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ORIGINAL PAPER

Alcohol Consumption and Urinary Estrogens and Estrogen Metabolites in Premenopausal Women

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Abstract In a cross-sectional analysis, we evaluated the associations of usual total alcohol and wine intake with a comprehensive profile of mid-luteal phase urinary estrogens and estrogen metabolites (referred to jointly as EM) in a sample of 603 premenopausal women participating in the Nurses' Health Study II (NHSII). A total of 15 individual EM (pmol/mg creatinine) were measured by a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method with high accuracy and reproducibility. We used linear mixed models to calculate the adjusted geometric means of individual EM, EM grouped by metabolic pathways, and pathway ratios by category of alcohol intake with non-drinkers of alcohol as the referent. Total alcohol intake was not associated with total EM but was positively associated with estradiol (26 % higher among women consuming >15 g/day vs. non-drinkers; P trend = 0.03). Wine consumption was positively associated with a number of EM measures including estradiol (22 % higher among women consuming ≥5 drinks/week vs. non-drinkers, P trend < 0.0001). In conclusion, the total alcohol intake was positively and significantly associated with urinary estradiol levels. Some differences in urinary estrogen metabolites were observed with wine drinking, when compared with non-drinkers. This study strengthens the evidence that alcohol consumption might play a role in breast cancer and other estrogen-related conditions. Additional studies of premenopausal women are needed to further explore the association of alcohol, particularly the specific types of alcohol, on patterns of estrogen metabolism in blood, urine, and tissue.

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Introduction

Alcohol consumption is common among women in many countries [1] and has been positively and consistently associated with increased breast cancer risk in epidemiologic studies [2–5]. In 2010, the World Cancer Research Fund/American Institute for Cancer Research Continuous Update Project Panel judged that there was convincing evidence that consuming alcoholic drinks increases the risk for both pre- and postmenopausal breast cancer [6]. Meta-analyses of cohort studies showed a 9 % increased risk for premenopausal and an 8 % increased risk for postmenopausal breast cancer per 10 g ethanol/day [6]. To date, a biological mechanism for the association has not been clearly identified. There is some evidence that alcohol may influence the levels of sex hormones associated with increased risk of breast cancer, even at lower levels of alcohol intake; however, results have not been consistent across studies [7–10]. Supporting evidence derives from

studies suggesting that alcohol ingestion has substantial effects on menstrual cycle characteristics, with non-drinkers more likely to experience longer and more irregular cycles [11–13].

The parent estrogens, estrone, and estradiol are metabolized by irreversible hydroxylation at the 2-, 4-, or 16-positions on the steroid ring [14]. In part, interest in estrogen metabolites (EM) may derive from the evidence that they may have different roles in breast carcinogenesis. Laboratory experiments have suggested several mechanisms by which individual estrogen metabolites might be carcinogenic. For example, EM have demonstrated distinctive biologic effects on the proliferation, apoptosis, and markers of metastasis in human breast cancer cell lines [15]. In laboratory experiments, 2-hydroxyestradiol did not affect proliferation or apoptosis, yet 4-hydroxyestradiol and 16α-hydroxyestrone increased proliferation and decreased apoptosis similarly to estradiol, albeit at noticeably higher concentrations [15]. Estrogen metabolites are also thought to differ in their capacity to promote DNA damage. In human mammary epithelial cells, 2- and 4-catechols can generate reactive oxygen species leading to DNA damage [16] while methylated catechol estrogens may induce apoptosis, thereby inhibiting tumor growth [17]. Understanding the role of modifiable lifestyle factors, like alcohol ingestion, on estrogen metabolism may be central to fully comprehending their involvement in the risk of breast carcinogenesis. Studies of the influence of alcohol consumption have focused on parent estrogens but neglected estrogen metabolism. Recent scientific advances have led to the precise and accurate quantification of 15 estrogens and estrogen metabolites (all 15 referred to as EM) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [18, 19]. We evaluated the relationship of alcohol consumption, including the types of alcoholic beverages, with luteal phase urinary concentrations of individual EM, EM grouped by metabolic pathway, and selected pathway ratios in a large well-characterized cross-sectional sample of premenopausal women participating in the Nurses' Health Study II (NHSII).

Methods

Study Population

This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital (Boston, MA). Briefly, the NHSII was established in 1989 when 116,430 female registered nurses 25–42 years of age were enrolled in the cohort and completed baseline questionnaires [20–26]. Participants have completed biennial questionnaires to update exposures and collect health and disease information since enrollment. Between 1996 and 1999, cancer-free participants between the ages of 32 and 54 were asked to provide blood and urine samples. Of the 29,611 samples collected, 18,521 were from premenopausal women who had not used oral-contraceptive agents, been pregnant, or breastfed in the past 6 months before collection, and were timed within the menstrual cycle. Blood samples were collected in the follicular and luteal phase, while urine samples were collected in the mid-luteal phase, approximately 7–9 days before the expected onset of the next cycle [27]. Approximately 97 % of participants returned a postcard reporting the first day of their next cycle after the urine collection to facilitate the calculation of the luteal day of collection (date of next menstrual cycle minus that of urine collection). Urine samples were packaged with an ice pack and returned to our laboratory via overnight courier, with 93 % of samples arriving within 26 h of collection. Urine samples were aliquoted into cryotubes without preservatives and stored in liquid nitrogen freezers. This cross-sectional analysis includes premenopausal women who provided a luteal urine sample and were participants in a reproducibility study ($n = 110$) or were selected as controls in a nested case-control study ($n = 493$) [24, 25]. At the time of urine collection, women were also free of other cancers (with the exception of non-melanoma skin cancer).

