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ORIGINAL ARTICLE

Alcohol intake and endogenous sex hormones in women: Meta-analysis of cohort studies and Mendelian randomization

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Abstract

Background: The mechanisms underlying alcohol-induced breast carcinogenesis are not fully understood but may involve hormonal changes.

Methods: Cross-sectional associations were investigated between self-reported alcohol intake and serum or plasma concentrations of estradiol, estrone, progesterone (in premenopausal women only), testosterone, androstenedione, dehydroepiandrosterone sulfate, and sex hormone binding globulin (SHBG) in 45 431 premenopausal and 173 476 postmenopausal women. Multivariable linear regression was performed separately for UK Biobank, European Prospective Investigation into Cancer and Nutrition, and Endogenous Hormones and Breast Cancer Collaborative Group, and meta-analyzed the results. For testosterone and SHBG, we also conducted Mendelian randomization and colocalization using the *ADH1B* (alcohol dehydrogenase 1B) variant (rs1229984).

Results: Alcohol intake was positively, though weakly, associated with all hormones (except progesterone in premenopausal women), with increments in concentrations per 10 g/day increment in alcohol intake ranging from 1.7% for luteal estradiol to 6.6% for postmenopausal dehydroepiandrosterone sulfate. There was an inverse association of alcohol with SHBG in postmenopausal women but a small positive association in premenopausal women. Two-sample randomization identified positive associations of alcohol intake with total testosterone (difference per 10 g/day increment: 4.1%; 95% CI, 0.6–7.6) and free testosterone (7.8%; 4.1–11.5), and an inverse association with SHBG (−8.1%; −11.3% to −4.9%). Colocalization suggested a shared causal locus at *ADH1B* between alcohol intake and higher free testosterone and lower SHBG (posterior probability for H4, 0.81 and 0.97, respectively).

Conclusions: Alcohol intake was associated with small increases in sex hormone concentrations, including bioavailable fractions, which may contribute to its effect on breast cancer risk.

KEYWORDS
alcohol drinking, sex hormones, estrogens, androgens, breast cancer

INTRODUCTION

Alcoholic beverages are commonly consumed in many populations and are known to be causally associated with increased risk of several diseases, including breast cancer.[1,2] The mechanisms underlying alcohol-induced carcinogenesis are not fully understood; the mutagenic alcohol metabolite acetaldehyde may be the causal factor for some cancers such as those of the upper gastrointestinal tract,[2,3] but the effect on breast cancer may involve hormonal changes.[4]

Alcohol may influence concentrations of endogenous sex hormones by altering their secretion, metabolism, and/or clearance.[5–8] Earlier intervention studies have reported an acute increase in serum/plasma concentrations of estrogens and/or androgens within hours after intake of alcohol[5,9–13] in pre- and/or postmenopausal women, although others found no clear effect.[6,7,14] Other intervention studies have also found an increase in sex hormone concentrations after daily intake of alcohol for 2 to 3 months.[15–18] Similarly, more recent cross-sectional observational studies have associated habitual alcohol intake with higher sex hormone concentrations as well as differences in sex hormone binding globulin (SHBG), a glycoprotein that binds to estrogens and androgens.[19–21]

In this study, we combined data from 14 prospective cohort studies and conducted cross-sectional analyses to provide the most comprehensive evidence to date on the associations of usual alcohol intake (average alcohol intake over a period defined in each study) with serum or plasma concentrations of estradiol, estrone, testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), and SHBG in pre- and postmenopausal women, and with progesterone in premenopausal women only. To examine the potential causal associations with testosterone and SHBG, we also conducted two-sample Mendelian randomization (MR) and colocalization analyses.

MATERIALS AND METHODS

Observational analyses

We conducted literature searches using PubMed to identify cohort studies or consortia with data on alcohol and one or more sex hormones for at least 5000 women; these were the UK Biobank, the European Prospective Investigation into Cancer and Nutrition (EPIC), and the Endogenous Hormones and Breast Cancer Collaborative Group (EHBCCG, providing harmonized data from 12 constituent cohorts).

