when estradiol concentrations were averaged across the menstrual cycle (46). A smaller study of 107 women found no associations of alcohol with estrone, estradiol, and estrone sulfate regardless of menstrual cycle phase (47). In a small controlled feeding study of 34 women, positive associations were only evident between alcohol and ovulatory (days 12–15 of menstrual cycle) estrogens, but not follicular or luteal samples (45). Our study is the largest, to our knowledge, to evaluate associations by menstrual timing, and our findings of a positive association with estrogens are consistent with the larger cross-sectional studies and with some of the suggestive luteal estrogen associations in the smaller studies. Although our findings support a positive association between alcohol and luteal estrogens, results for follicular measures were generally similar, although not necessarily statistically significant. Furthermore, we did not find any evidence of interaction by menstrual cycle phase for the associations between alcohol and estradiol, estrone, and estrone sulfate. Although follicular and luteal estrogen levels are not similar, and may reflect different sources (59–61), it is unclear why alcohol would have differential effects by phase and specific estrogens. Thus, additional studies are warranted to better understand whether associations of alcohol and estrogens differ by menstrual cycle phase.

Positive associations between alcohol and androstenedione, DHEAS, and testosterone have been observed in several large studies among premenopausal women (41, 42), while smaller studies, although also supportive, have varied in the specific androgen that was significant (44, 46, 47). In addition, acute effects of alcohol were evident for at least one of the androgens in the small controlled feeding study (45). However, our study did not observe any positive associations between overall alcohol intake and androgens. Furthermore, while a positive association has been observed in other studies for free testosterone (41, 42), we observed lower concentrations of free testosterone among drinkers in our study population. The inverse association we observed with free testosterone likely was driven by the positive association between alcohol intake and SHBG concentrations, while studies

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Table 4. Adjusted geometric mean concentration of hormones[a] by categories of average alcohol type among 2,000 premenopausal women in the NHSII