Exposure and Covariate Data

Alcohol consumption data were collected as part of detailed food frequency questionnaires (FFQs) administered in 1991 and every 4 years thereafter. Participants estimated food and beverage intakes, including alcohol consumption, using the previous year as a time referent. The 1995 and 1999 data were considered; however, the 1999 alcohol data were available for more participants and aligned more closely with the urine sample collection and were used in our analyses. When we evaluated the average of 1995 and 1999, our results were unchanged.

Participants indicated their average intake over the past year of white wine (4 oz glass), red wine (4 oz glass), regular beer (one glass, beer, can), light beer (one glass, beer, can), and liquor (one shot or drink), with possible choices ranging from "never or less than once a month" to "6+ per day". Total alcohol intake (g/day) was estimated by summing daily servings of each beverage multiplied by the average alcohol content of each beverage (12.8 g for regular beer, 11.3 g for light beer, 11.0 g for wine, 14.0 g for spirits). Wine intake was analyzed without differentiation as white or red because separate analyses would have led to small numbers at the higher intake levels and substantial overlap especially at lower intakes. Liquor and beer were included in total alcohol consumption but consumption was low with limited variability; therefore, these were not analyzed as sub-types of alcohol exposure. Total alcohol (g/day) and wine (drinks/week) were each categorized for analysis with non-drinkers of any alcohol as the referent group throughout the analyses. Total alcohol

intake was modeled in 5-g/day categories while wine was modeled as servings per week or day.

Data on potential covariates were collected at baseline, on the biennial study questionnaires, and at the time of the urine collection. These included height (1989), usual menstrual cycle length and pattern (1993), parity (1997), age at first birth (1997), physical activity (mean of 1997, 2000; MET-h/week), caffeine intake (mean of 1995, 1999), and smoking status (1997). The biospecimen questionnaire provided information on current age (continuous) and weight (to calculate BMI, kg/m^2), fasting status, date and time of the urine collection, and whether the collection was a first morning void (most were).

Urinary Laboratory Procedures

A 500-μL frozen urine sample was shipped to the Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc. (Frederick, MD) for the estrogen metabolism (EM) assay. The details of the assay have been reported previously [18, 19, 26]. Briefly, the assay includes an initial enzymatic hydrolysis step with β-glucuronidase/sulfatase from *Helix pomatia* and quantitation by liquid chromatography-tandem mass spectrometry (LC-ML/MS). The lower limit of detection for each of the estrogen metabolites (EM) is approximately 150 pmol/mL urine. Laboratory coefficients of variation (CV), including within- and between-batch variation, were less than 7 %, with the exception of the two estrogen metabolites with the lowest concentrations, 4-methoxyestrone (17 %) and 4-methoxyestradiol (15 %).

Urinary creatinine was measured at one of three laboratories with an overall CV of ≤9.2 % and used to adjust estrogen metabolite concentrations (pmol/mL) for urine volume resulting in picomoles per milligram (pmol/mg) creatinine for the total, individual, and metabolic pathway groups of EM.

Statistical Analyses

We calculated total EM levels by summing all 15 individual EM. Metabolites were also evaluated individually, grouped by metabolic pathway (e.g., methylated catechols, 16-hydroxylation pathway), and as pathway ratios. All values were log-transformed to improve normality. Statistical outliers were identified using the extreme studentized many-deviate procedure [28], resulting in the exclusion of 0–9 outliers for most EM, except 2-methoxyestradiol (16 outliers).

We used linear mixed models to calculate the adjusted geometric means of EM, pathway groups, and ratios by category of alcohol intake with non-drinkers of any type of alcohol as the referent. Final models were adjusted for age (continuous), body mass index (BMI, kg/m^2, continuous), height (continuous), first morning urine sample (yes/no), luteal day at urine collection (≤5, 6–7, 8–9, ≥10 days before next menstrual period), ovulatory cycle (yes/no), menstrual cycle pattern (extremely regular, very regular, regular, usually/always irregular), caffeine intake (quartiles), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h/week), tobacco use (current/former or never), and menstrual cycle length (<26, 26–31, ≥32 days). Tests for linear trend were conducted by modeling the median of the exposure categories as a continuous variable. In addition, we examined potential effect modification by creating interaction terms between dichotomous age (<43 vs. ≥ 43 years) and BMI (<25 vs. ≥25 kg/m^2), separately, and medians of exposure categories. The statistical significance of these interaction terms was determined by evaluating the Wald statistic in models that included all women. Finally, we conducted sensitivity analyses restricted, separately, to ovulatory women based on luteal plasma progesterone levels (≥400 ng/dL) measured at the time of urine collection ($n = 537$), women who had not smoked in the 30 days prior to urine collection or reported current smoking in 1997 or 1999 ($n = 560$), and women who provided samples collected during luteal days 4–10 ($n = 516$). All analyses were conducted using SAS version 9 (SAS Institute, Cary, NC). Reported P values are two-sided and considered statistically significant if $P < 0.05$.