UK Biobank: This is a prospective cohort study involving approximately 500,000 adults, including more than 270,000 women aged 40 to 69 years when recruited between 2006 and 2010. At the initial assessment visit, usual alcohol intake was assessed using a touchscreen questionnaire, and blood samples were collected, from which serum was prepared and concentrations of hormones and SHBG were measured using chemiluminescent immunoassays. The current analysis included premenopausal women, who reported they had not undergone menopause (i.e., periods had not stopped), and were younger than 50 years of age, and postmenopausal women, who reported they had gone through menopause, or were 55 years or older, or reported a bilateral oophorectomy; those who had a history of cancer (except for nonmelanoma skin cancer) or reported currently using hormone therapy (hormone replacement therapy [HRT] or oral contraceptives [OCs]) were excluded. Detailed information on the study design and methodology,[22] calculation of alcohol intake in grams per day,[21] and the assay data processing[23] has been reported elsewhere.

EPIC: This is a prospective cohort study involving approximately 520,000 adults, including more than 360,000 women, aged 25 to 70 years when recruited from 23 centers across 10 European countries between 1992 and 2000. Diet, including usual alcohol intake, was measured by country-specific questionnaires that were validated against reference measurements based on 12 24-hour diet recall interviews.[24] Blood samples were collected from approximately 74% of the participants. The current analysis included pre- and postmenopausal women from nested case-control studies on breast, ovarian, endometrial, cervical, liver, and thyroid cancer risk for whom serum (in most of these studies) or plasma concentrations of sex hormones and SHBG were measured. Both precases (women who were cancer-free at the time of blood collection but were subsequently diagnosed with the cancer of interest during follow-up) and controls were included, except for the liver cancer study, in which only controls were included. Participants were categorized as premenopausal if they reported regular menstrual cycles over the 12 months before blood collection or were younger than 42 years at recruitment, and as postmenopausal if they reported having had no menses over the past 12 months, were 55 years or older, or reported a bilateral oophorectomy. Women who reported currently using hormone therapy (HRT or OCs) were excluded, as well as those from Greece (because of a restriction concerning information governance). Detailed information on the study design and methodology,[25] calculation of alcohol intake in grams per day, and the assay data[26] have been reported elsewhere.

The EPIC study data for breast cancer were included in the EHBCCG in previous publications on cancer risk, but the EPIC data were analyzed separately here because, since the publication of the collaborative analyses, more nested case-control studies of other cancer sites have been conducted and hormone assay data are now available for a larger sample of women.

EHBCCG: This is a consortium established to conduct pooled analyses of endogenous hormones in relation to breast cancer risk. Studies were eligible for inclusion if they had published data on endogenous hormone concentrations and breast cancer risk using prospectively collected blood samples from pre- and/or postmenopausal women. Detailed information on the design and methodology of EHBCCG has been reported elsewhere.[19,27,28]

Of the seven prospective studies of premenopausal women, information on usual alcohol intake was available for three: Nurses' Health Study II, USA; New York University Women's Health Study, USA; and the Study of Hormones and Diet in the Etiology of Breast Tumors, Italy. Of the 18 studies of postmenopausal women, 11 were included: Cancer Prevention Study-II Nutrition Cohort, USA; Malmö/Umeå, Sweden; the Melbourne Collaborative Cohort Study, Australia; the Multiethnic Cohort, USA; Nurses' Health Study, USA; New York University Women's Health Study, USA; Study of Hormones and Diet in the Etiology of Breast Tumors, Italy; Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial cohort, USA; Study of Osteoporotic Fractures, USA; United Kingdom Collaborative Trial of Ovarian Cancer Screening, UK; and the Women's Health Initiative, Observational Study, USA; menopausal status was taken as defined in each individual study. Women who reported currently using hormone therapy (HRT or OCs) were excluded. Alcohol intake was measured by study-specific questionnaires, mainly using frequency questions, and validated against 24-hour recalls or diet records in most studies; intake in grams per day was calculated using country- or study-specific food composition tables. References for the individual studies and measurement of usual alcohol intake are provided in Tables S1 and S2.