		Total alcohol intake by type[b] (No. of drinks per week)					
Hormone and category	**N**	**Nondrinker**	**<1**	**1–4**	**5+[g]**	**P_{trend} adjusted for other type[c]**	**Percentage difference[d] and 95% CI**
A. Total wine							
Range, *N*		(130–618)	(99–452)	(117–524)	(62–225)		
Follicular estradiol, pg/mL	1,403	80.9	74.7	72.9	91.3	0.31	12.9% (−8.3%–38.9%)
Luteal estradiol, pg/mL	1,531	142	159	153	168	0.05	17.3% (6.4%–29.3%)
Follicular free estradiol, pg/mL	1,367	0.82	0.79	0.75	0.86	0.24	5.5% (−9.3%–22.8%)
Luteal free estradiol, pg/mL	1,515	1.79	1.97	1.85	1.93	0.51	7.3% (−2.3%–17.8%)
Follicular estrone, pg/mL	1,423	49.4	48.4	47.9	49.9	0.79	1.1% (−8.4%–11.5%)
Luteal estrone, pg/mL	1,578	83.7	91.7	92.6	95.2	0.13	12.7% (4.2%–21.8%)
Follicular estrone sulfate, pg/mL	444	795	826	915	961	0.28	20.9% (−3.6%–51.6%)
Luteal estrone sulfate, pg/mL	449	1,524	1,655	1,585	1,706	0.63	13.0% (−12.6%–46.0%)
Luteal progesterone, ng/dL	1,595	1,217	1,214	1,304	1,217	0.53	−1.0% (−10.8%–9.9%)
DHEA, ng/dL[e]	386	916	887	836	847	0.20	−8.2% (−21.9%–7.9%)
DHEAS, μg/dL[e]	1,085	128	119	127	130	0.72	1.1% (−9.3%–12.8%)
Testosterone, ng/dL[f]	1,967	24.0	23.7	23.7	24.2	0.86	0.7% (−5.2%–7.0%)
Free testosterone, ng/dL[f]	1,906	0.20	0.19	0.19	0.18	0.02	−9.1% (−16.1% to −1.4%)
Androstenedione, ng/dL[f]	627	131	131	124	132	0.88	0.9% (−8.6%–11.3%)
Prolactin, ng/dL[f]	1,301	22.0	22.0	20.6	23.1	0.85	4.7% (−7.0%–18.0%)
SHBG, mol/L[f]	1,682	73.0	75.7	74.9	77.9	0.36	6.1% (−1.5%–14.2%)
B. Total beer							
Range, *N*		(130–618)	(64–310)	(78–368)	(26–120)		
Follicular estradiol, pg/mL	1,402	78.5	82.0	69.9	86.8	0.94	10.6% (−21.0%–54.9%)
Luteal estradiol, pg/mL	1,530	144	150	161	166	0.23	15.7% (2.7%–30.4%)
Follicular free estradiol, pg/mL	1,366	0.81	0.80	0.75	0.75	0.26	−7.9% (−26.7%–15.9%)
Luteal free estradiol, pg/mL	1,514	1.81	1.83	1.93	1.99	0.35	10.3% (−4.3%–27.0%)
Follicular estrone, pg/mL	1,422	48.9	52.3	46.6	50.4	0.76	3.1% (−9.3%–17.2%)
Luteal estrone, pg/mL	1,577	84.4	92.4	95.2	95.3	0.15	13.0% (1.8%–25.4%)
Follicular estrone sulfate, pg/mL	444	763	912	1,032	836	0.49	9.6% (−16.8%–44.4%)
Luteal estrone sulfate, pg/mL	449	1,502	1,702	1,606	1,690	0.76	12.5% (−15.5%–49.9%)
Luteal progesterone, ng/dL	1,594	1,229	1,211	1,281	1,352	0.10	10.0%(−3.2%–25.1%)
DHEA, ng/dL[e]	386	913	804	943	960	0.04	5.1% (−13.5%–27.7%)
DHEAS, μg/dL[e]	1,085	127	125	130	124	0.62	−2.2% (−16.6%–14.9%)
Testosterone, ng/dL[f]	1,966	23.8	24.4	24.7	23.8	0.36	−0.1% (−8.0%–8.5%)
Free testosterone, ng/dL[f]	1,905	0.20	0.20	0.20	0.18	0.87	−9.7%(−18.3% to −0.3%)
Androstenedione, ng/dL[f]	627	130	134	135	147	0.01	12.3% (−3.9%–31.1%)
Prolactin, ng/dL[f]	1,301	21.6	23.3	21.3	24.0	0.08	11.1% (−4.5%–29.4%)
SHBG, nmol/L[f]	1,681	73.5	74.4	75.1	79.2	0.39	7.8% (−1.6%–18.1%)
C. Liquor							
Range, *N*		(130–618)	(81–374)	(46–169)	(20–34)		
Follicular estradiol, pg/mL	1,400	78.5	71.5	83.3	103	0.39	30.8% (−35.9%–167%)
Luteal estradiol, pg/mL	1,528	143	163	154	172	0.42	20.1% (0.6%–43.2%)
Follicular free estradiol, pg/mL	1,364	0.81	0.74	0.85	0.64	0.41	−21.4% (−40.8%–4.2%)
Luteal free estradiol, pg/mL	1,512	1.80	1.93	1.82	1.96	0.97	8.7% (−9.8%–31.1%)
Follicular estrone, pg/mL	1,420	49.1	47.3	53.5	47.6	0.54	−3.1% (−20.7%–18.5%)
Luteal estrone, pg/mL	1,575	84.3	94.8	91.6	107	0.13	27.2% (2.5%–57.7%)
Follicular estrone sulfate, pg/mL	443	801	833	960	—	0.63	19.8% (−3.9%–49.5%)

(Continued on the following page)

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Table 4. Adjusted geometric mean concentration of hormones[a] by categories of average alcohol type among 2,000 premenopausal women in the NHSII (Cont'd)