Results

In this population of 603 premenopausal women, approximately one third ($n = 206$) reported being non-drinkers. Similarly, 45 % ($n = 260$) of women reported not drinking wine. Alcohol consumption ranged from 0 to approximately 60 g/day (approximately four drinks/day). Participants were 43 years of age, on average, at the time of urine collection and 97 % were Caucasian. Participants reported few comorbidities overall at urine collection; 8 (1.3 %) women reported being diagnosed with diabetes, 35 (5.8 %) reported high blood pressure, and 58 (9.6 %) reported hypercholesterolemia. As reported previously in this study population [21], those who consumed higher alcohol intakes also reported higher levels of overall physical activity and had lower BMIs than non-drinkers (Table 1). Women who consumed more alcohol tended to consume more caffeine (e.g., 292 mg/day for those consuming >15 g/day of alcohol vs. 175 mg/day for non-drinkers), smoke (current smokers 9 vs. 4 %), and have irregular menstrual cycles (regular 89 vs. 95 %) (Table 1).

Total alcohol intake was positively associated with urinary estradiol levels (26 % higher among women consuming >15 g/d vs. non-drinkers; P trend = 0.03) (Table 2). No significant associations were observed for total estrogen metabolites (EM) or any other individual EM, including estrone, the other parent estrogen. Two of

Table 1 Characteristics of the premenopausal study population in the Nurses' Health Study II ($n = 603$) by category of total alcohol intake

Number	Non-drinkers 206	≤5 g/day 211	>5–10 g/day 70	>10-15 g/day 55	>15 g/day 35
Age, years	42.8 (4.0)	43.1 (3.7)	43.0 (3.9)	42.4 (3.4)	43.4 (3.8)
Body mass index, kg/m^{2a}	25.7 (5.8)	25.6 (5.3)	24.2 (5.1)	23.1 (3.9)	24.4 (4.5)
Height, in.	64.9 (2.8)	65.2 (2.6)	65.1 (2.6)	65.5 (2.4)	65.2 (2.7)
Caucasian, %	95	99	94	96	100
Ovulatory cycle, %	88	90	91	95	86
First-morning urine sample, %	81	78	81	82	80
Sample collected 4–10 days before the next period, %	87	85	77	93	86
Regular menstrual cycles, %	95	96	96	98	89
Cycle length 26-31 day, %	72	59	66	73	71
Urinary creatinine, mg/L	1201 (633)	1073 (560)	1080 (559)	1101 (593)	1221 (666)
Caffeine intake, mg/day	175 (194)	240 (197)	310 (210)	306 (177)	292 (141)
Physical activity, MET-h/week	17.6 (16.9)	21.6 (22.0)	22.7 (16.1)	26.7 (21.0)	32.0 (20.4)
Current smoker, %	4	5	9	9	9

Values are means (SD) or percentages. Numbers of subjects do not sum to 603 due to missing values for exposure

[a] Measured at time of urine collection

the methylated 2-catechol EM, 2-methyoxyestradiol and 2-hydroxyestrone-3-methyl ether, showed positive but non-significant trends with increasing total alcohol intake (P trend = 0.09 and 0.08, respectively). Finally, total alcohol intake was inversely associated with the 2-catechols/methylated 2-catechols ratio (15 % lower, P trend = 0.05) and non-significantly inversely associated with the 2-pathway/parent estrogens ratio (18 % lower; P trend = 0.09).

In contrast to total alcohol, wine consumption was positively associated with a number of EM measures (Table 3). Compared to non-drinkers, those who reported consuming five or more drinks/week of wine had significantly higher levels of total parent estrogens (17 % higher, P trend = 0.01), specifically estradiol (22 % higher, P trend < 0.0001). The 4-hydroxylation pathway was elevated among the heaviest wine drinkers (14 % higher; P trend = 0.04), but no significant associations were observed for the other two estrogen hydroxylation pathways or total estrogens and metabolites. Higher levels of 2-hydroxyestradiol (15 % higher, P trend = 0.03) and two of the methylated 2-catechol EM, 2-methyoxyestradiol (24 % higher, P trend = 0.001) and 2-hydroxyestrone-3-methyl ether (34 % higher, P trend = 0.02), and 4-hydroxyestrone (12 % higher, P trend =0.049) were also observed among those reporting the highest levels of wine intake. Wine consumption was inversely associated with 17-epiestriol (16 % decrease, $P = 0.03$), a metabolite in the 16-hydroxylation pathway; however, there was not a consistently inverse trend across increasing categories of intake. Finally, only one metabolic pathway ratio, the ratio of parent estrogens to 2-, 4-, and 16-pathway estrogen metabolites was associated with wine consumption (15 % higher, P trend = 0.01).

We conducted separate sensitivity analyses restricted to women (1) whose urine samples were collected during an ovulatory cycle (mid-luteal plasma progesterone levels ≥400 ng/dL), (2) whose urine samples were collected within 4–10 days of their next menstrual cycle, and (3) who were non-smokers. For the total alcohol analyses, some of the borderline positive associations became significant (e.g., 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether) as did some of the borderline inverse associations (e.g., 17-epiestriol, 2-catechols/methylated 2-catechols ratio) when the analyses were restricted to ovulatory women. Similar strengthening of the observed associations with alcohol intake was noted when analyses were restricted to women whose urine samples were collected within 4–10 days of their next cycle. In general, the positive associations observed for wine consumption became stronger in analyses restricted to ovulatory women or restricted to women with samples collected during the 4–10-day window. In analyses of non-smokers only, we observed similar patterns to those observed in all subjects, although the absolute levels of 2-catechols tended to be lower and levels of several 16-pathway EM higher among non-smokers. Lastly, we did not observe any evidence of significant and meaningful effect modification by age or BMI for total alcohol intake (data not presented). We observed significant interactions between age and BMI with wine

Table 2 Adjusted geometric means (pmol/mg creatinine) of urinary estrogen and estrogen metabolite measures by categories of total alcohol intake in the Nurses' Health Study II