The number of women who contributed to each hormone analysis is presented in Table S3, and blood sample (serum vs. plasma), type of assay, and coefficients of variation for hormones and SHBG measured in each study are presented in Table S4. In all studies, concentrations of free estradiol and testosterone were calculated from those of total estradiol and testosterone, respectively, and of SHBG, assuming that the binding of these hormones to serum SHBG and albumin follows the law of mass action as below:[29,30]

$$[T] = [F](1+K^A\,[A] + \frac{K^S[S]}{1+K^S[F]}$$

where *[T]* is total hormone concentration, *[F]* is free hormone concentration, *[A]* is albumin concentration, *[S]* is SHBG concentration, and K^A and K^S are association constants for the binding of hormone to albumin and SHBG, respectively. The association constants were

assumed to be: $K^A = 6.0 \times 10^4$ and 3.6×10^4, respectively, and $K^S = 0.68 \times 10^9$ and 1.0×10^9, respectively for estradiol and testosterone. Because albumin concentration was not measured in EPIC and EHBCCG, it was assumed to be constant at 40 g/L.[31]

Statistical analysis: Analyses were undertaken separately for pre- and postmenopausal women in UK Biobank, EPIC, and EHBCCG. STATA 17 (StataCorp, College Station, Texas) was used for all analyses.

Hormone concentrations were logarithmically transformed. In premenopausal women, concentrations were standardized for phase of the menstrual cycle (early follicular, late follicular, mid-cycle, early luteal, mid-luteal, and late luteal) with residuals from the mean for each cycle phase. The cycle phase was determined using forward dating (UK Biobank[21]), or both forward and backward dating, with the latter used where possible (EPIC[32] and EHBCCG[19]).

Alcohol intake was categorized as 0 g/day (nondrinkers), 1 to 5 g/day, 6 to 10 g/day, 11 to 20 g/day, 20 to 30 g/day, and >30 g/day. Using the median intake in each category, multivariable linear regression analysis was performed for each study to estimate trends in hormone concentrations and 95% CIs per 10 g/day (approximately one standard drink/day). The models were adjusted for individual component studies (EPIC and EHBCCG), case-control status (EPIC and EHBCCG), age at blood collection (in 2-year categories for premenopausal women and 5-year categories for postmenopausal women), previous alcohol use among noncurrent drinkers (UK Biobank and EPIC), smoking (never, former, current), body mass index (BMI) (<22.5 kg/m^2, 22.5–24.9 kg/m^2, 25–27.4 kg/m^2, 27.5–29.9 kg/m^2, 30–34.9 kg/m^2, ≥35 kg/m^2), number of full-term pregnancies (0, 1, 2, 3, 4+), past use of hormone therapy (HRT or OCs; yes/no), age at menopause (in 3-year categories; postmenopausal women only), and menopause type (natural, surgical; postmenopausal women only). The study-specific results were then pooled using fixed-effect meta-analysis. Potential differences in the estimates by menopausal status were assessed using the chi-square test for heterogeneity.

In premenopausal women, subgroup analyses were undertaken for total estradiol, estrone, progesterone, and total testosterone by phase of the menstrual cycle (follicular, mid-cycle, and luteal). In both pre- and postmenopausal women, subgroup analyses were undertaken for total estradiol, estrone, and total testosterone by type of the assay used (direct immunoassay, extraction immunoassay, and mass spectrometry); the individual studies that contributed to each assay type are presented in Table S5. Sensitivity analyses were undertaken by restricting the sample to those who reported alcohol intake of <15 g/day, to those who reported intake of <30 g/day (i.e., excluding heavy drinkers), and to those whose blood samples were collected during an ovulatory cycle (progesterone concentrations measured in the mid-luteal phase ≥ 12.72 nmol/L [~400 ng/dL]).[33]

MR and colocalization analyses

Data on alcohol intake: A genetic instrument in the *ADH1B* (alcohol dehydrogenase 1B) gene (rs1229984) for self-reported alcohol intake (number of drinks per week) was extracted from a genome-wide association study (GWAS) meta-analysis undertaken by the GWAS and Sequencing Consortium of Alcohol and Nicotine Use (GSCAN).[34] This variant was used because of its highly biologically plausible association with alcohol intake.[35] The minor A allele of this variant increases the activity of *ADH1B* that oxidizes ethanol to acetaldehyde, resulting in unpleasant reactions and limiting further drinking.[36] Although this polymorphism is uncommon in people of White European ancestry with a frequency of <5% (90% in East Asians), it is nonetheless a strong genetic predictor of alcohol intake in this population.[36] Estimates were available per 1 SD (approximately 9 drinks/week) increment in alcohol intake and extracted from the GWAS meta-analysis, excluding the UK Biobank ($n = 226{,}223$) to avoid sample overlap between the GWAS for alcohol intake and that for hormone concentrations. The *ADH1B* variant explains 0.19% of the variance in alcohol intake.