		Total alcohol intake by type[b] (No. of drinks per week)					
Hormone and category	N	Nondrinker	<1	1–4	5+[g]	P_{trend} adjusted for other type[c]	Percentage difference[d] and 95% CI
Luteal estrone sulfate, pg/mL	448	1,503	1,646	1,732	—	0.48	15.2% (−11.6%–50.3%)
Luteal progesterone, ng/dL	1,592	1,226	1,236	1,266	1,231	0.89	0.4% (−20.8%–27.3%)
DHEA, ng/dL[e]	385	943	892	935	—	0.41	−0.9% (−15.8%–16.8%)
DHEAS, μg/dL[e]	1,082	131	139	139	122	0.44	−6.8% (−25.8%–17.1%)
Testosterone, ng/dL[f]	1,963	23.9	24.7	23.8	23.4	0.93	−2.1% (−14.1%–11.5%)
Free testosterone, g/dL[f]	1,902	0.20	0.20	0.19	0.19	0.78	−5.0% (−19.2%–11.6%)
Androstenedione, ng/dL[f]	627	130	133	124	—	0.40	−4.7% (−14.9%–6.6%)
Prolactin, ng/dL[f]	1,298	21.7	21.0	20.6	26.3	0.71	21.0% (−17.0%–76.3%)
SHBG, nmol/L[f]	1,678	73.3	77.4	75.7	80.0	0.39	9.1% (−6.2%–26.9%)

[a]All geometric mean concentrations are adjusted for age at blood collection (continuous), BMI at blood collection (continuous), smoking (never smoker, past smoker with ≥5 years since quitting, past smoker with <5 years since quitting, current smoker of <15 cigarettes per day, current smoker of 15+ cigarettes per day), duration of past oral contraceptive use (never, <4 years, 4+ years), age at first birth/parity (nulliparous, 1–2 children and age at first birth < 25 years, 1–2 children and age at first birth > 25 years, >3 children and age at first birth < 25 years, >3 children and age at first birth > 25 years), age at menarche (<12, 12–13, >13 years), physical activity in MET-hours/week (continuous), date of blood collection (month/year, ≤1/97, 2/97–1/98, 2/98–1/99, ≥2/99), time of day of blood collection (1–8 a.m., 9 a.m. to noon, 1–4 p.m., 5 p.m. to midnight), and fasting status (<10, ≥10 hours). Models that included luteal or average of timed samples also were adjusted for the difference between luteal blood draw date and date of next menstrual period (3–7, 8–12, 13–17, 18–21 days, unknown/untimed).

[b]Total alcohol by type excludes women who only drank other alcohol types.

[c]P_{trend} across alcohol category including all women and adjusting for other alcohol types.

[d]Percentage difference for highest category of alcohol use vs. no alcohol use.

[e]Luteal/untimed.

[f]Average of follicular and luteal measures, or untimed.

[g]Geometric mean concentration of follicular estrone sulfate, luteal estrone sulfate, DHEA, and androstenedione reflect liquor intake of 1+ drinks per week in highest category of alcohol use.

that observed positive associations with free testosterone did not report elevated concentrations of SHBG among drinkers. Inconsistencies in results from other studies may be partially explained by differences in alcohol type consumed. Although we did not observe associations with androstenedione and DHEA overall, these androgens were higher among beer drinkers compared with nondrinkers. Although prior studies have not examined associations by alcohol type, our results suggest differences in the type of alcohol consumed may be important.

Alcohol consumption has been inversely related to insulin sensitivity markers (62), which are inversely associated with SHBG concentrations (63). However, the positive association we observed between alcohol and SHBG was only slightly attenuated when we adjusted for concentrations of c-peptide and IGF1, suggesting that our findings are not entirely explained by alcohol-induced alterations in these markers. SHBG concentrations are positively correlated with estradiol levels in premenopausal women (49, 64), and the previous studies that have not observed an association between alcohol and SHBG levels among premenopausal women (41, 42, 47, 65, 66), also have not observed associations with estrogen levels. Furthermore, SHBG concentrations among premenopausal women have not been associated with breast cancer risk (19, 42). Thus, our finding of a positive association between alcohol and SHBG is not likely to be mediating the effect of alcohol on breast cancer risk, but rather it may be correlated with the higher estradiol concentrations evident among drinkers in our study population.