	Non-drinker	≤5 g/day	>5–10 g/day	>10–15 g/day	>15 g/day	*P* trend	Percent difference
N	206	211	70	55	35		
Total estrogens and metabolites	200.4	197.8	189.4	210.7	196.8	0.91	−2 %
Parent estrogens	38.4	42.1	37.1	43.3	42.9	0.38	12 %
Estrone	25.2	28.1	23.9	28.7	25.6	0.99	2 %
Estradiol	12.3	13.3	11.6	14.7	15.5	0.03	26 %
Catechols	69.2	64.9	58.7	72.7	64.0	0.84	−8 %
2-catechols	58.8	56.2	50.2	60.7	53.7	0.68	−9 %
2-Hydroxyestrone	51.9	49.6	43.9	52.9	47.2	0.62	−9 %
2-Hydroxyestradiol	5.9	5.9	5.6	6.4	5.6	0.97	−5 %
4-catechols							
4-Hydroxyestrone	6.6	6.2	6.1	6.9	7.3	0.46	10 %
Methylated catechols	10.5	10.8	10.6	11.3	10.4	0.94	−1 %
Methylated 2-catechols	10.1	10.5	10.3	10.9	10.1	0.85	1 %
2-Methoxyestrone	7.9	8.3	7.7	8.5	7.4	0.74	−6 %
2-Methoxyestradiol	0.72	0.69	0.67	0.78	0.89	0.09	24 %
2-Hydroxyestrone-3-methyl ether	1.2	1.3	1.4	1.4	1.5	0.08	24 %
Methylated 4-catechols	0.23	0.23	0.26	0.30	0.21	0.59	−7 %
4-Methoxyestrone	0.14	0.15	0.17	0.16	0.13	0.98	−8 %
4-Methoxyestradiol	0.05	0.05	0.06	0.09	0.05	0.34	−11 %
2-Hydroxylation pathway	70.2	68.0	62.5	73.7	65.9	0.84	−6 %
4-Hydroxylation pathway	7.4	7.0	6.4	7.9	8.0	0.50	9 %
16-Hydroxylation pathway	62.6	62.2	61.7	67.6	69.2	0.30	10 %
16α-Hydroxyestrone	10.9	10.6	11.3	11.1	11.3	0.68	4 %
Estriol	27.4	26.9	26.5	30.2	28.7	0.49	5 %
17-Epiestriol	1.7	1.8	1.9	1.5	1.3	0.11	−24 %
16-Ketoestradiol	13.6	13.5	12.4	14.1	11.1	0.16	−18 %
16-Epiestriol	5.5	5.6	5.2	7.0	5.9	0.12	8 %
Ratios of metabolic pathways							
2-Hydroxyestrone/16α-hydroxyestrone	4.6	4.6	3.9	4.7	3.9	0.39	−15 %
4-Pathway/2-pathway	0.10	0.10	0.10	0.10	0.11	0.51	9 %
2-Pathway/16-pathway	1.07	1.08	1.01	0.99	0.94	0.30	−12 %
2,4-Pathway/16-pathway	1.2	1.2	1.2	1.2	1.1	0.39	−11 %
2-Catechols/methylated 2-catechols	5.8	5.4	4.8	5.0	4.9	0.05	−15 %
4-Catechols/methylated 4-catechols	30.4	28.5	20.4	25.9	36.8	0.90	21 %
Catechols/methylated catechols	6.6	6.1	5.4	5.8	5.8	0.16	−12 %
Parent estrogens/estrogen metabolites	0.25	0.28	0.26	0.27	0.30	0.18	19 %
2-Pathway/parent estrogens	1.8	1.6	1.7	1.6	1.5	0.09	−18 %
4-Pathway/parent estrogens	0.18	0.16	0.16	0.17	0.18	0.85	−1 %
16-Pathway/parent estrogens	1.6	1.5	1.6	1.6	1.5	0.97	−7 %

Adjusted for age (continuous), BMI (continuous), height (continuous), ovulatory cycle (yes/no), first morning urine sample (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days before the next menstrual period), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), menstrual cycle length (<26, 26–31, ≥32 days), caffeine intake (quartiles), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h/week), and smoking status (current/former or never)

intake for several EM where there were significant main effects, including estradiol (age, *P* for interaction (*P*-int) = 0.02), 2-hydroxyestradiol (age, *P*-int = 0.03), 4-hydroxyesterone (age, *P*-int = 0.01), the 4-hydroxylation

Table 3 Adjusted geometric means (pmol/mg creatinine) of urinary estrogen and estrogen metabolite levels by categories of total wine intake in the Nurses' Health Study II