Data on testosterone and SHBG: Summary statistics for the association of rs1229984 with SD increments in the concentrations of hormones and SHBG were obtained from a publicly available GWAS of all women, regardless of menopausal status, from the UK Biobank, extracted from the OpenGWAS platform[37] (data set used for total testosterone: ieu-b-4864 involving 199,569 women; free testosterone: ieu-b-4869 involving 180,386 women; and SHBG: ieu-b-4870 involving 214,989 women). Data on estradiol were available but were not used because of the potential limitations related to measurement of this hormone in the UK Biobank (see details in the Discussion); data on the other sex hormones were not available.

MR analyses: MR assesses the associations between exposure(s) and outcome(s) using genetic variants associated with the exposure of interest as instrumental variables. The *ADH1B* variant was used as the instrumental variable, and a Wald ratio was calculated using the "TwoSampleMR"[38] package in R. A one-sample MR was also performed in the UK Biobank separately for pre- and postmenopausal women, using the two-stage least squares method. To be able to present the MR results in a way that is directly comparable to the observational results, assuming that one standard drink contains 10 g of alcohol, the β estimates generated from the Wald ratio (per 1 SD increment in alcohol intake) were converted to the estimates per 10 g/day increment. The results were then multiplied by 0.341 (assuming that, for a normal distribution, 1 SD is 34.1% of the range) to convert the difference in hormone concentrations from units expressed as SD to percentages.

Colocalization analyses: Colocalization assesses the probability that two traits are affected by the same genetic variants at a given locus. Using the *ADH1B* variant, colocalization analyses were conducted to identify the presence of a shared causal locus between alcohol intake and concentrations of testosterone and SHBG, in which a conventionally significant association was observed in MR analyses. The "coloc" package[39] in R was used to estimate the posterior probability for two traits sharing the same causal variant (PP4) in a 150-kb linkage disequilibrium window centered on rs1229984, with PP4 > 0.70 corresponding to strong evidence of colocalization.[40] Priors chosen were: p1 = 10^{-3}, p2 = 10^{-4}, and p12 = 10^{-5}, or

approximately a 75% prior belief that a signal will only be observed in the GSCAN GWAS and <0.01% prior belief in favor of colocalization between the two traits at a given locus.[41]

RESULTS

Observational analyses

In total, 45,431 premenopausal (39,188 in UK Biobank, 2343 in EPIC, and 3900 in EHBCCG) and 173,476 postmenopausal (160,363 in UK Biobank, 4371 in EPIC, and 8742 in EHBCCG) women were included in this analysis. Table 1 presents characteristics of the study participants.

Estrogens: Alcohol intake was positively associated with concentrations of total and calculated free estradiol in postmenopausal women but not in premenopausal women ($p_{heterogeneity}$ = .04 for total estradiol and 0.0002 for calculated free estradiol). The concentrations were 2.2% (95% CI, 0.8–3.6) and 3.8% (2.2–5.5) higher, respectively, per 10 g/day increment in alcohol intake (approximately one drink/day) in postmenopausal women (Figure 1).

Alcohol intake was positively associated with estrone concentration in both pre- and postmenopausal women (Figure 1). The concentrations were 6.2% (3.4–9.0) and 4.2% (2.7–5.6) higher, respectively, in pre- and postmenopausal women per 10 g/day increment in alcohol intake.

There was no significant difference in the associations across the studies (UK Biobank, EPIC, and EHBCCG).

TABLE 1 Participant characteristics.