Our study is the first, to our knowledge, to examine associations between specific types of alcohol and premenopausal hormone levels. Although the majority of women in our cohort were wine drinkers, we nevertheless observed significant associations with androstenedione and DHEA concentrations among beer drinkers. Beer drinkers were not substantially different from wine drinkers in our population, though they were more likely to smoke cigarettes. Although few women in this study consumed liquor, our differential findings between wine and beer drinkers suggest that the type of alcohol beverage may be important. Consumption by alcohol type is not reported in prior studies, and it is possible that differences in the distribution of beer and wine consumption across

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study populations could partially explain discrepancies between our findings and other studies with regard to androgens. Thus, further exploration of potential differences in associations of sex steroid concentrations by alcohol type is warranted.

Although postmenopausal hormone concentrations have been consistently linked with breast cancer risk (9–16), the role of premenopausal hormone concentrations in relation to breast cancer is not well understood (17–24). In our recent analysis, we observed suggestive positive associations between luteal estradiol concentrations with estrogen receptor (ER)–positive and progesterone receptor (PR)–positive breast cancers, but no strong associations for other estrogens (18). A recent pooled analysis of data from seven prospective studies also demonstrated a positive association between estrogen concentrations and breast cancer risk among premenopausal women (42). Therefore, our findings of increased concentrations of luteal estrogens among premenopausal women who consumed alcohol suggest that the association between alcohol consumption and breast cancer risk may be partially mediated by alterations in sex steroid concentrations.

Strengths of this study include the large sample size, the ability to evaluate the association between alcohol and estrogens in both the follicular and luteal phases of the menstrual cycle, and our examination by type of alcohol consumed. The cross-sectional nature of this study limits our ability to attribute causality to the observed associations. Although women in this study did not consume high amounts of alcohol and we were unable to assess how very high alcohol consumption affects sex steroid concentrations, we observed significant associations even among women with lower levels of alcohol consumption. Although alcohol was self-reported, this assessment has been validated against dietary records, with Spearman correlation coefficients of 0.9 (67). Although we only had one or two (for timed samples) hormone measures per participant to capture the association with habitual alcohol consumption, one androgen measure is reproducible in our population of premenopausal women over 2 to 3 years [intraclass correlations (ICC), 0.58-0.94]. Although ICCs for estrogens were lower (0.38–0.69; ref. 49), we still observed statistically significant associations. Drinking patterns were not assessed in our study, so we were unable to examine whether plasma hormone levels differ between binge drinkers and those with moderate drinking patterns. Unmeasured confounding is always a possibility; however, we were able to adjust our models for many potential confounders.

In this large study of premenopausal women, we observed higher luteal estrogen and SHBG concentrations and lower levels of free testosterone among women who consumed alcohol compared with nondrinkers. We did not observe significant associations between alcohol consumption and plasma androgen levels, nor estrone or estradiol measured in the follicular phase. Although the observed associations were most consistent for wine consumption, specific types of alcohol should be explored further in future studies. Differences in premenopausal estrogen levels may contribute to the well-confirmed positive association between alcohol intake and breast cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: W.C. Willett, S.E. Hankinson, A.H. Eliassen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.C. Willett, S.E. Hankinson, A.H. Eliassen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.A. Hirko, D. Spiegelman, S.E. Hankinson, A.H. Eliassen
Writing, review, and/or revision of the manuscript: K.A. Hirko, D. Spiegelman, W.C. Willett, S.E. Hankinson, A.H. Eliassen
Study supervision: A.H. Eliassen

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