	Non-drinker[a]	≤1 drink/week	2–4 drinks/week	≥5 drinks/week	P trend	Percent difference
N	206	168	72	76		
Total estrogens and metabolites	202.8	199.2	198.5	210.6	0.09	4 %
Parent estrogens	40.0	43.3	41.6	46.9	0.01	17 %
Estrone	26.3	29.1	26.4	29.4	0.27	12 %
Estradiol	13.0	13.9	13.3	15.9	<0.0001	22 %
Catechols	72.4	70.0	64.1	73.4	0.53	1 %
2-Catechols	61.7	60.4	55.5	61.6	0.77	0 %
2-Hydroxyestrone	54.6	53.6	48.7	53.9	0.94	−1 %
2-Hydroxyestradiol	6.1	5.9	6.4	7.0	0.03	15 %
4-catechols						
4-Hydroxyestrone	7.1	6.8	6.6	8.0	0.049	12 %
Methylated catechols	10.8	10.9	10.8	12.4	0.14	15 %
Methylated 2-catechols	10.3	10.6	10.5	12.1	0.13	17 %
2-Methoxyestrone	8.1	8.4	8.1	9.1	0.32	13 %
2-Methoxyestradiol	0.73	0.70	0.77	0.91	0.001	24 %
2-Hydroxyestrone-3-methyl ether	1.2	1.3	1.4	1.7	0.02	34 %
Methylated 4-catechols	0.23	0.25	0.24	0.27	0.52	14 %
4-Methoxyestrone	0.15	0.17	0.15	0.16	0.86	7 %
4-Methoxyestradiol	0.06	0.05	0.06	0.07	0.27	27 %
2-Hydroxylation pathway	73.5	73.0	67.5	75.9	0.51	3 %
4-Hydroxylation pathway	7.8	7.74	6.9	8.84	0.04	14 %
16-Hydroxylation pathway	63.1	60.7	65.7	66.7	0.12	6 %
16α-Hydroxyestrone	10.6	10.1	10.3	10.5	0.64	−1 %
Estriol	27.5	25.8	29.3	28.3	0.06	3 %
17-Epiestriol	1.6	1.7	1.8	1.4	0.03	−16 %
16-Ketoestradiol	13.6	13.17	13.6	12.7	0.16	−7 %
16-Epiestriol	5.6	5.4	6.0	6.2	0.08	12 %
Ratios of metabolic pathways						
2-Hydroxyestrone/16α-hydroxyestrone	5.1	5.2	4.8	5.1	0.62	0 %
4-Pathway/2-pathway	0.10	0.10	0.09	0.10	0.83	−2 %
2-Pathway/16-pathway	1.1	1.2	1.1	1.2	0.83	3 %
4-Pathway/16-pathway	1.3	1.3	1.2	1.3	0.99	4 %
2-Catechols/methylated 2-catechols	5.9	5.5	5.2	5.0	0.17	−15 %
4-Catechols/methylated 4-catechols	30.0	27.6	25.5	30.2	0.33	1 %
Catechols/methylated catechols	6.7	6.2	5.8	5.8	0.38	−12 %
Parent estrogens/estrogen metabolites	0.26	0.29	0.28	0.30	0.01	15 %
2-Pathway/parent estrogens	1.8	1.6	1.6	1.6	0.16	−11 %
4-Pathway/parent estrogens	0.18	0.17	0.16	0.18	0.59	−2 %
16-Pathway/parent estrogens	1.5	1.4	1.5	1.4	0.07	−11 %

Adjusted for age (continuous), BMI (continuous), height (continuous), ovulatory cycle (yes/no), first morning urine sample (yes/no), luteal day (≤5, 6–7, 8–9, ≥10 days before the next menstrual period), menstrual cycle regularity (extremely regular, very regular, regular, usually/always irregular), menstrual cycle length (<26, 26–31, ≥32 days), caffeine intake (quartiles), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h/week), and smoking status (current/former or never)

[a] Referent category is non-drinkers of any alcohol

pathway (age, P-int = 0.01), and the parent estrogens/estrogen metabolites ratio (BMI, P-int = 0.01). In stratified analyses, stronger associations were observed among older and heavier women (data not presented).

Discussion

We evaluated the cross-sectional relationship between alcohol consumption and luteal phase urinary concentrations of individual and grouped EM and the ratios of selected metabolic pathways. This was the first study in a sample of premenopausal women to evaluate associations between alcohol and 15 EM in detail. Overall, total alcohol intake was not strongly associated with individual or grouped urinary EM, though a positive association with estradiol was observed. We observed that wine consumption, the type of alcohol most frequently consumed in our study population, was associated with higher urinary concentrations of estradiol, one of the 2-hydroxylation pathway catechols (2-hydroxyestradiol), two 2-pathway methylated catechols (2-methyoxyestradiol and 2-hydroxyestrone-3-methyl ether), the only 4-pathway catechol (4-hydroxyestrone), and the 16-hydroxylation pathway EM, 17-epiestriol. In addition, the ratio of parent estrogens/estrogen metabolites increased with wine consumption.

Results for the associations between alcohol consumption and plasma sex hormone levels have recently been reported for a larger sample of premenopausal women ($n = 2000$) participating in the NHSII [10]. This larger sample, which analyzed selected plasma estrogen concentrations (estrogen, estradiol, estrone, and estrone sulfate), had the advantage of a greater range of alcohol intakes with more women in the upper tail of the distribution compared to the present study. Total alcohol and wine consumption (but not beer) were both significantly positively associated with plasma luteal estradiol concentrations. Compared to non-drinkers, those with the highest total alcohol and wine consumption had plasma luteal estradiol levels 27.2 and 17.3 % higher, respectively. Luteal estrone concentrations were also significantly positively associated with total alcohol ingestion. Luteal estrone concentrations were positively but not significantly associated with either wine or beer consumption. In the present study, we observed significant positive associations of total alcohol and wine with urinary luteal estradiol, though not estrone.