	Premenopausal women			Postmenopausal women		
	UK Biobank (n = 39,188)	EPIC (n = 2343)	EHBCCG excluding EPIC (n = 3900)	UK Biobank (n = 160,363)	EPIC (n = 4371)	EHBCCG excluding EPIC (n = 8742)
Age at recruitment (years), mean (SD)	44.7 (2.7)	42.8 (4.3)	43.3 (4.5)	60.5 (5.3)	60.0 (5.4)	62.5 (7.0)
Cases, %		40.5	30.6		42.6	35.2
Usual alcohol intake (grams/day), median (IQR)	6.9 (13.3)	2.9 (11.5)	2.0 (8.0)	5.7 (12.9)	2.8 (10.6)	1.0 (7.0)
Usual alcohol intake (grams/day), mean (SD)	10.8 (12.9)	7.9 (11.7)	6.5 (11.7)	9.4 (11.4)	7.6 (11.2)	5.6 (10.6)
Current smoker, %	11.1	23.6	14.4	8.0	17.6	9.8
Body mass index (kg/m^2), mean (SD)	26.3 (5.3)	24.7 (4.2)	24.7 (4.9)	27.3 (5.1)	26.5 (4.6)	26.7 (5.0)
Nulliparous, %	26.8	16.7	22.5	15.4	13.9	12.6
Past use of hormones, %	87.7	66.7	28.6	86.5	45.5	32.4
Age at menopause (years), mean (SD)				49.5 (5.7)	49.4 (4.6)	48.8 (5.2)
Natural menopause, %				84.0	96.7	68.1
Total estradiol (pmol/L), median (IQR)	346.1 (365.4)	269.6 (230.3)	414.8 (289.4)		73.47 (63.31)	33.04 (41.66)
Calculated free estradiol (pmol/L), median (IQR)	4.05 (4.20)	3.66 (3.18)	5.10 (3.46)		1.10 (1.12)	0.54 (0.64)
Estrone (pmol/L), median (IQR)		312.7 (236.2)	307.0 (166.4)		138.34 (79.44)	88.76 (72.65)
Progesterone (nmol/L), median (IQR)[a]		3.72 (5.48)	42.77 (38.10)			
Total testosterone (nmol/L), median (IQR)	1.12 (0.71)	1.28 (0.99)	0.90 (0.52)	0.85 (0.74)	1.15 (0.86)	0.80 (0.62)
Calculated free testosterone (pmol/L), median (IQR)	12.53 (10.32)	16.38 (16.59)	10.70 (7.85)	10.50 (10.78)	17.18 (16.32)	11.13 (9.87)
Androstenedione (nmol/L), median (IQR)		4.41 (3.24)	4.02 (2.49)		2.88 (2.27)	2.19 (1.70)
DHEAS (nmol/L), median (IQR)		3,314.9 (2365.9)	2794.0 (2206.7)		1917.8 (1705.9)	1951.0 (1990.0)
SHBG (nmol/L), median (IQR)	62.91 (38.02)	51.97 (36.68)	58.51 (38.00)	53.52 (33.59)	40.71 (31.90)	47.71 (31.70)

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; EHBCCG, Endogenous Hormones and Breast Cancer Collaborative Group; EPIC, European Prospective Investigation into Cancer and Nutrition; IQR, interquartile range; SHBG, Sex hormone binding globulin.

[a]Luteal phase progesterone measured in EHBCCG excluding EPIC.

Progesterone: Alcohol intake was not associated with progesterone concentration in premenopausal women (Figure 1). No data were available for postmenopausal women.

Androgens: Alcohol intake was positively associated with testosterone concentrations in both pre- and postmenopausal women (Figure 1). Per 10 g/day increment in alcohol intake, the concentrations of total testosterone were 4.3% (3.7%–4.9%) and 2.8% (2.4%–3.2%) higher, respectively, in pre- and postmenopausal women, and those of calculated free testosterone were 4.0% (3.4%–4.7%) and 4.5% (4.0%–4.9%) higher, respectively; the associations were weaker in UK Biobank in comparison to EPIC and EHBCCG (Table S6). The associations for total testosterone were larger in premenopausal than postmenopausal women ($p_{heterogeneity} < .0001$), but this difference was significant only in the UK Biobank (Table S6).

Similarly, alcohol intake was positively associated with concentrations of androstenedione and DHEAS in both pre- and postmenopausal women (Figure 1). Per 10 g/day increment in alcohol intake, the concentrations of androstenedione were 3.5% (1.4%–5.5%) and 3.7% (1.9%–5.5%) higher, and those of DHEAS were 6.0% (3.7%–8.3%) and 6.6% (4.4%–8.8%) higher, respectively, in pre- and postmenopausal women. There were no differences in the associations by menopausal status.

SHBG: Alcohol intake was positively associated with SHBG concentration in premenopausal women but inversely associated in postmenopausal women ($p_{heterogeneity} < .0001$; Figure 1); the concentration was 0.7% (0.3%–1.1%) higher in premenopausal women but was 2.4% (2.2%–2.6%) lower in postmenopausal women per 10 g/day increment in alcohol intake.

The positive association with SHBG in premenopausal women was observed in the UK Biobank but not in EPIC and EHBCCG (Table S6). The inverse association in postmenopausal women was weaker in UK Biobank compared with EPIC and EHBCCG.

Associations by phase of the menstrual cycle in premenopausal women: Alcohol intake was inversely associated with total estradiol (−1.3%; −2.5% to −0.1%) in the follicular phase but positively associated (1.7%; 0.7%–2.7%) in the luteal phase ($p_{heterogeneity\ by\ cycle\ phase} = .0008$; Figure 2). The associations for estrone, progesterone, and total testosterone did not differ by cycle phase.

Associations by assay type: There were no differences in the associations by assay type for total estradiol, estrone, and total testosterone (Figure 3).

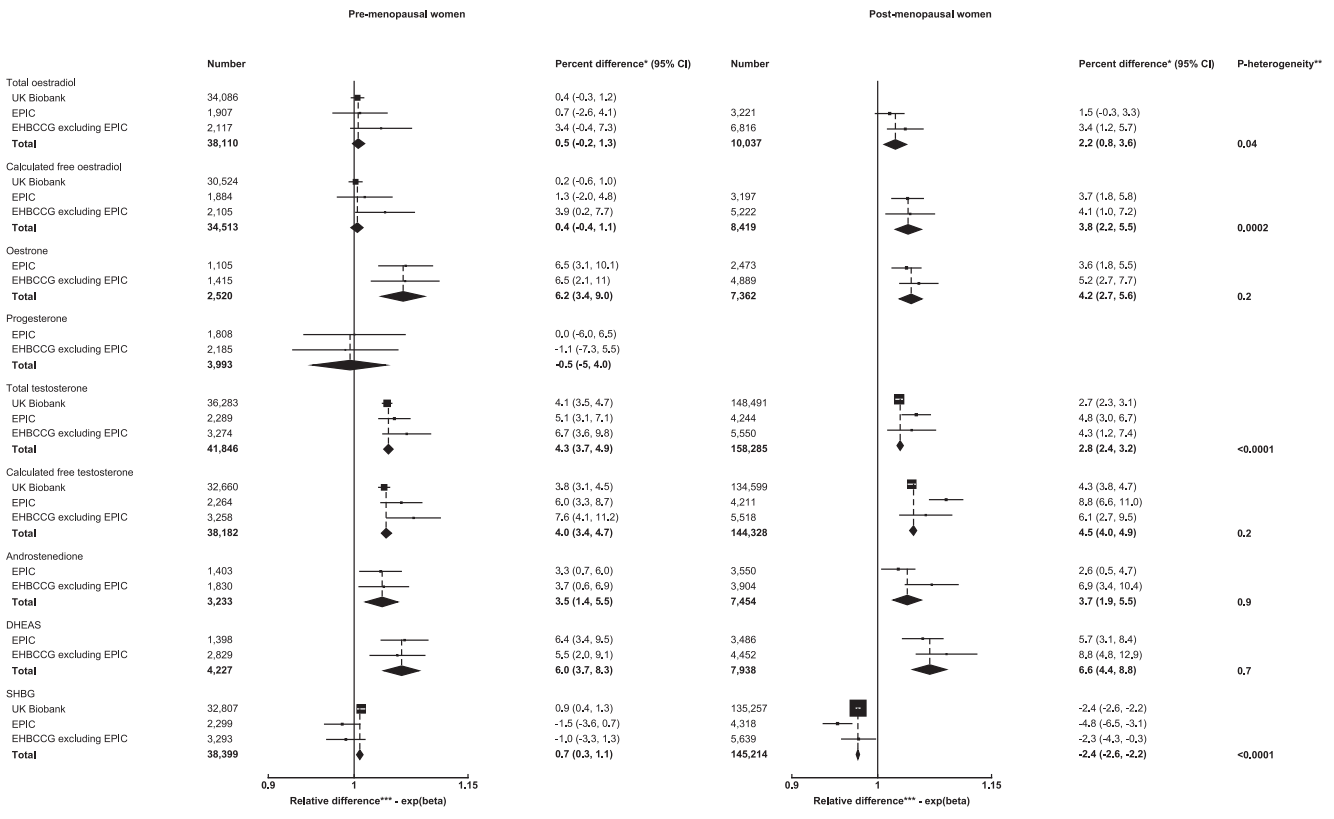

FIGURE 1 Associations of usual alcohol intake (per 10 g/day increment) with hormones and SHBG in pre- and postmenopausal women. Estimates adjusted for individual component studies (EPIC and EHBCCG), case-control status (EPIC and EHBCCG), age at blood collection, previous alcohol use among noncurrent drinkers (UK Biobank and EPIC), smoking, BMI, number of full-term pregnancies, past use of hormone therapy, age at menopause (postmenopausal women only), and menopausal type (postmenopausal women only). BMI indicates body mass index; DHEAS, dehydroepiandrosterone sulfate; EHBCCG, Endogenous Hormones and Breast Cancer Collaborative Group; EPIC, European Prospective Investigation into Cancer and Nutrition; SHBG, sex hormone binding globulin. *Percent difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake. **p value for heterogeneity by menopausal status. ***Relative difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake.