Few studies have evaluated the association between alcohol consumption and urinary estrogens or estrogen metabolites among premenopausal women utilizing urine collections timed within the menstrual cycle. A cross-sectional analysis from the Study of Women's Health Across the Nation (SWAN), including a racially diverse sample of 1881 women ages 42–52, did not observe an association between total alcohol consumption and the urinary follicular phase levels of 2-hydroxyestrone and 16α-hydroxyestrone [29]. Wine consumption was positively and significantly associated with urinary levels of 2-hydroxyestrone. In contrast, we observed a positive association between wine and luteal 2-hydroxyestradiol levels. Unfortunately, the SWAN did not collect any urine samples during the luteal phase of the menstrual cycle. Reichman and colleagues [9] conducted a controlled cross-over feeding study where premenopausal women ($n = 34$) were provided with either no alcohol or 30 g/day of alcohol (95 % ethanol in fruit juice) in random order. All food and other beverages consumed were also provided and body weight was monitored. This high level of alcohol consumption was associated with significant increases in luteal urinary estrone, estradiol, and estriol (15.2, 21.6, and 29.1 %, respectively), which were the only estrogens and estrogen metabolites measured.

Our results suggest that the type of alcoholic beverage may be important when studying associations with estrogen metabolism profiles. The recent study of 2000 women in NHSII also observed differences in associations between plasma luteal phase sex steroid concentrations and different types of alcohol consumption [10]. Unfortunately there are few available data to address the effects of long-term exposure to different alcoholic beverage types, including detailed frequency and quantity information on biological parameters (e.g., lipids, insulin, sensitivity, sex hormones) among premenopausal women. It is possible that the pattern of alcohol consumption differs by beverage type. For example, perhaps wine drinkers are more frequent drinkers (number of drinking days) at a similar overall quantity (number of drinks consumed) and higher frequency tends to influence estrogen metabolism profiles. Our data do not allow us to comprehensively explore this possibility. It is also possible that there is a dietary constituent other than ethanol that varies across types of alcoholic beverages and influences sex hormone metabolism. Wine, which is often consumed with a meal, is more accurately reported than other types of alcohol and there is less misclassification of this exposure in some cohorts; however, that has not been observed in the NHS cohorts [30]. Finally, there may be residual confounding by other lifestyle characteristics that differ among those who consume different types of alcoholic beverages; however, our adjustment for several lifestyle factors associated with alcohol use, such as smoking and caffeine intake, did not appreciably change the results. These results deserve further study in large samples with more variation in alcoholic beverage choice.

Estrogens are involved in several mechanisms which may affect hormone-related cancer risk including cellular proliferation, activation of the cytochrome P450 pathway thought to promote mutations, and induction of aneuploidy or aberrant cell replication that results in cells with the wrong number of chromosomes [31]. The interpretation of the findings in the current study relative to the role of alcohol in premenopausal breast cancer risk is difficult in part because the role of circulating and excreted estrogen metabolites in premenopausal breast cancer risk is not completely understood. Four prospective studies and a combined analysis of published studies have evaluated estrogen metabolites and breast cancer risk among premenopausal women and have reported conflicting results [14, 25, 32, 33]. A combined analysis of five published studies

including premenopausal women by Dallal et al. [33] reported that higher urinary 2-hydroxyestrone:16α-hydroxyestrone was associated with a non-significant reduction in breast cancer risk. As reviewed by Ziegler and colleagues [34], unfortunately, the early studies of estrogen metabolism measured only 2-hydroxyestrone and 16α-hydroxyestrone and used direct enzyme immunoassay methods which did not include purification steps. This likely contributed to the inconsistent results observed among early studies. In our prior nested case-control study conducted in the NHSII that included many of the participants from the current study, we reported that concentrations of urinary luteal phase parent estrogens and the ratio of parent estrogens to estrogen metabolites were inversely associated with risk for premenopausal breast cancer [25]. The present observation of higher levels of most EM with alcohol consumption would suggest that this may not be a mechanism by which the consumption of alcohol increases breast cancer risk. Alcohol could influence breast cancer through other mechanisms other than estrogen response, including producing acetaldehyde, a highly toxic metabolite that promotes fatty acid synthesis and reduces lipid oxidation, through the generation of reactive oxygen species, by altering the absorption and metabolism of protective antioxidant nutrients with the ability to reduce oxidative stress, and by affecting one-carbon metabolism pathways [35–38].

This study has a number of strengths including the large sample size of premenopausal women, the availability of first morning urine samples timed within the luteal phase of the menstrual cycle, and the use of a comprehensive LC-MS/MS assay that measures 15 estrogens and estrogen metabolites with high sensitivity, reproducibility, and accuracy with good precision. The assay has high reproducibility of luteal EM measurements over 3 years [26]. The alcohol data were collected by type allowing for the analysis of total alcohol and wine. In addition, many well-characterized potential confounding variables were considered in our analyses.

There are also several important considerations relative to this study. The measurement of a single urine sample may not accurately reflect true EM profiles nor is it known how well urinary EM concentrations represent levels in circulation or in the breast or other tissues. Among 110 participants in the Nurses' Health study II, correlations between luteal-phase urinary and plasma parent estrogens were 0.3–0.5 [26]. The lack of strong correlations between urinary and plasma estrogens suggests that it is possible that EM may provide additional insight into the relationship between estrogen and breast cancer risk. We only looked at luteal measures and cannot address the relationship between alcohol and EM measures at other phases of the menstrual cycle. Previous research supports the analysis of luteal phase estrogens among premenopausal women as reasonably reflective of long-term hormone levels, reflective of tissue levels, and more strongly associated with alcohol intake. For repeated samples collected over several years, the intraclass correlation coefficient (ICC; ratio of between-person variation/[between + within person variation]) for estrogen levels was higher during the luteal phase compared to the follicular phase [39]. A small study ($n = 22$) of premenopausal women reported that correlation between tissue and blood estrone levels was higher during the luteal phase (0.63) compared to the follicular phase (0.52) [40]. Hirko et al. [10] reported a positive association between alcohol intake and luteal plasma estrogens, but no association for follicular plasma estrogens. Lastly, Reichman and colleagues [9] assessed change in the levels of urinary estrone, estradiol, and estriol with alcohol consumption across the menstrual cycle but observed significant increases for urinary estrogens only during the luteal phase. In our study, alcohol consumption was self-reported and recalled over the previous year; however, in a validation study, alcohol intake collected by FFQ was very highly correlated (Spearmen $r = 0.90$) with that from dietary records [30]. Our data do not allow us to determine the timing of alcohol consumption during the day or week, the quantity consumed per drinking occasion or whether alcohol was consumed alone or with a meal, which may be relevant and may vary by beverage type. The study sample was primarily Caucasian and, compared to the overall population of US women, is of higher socioeconomic status (e.g., better educated, higher income) which potentially limits the generalizability of the results; however, underlying biology is not likely to differ by socioeconomic status or race/ethnicity. Our analyses were exploratory in nature; we looked at a number of different outcome variables without adjustment for multiple comparisons. If we had used a Bonferroni correction, at least some of our statistically significant results would not have reached statistical significance. Finally, compared to some other populations, alcohol consumption in this study was modest and relatively few women reported consuming spirits or beer. Available data suggest that overall alcohol consumption in our study was similar to reports for US women. In 2001–2002, the US National Institute for Alcohol Abuse and Alcoholism (NIAAA) administered a nationwide household survey to characterize alcohol consumption over the past year [41]. Among US adult women of all ages, approximately 60 % reported some current alcohol consumption (compared to 65.8 % in our study), and among those reporting alcohol consumption, approximately 13.3 % reported consuming more than one drink/day (compared to 9.4 % in our study). The data provided in the NIAAA report on the type of alcohol consumed are not directly comparable to our data; however, beverage preference (consuming ≥75 % of alcohol from a particular type) was highest for wine (~25 %) among US women [41]. Interestingly, wine consumption increased between 1995 and 2000 among US women and was positively associated with educational status [42]. Although intakes appear modest in our study, meta-analyses of cohort studies have shown a 9 % increased risk for premenopausal breast cancer per 10 g

ethanol/day (i.e., intakes similar to those in our two highest categories of total alcohol intake, >10–15 g/day and >15 g/day—see Table 2) [6]. Thus, the etiologic role of estrogen in breast cancer in combination with our results, that alcohol consumers of >10 g/d have 20–26 % higher estradiol compared to non-drinkers, suggest that higher estrogen levels may be a mechanism by which alcohol increases breast cancer risk.

Conclusions

In summary, total alcohol intake was positively and significantly associated with urinary estradiol levels. Some differences in urinary estrogen metabolites were observed with wine drinking, when compared with non-drinkers. Additional studies of premenopausal women are needed to further explore the association of alcohol, particularly specific types of alcohol, on patterns of estrogen metabolism in blood, urine, and tissue.

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Compliance with Ethical Standards

Conflict of Interest The content of this publication does not necessarily reflect the views or policies of the US Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

No potential conflicts of interest were disclosed by the other authors.

Ethics Approval This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital (Boston, MA).

References

1. Lee YC, Hashibe M (2014) Tobacco, alcohol, and cancer in low and high income countries. Ann Glob Health 80:378–383
2. World Cancer Research Fund \American Institute for Cancer Research (2007) Food, nutrition, physical activity, and the prevention of cancer: a global perspective. AICR, Washington
3. Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsum AR, Goldbohm RA et al (1998) Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 279:535–540
4. Allen NE, Beral V, Casabonne D, Kan SW, Reeves GK, Brown A, Green J (2009) Moderate alcohol intake and cancer incidence in women. J Natl Cancer Inst 101:296–305
5. Zhang SM, Lee IM, Manson JE, Cook NR, Willett WC, Buring JE (2007) Alcohol consumption and breast cancer risk in the Women's Health Study. Am J Epidemiol 165:667–676
6. World Cancer Research Fund/American Institute for Cancer Research (2010) Breast cancer report continuous update project: food, nutrition, physical activity, and the prevention of breast cancer. AICR, Washington
7. Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK et al (2001) Serum hormones and the alcohol-breast cancer association in postmenopausal women. J Natl Cancer Inst 93:710–715
8. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A et al (1994) The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women. Cancer Causes Control 5:53–60
9. Reichman ME, Judd JT, Longcope C, Schatzkin A, Clevidence BA, Nair PP et al (1993) Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. J Natl Cancer Inst 85:722–727
10. Hirko KA, Spiegelman D, Willett WC, Hankinson SE, Eliassen AH (2014) Alcohol consumption in relation to plasma sex hormones, prolactin, and sex hormone-binding globulin in premenopausal women. Cancer Epidemiol Biomarkers Prev 23:2943–2953
11. Lyngso J, Toft G, Hoyer BB, Guldbrandsen K, Olsen J, Ramlau-Hansen CH (2014) Moderate alcohol intake and menstrual cycle characteristics. Hum Reprod 29:351–358
12. Cooper GS, Sandler DP, Whelan EA, Smith KR (1996) Association of physical and behavioral characteristics with menstrual cycle patterns in women age 29–31 years. Epidemiology 7:624–628
13. Liu Y, Gold EB, Lasley BL, Johnson WO (2004) Factors affecting menstrual cycle characteristics. Am J Epidemiol 160:131–140
14. Samavat H, Kurzer MS (2015) Estrogen metabolism and breast cancer. Cancer Lett 356:231–243
15. Seeger H, Wallwiener D, Kraemer E, Mueck AO (2006) Comparison of possible carcinogenic estradiol metabolites: effects on proliferation, apoptosis and metastasis of human breast cancer cells. Maturitas 54:72–77
16. Hurh YJ, Chen ZH, Na HK, Han SY, Surh YJ (2004) 2-Hydroxyestradiol induces oxidative DNA damage and apoptosis in human mammary epithelial cells. J Toxicol Environ Health A 67(23–24):1939–1953
17. Liu ZJ, Zhu BT (2004) Concentration-dependent mitogenic and antiproliferative actions of 2-methoxyestradiol in estrogen receptor-positive human breast cancer cells. J Steroid Biochem Mol Biol 88:265–275
18. Ziegler RG, Faupel-Badger JM, Sue LY, Frhrman BJ, Falk RT, Boyd-Morin J, Henderson MK, Hoover RN, Beenstra TD, Keefer LK, Xu X (2010) A new approach to measuring estrogen exposure and metabolism in epidemiologic studies. J Steroid Biochem Mol Biol 121:538–545
19. Xu X, Veenstra TD, Fox SD, Roman JM, Isaaq HJ, Falk R, Saavedra JE, Keefer LK, Ziegler RG (2005) Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry. Anal Chem 77:6646–6654
20. Fortner RT, Hankinson SE, Schairer C, Xu X, Ziegler RG, Eliassen AH (2012) Association between reproductive factors and urinary estrogens and estrogen metabolites in premenopausal women. Cancer Epidemiol Biomarkers Prev 21:959–968
21. Matthews CE, Fortner RT, Xu X, Hankinson SE, Eliassen AH, Ziegler RG (2012) Association between physical activity and urinary estrogens and estrogen metabolites in premenopausal women. J Clin Endocrinol Metab 97:3724–3733