Sensitivity analyses: The associations did not differ substantially when restricted to those who reported usual alcohol intake of <15 g/day (Figure S1), to those who reported intake of <30 g/day (data not shown), or to samples collected during ovulatory cycles (data not shown).

MR and colocalization analyses

Effect estimates for the association of rs1229984 with alcohol intake and with concentrations of testosterone and SHBG are presented in Table S7.

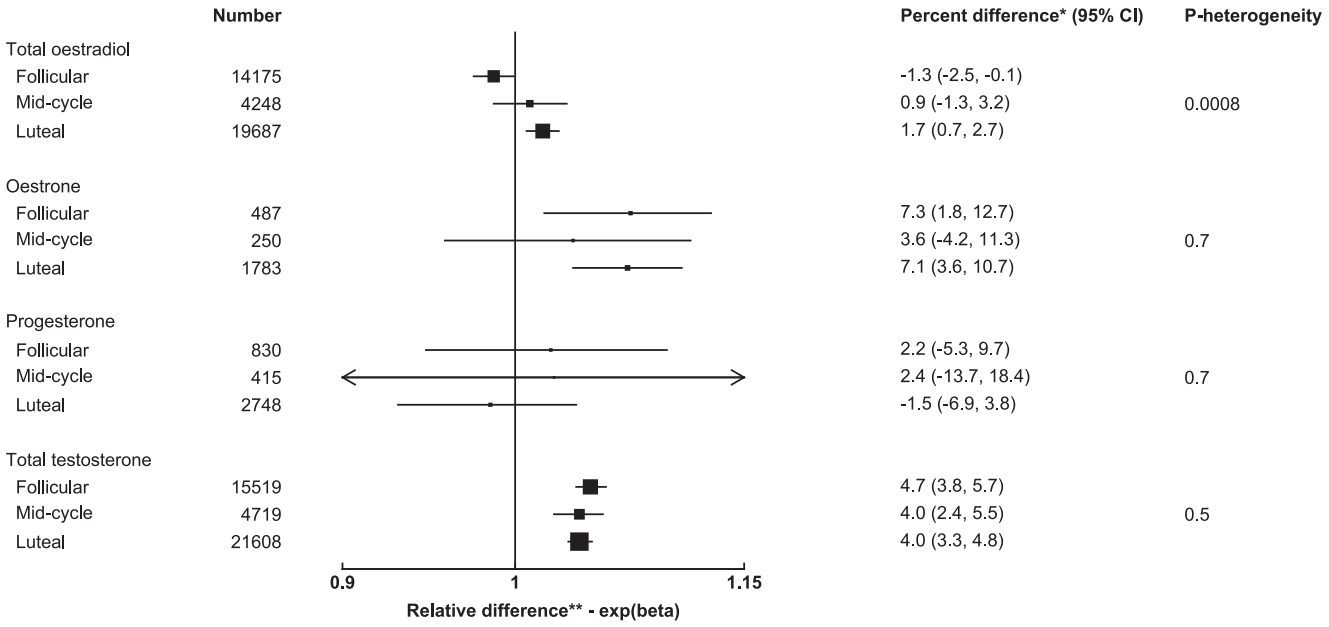

FIGURE 2 Associations of usual alcohol intake (per 10 g/day increment) with hormones and SHBG by phase of the menstrual cycle in pre-menopausal women. Estimates adjusted for individual component studies (EPIC and EHBCCG), case-control status (EPIC and EHBCCG), age at blood collection, previous alcohol use among noncurrent drinkers (UK Biobank and EPIC), smoking, BMI, number of full-term pregnancies and past use of hormone therapy. *Percent difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake. **Relative difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake. BMI indicates body mass index; DHEAS, dehydroepiandrosterone sulfate; EHBCCG, Endogenous Hormones and Breast Cancer Collaborative Group; EPIC, European Prospective Investigation into Cancer and Nutrition; SHBG, sex hormone binding globulin.