22. Gu F, Caporaso NE, Schairer C, Fortner RT, Xu X, Hankinson SE et al (2013) Urinary concentrations of estrogens and estrogen metabolites and smoking in Caucasian women. Cancer Epidemiol Biomarkers Prev 22:58–68
23. Fortner RT, Oh H, Daugherty SE, Xu X, Hankinson SE, Ziegler RG, Eliassen AH (2014) Analgesic use and patterns of estrogen metabolism in premenopausal women. Horm Cancer 5:104–112
24. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, Hankinson SE (2006) Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 98:1406–1415
25. Eliassen AH, Spiegelman D, Xu X, Keefer LK, Veenstra TD, Barbieri RL et al (2012) Urinary estrogens and estrogen metabolites and subsequent risk of breast cancer among premenopausal women. Cancer Res 72:696–706
26. Eliassen AH, Ziegler RG, Rosner B, Veenstra TD, Roman JM, Xu X, Hankinson SE (2009) Reproducibility of fifteen urinary estrogens and estrogen metabolites over a 2- to 3-year period in premenopausal women. Cancer Epidemiol Biomarkers Prev 18:2860–2868
27. Tworoger SS, Sluss P, Hankinson SE (2006) Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 66:2476–2482
28. Rosner B (1983) Percentage points for a generalized ESD many-outlier procedure. Technometrics 25(2):165–172
29. Sowers MR, Crawford S, McConnell DS, Randolph JF, Gold EB, Wilkin MK et al (2006) Selected diet and lifestyle factors are associated with estrogen metabolites in a multiracial/ethnic population of women. J Nutr 136:1588–1595
30. Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L et al (1991) The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol 133: 810–817
31. Dumitrescu RG, Shields PG (2005) The etiology of alcohol-induced breast cancer. Alcohol 35:213–225
32. Hankinson SE, Eliassen AH (2010) Circulating sex steroids and breast cancer risk in premenopausal women. Horm Cancer 1:2–10
33. Dallal CM, Stone RA, Cauley JA, Ness RB, Vogel VG, Fentiman IS et al (2013) Urinary estrogen metabolites and breast cancer: a combined analysis of individual level data. Int J Biol Markers 28:3–16
34. Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE (2015) Epidemiologic studies of estrogen metabolism and breast cancer. Steroids 99:67–75
35. Lieber CS (2004) The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. Drug Metab Rev 36:511–529
36. Novak RF, Woodcroft KJ (2000) The alcohol-inducible form of cytochrome P450 (CYP 2E1): role in toxicology and regulation of expression. Arch Pharm Res 23:267–282
37. Hartman TJ, Baer DJ, Graham LB, Stone WL, Gunter EW, Parker CE et al (2005) Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. Eur J Clin Nutr 59:161–168
38. Laufer EM, Hartman TJ, Baer DJ, Gunter EW, Dorgan JF, Campbell WS et al (2004) Effects of moderate alcohol consumption on folate and vitamin B(12) status in postmenopausal women. Eur J Clin Nutr 58:1518–1524
39. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE (2006) Reproducibility of plasma hormone and growth factor levels among premenopausal women over a 2–3 year period. Cancer Epidemiol Biomarkers Prev 15:972–978
40. Mady EA, Ramadan EE, Ossman AA (2000) Sex steroid hormones in serum and tissue of benign and malignant breast tumor patients. Dis Markers 16:151–157
41. National Institute on Alcohol Abuse and Alcoholism (2006) Alcohol use and alcohol disorders in the United States: main findings from the 2001–2002 National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). National Institutes of Health, Bethesda, NIH Publication No. 05–5737
42. Kerr WC, Greenfield TK, Bond J, Ye Y, Rehm J (2004) Age, period and cohort influences on beer, wine and spirits consumption trends in US National Alcohol Surveys. Addiction 99:1111–1120