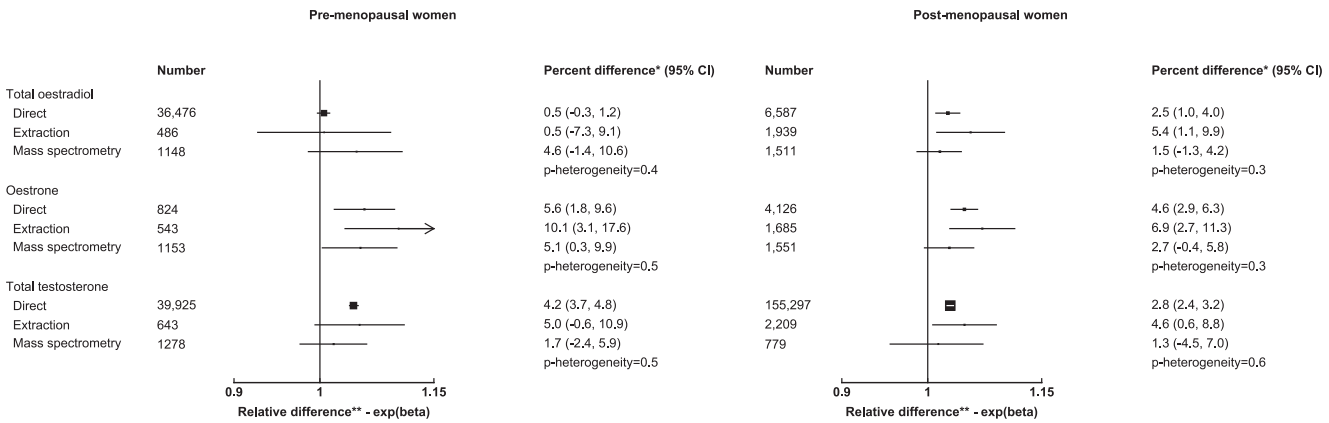

FIGURE 3 Associations of usual alcohol intake (per 10 g/day increment) with hormones and SHBG by assay type. Estimates adjusted for individual component studies (EPIC and EHBCCG), case-control status (EPIC and EHBCCG), age at blood collection, previous alcohol use among noncurrent drinkers (UK Biobank and EPIC), smoking, BMI, number of full-term pregnancies, past use of hormone therapy, age at menopause (postmenopausal women only) and menopausal type (postmenopausal women only). BMI indicates body mass index; DHEAS, dehydroepiandrosterone sulfate; EHBCCG, Endogenous Hormones and Breast Cancer Collaborative Group; EPIC, European Prospective Investigation into Cancer and Nutrition; SHBG, sex hormone binding globulin. *Percent difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake. **Relative difference in concentrations of hormones and SHBG per 10 g/day increment in usual alcohol intake.

In two sample MR analyses, a 10 g/day increment in genetically predicted alcohol intake was associated with higher concentrations of total testosterone (4.1%; 0.6%–7.6%) and free testosterone (7.8%; 4.1%–11.5%), and lower concentration of SHBG (−8.1%; −11.3% to −4.9%) (Table 2). Similar results were observed in one sample MR analyses with slightly larger associations in premenopausal women but the difference by menopausal status was not significant. Colocalization analyses showed strong evidence in favor of a shared causal locus between alcohol intake and free testosterone (PP4 = 0.81) and SHBG (PP4 = 0.97) at *ADH1B* (Table 3, Figure S2).

TABLE 2 Mendelian randomization estimates, instrumented by rs1229984, for usual alcohol intake (per 10 g/day increment) with hormones and SHBG in women.

	Percent difference (95% CI)	*p*
Two-sample MR		
Total testosterone	4.1 (0.6–7.6)	.02
Free testosterone	7.8 (4.1–11.5)	.00003
SHBG	−8.1 (−11.3 to −4.9)	.000001
One sample MR—premenopausal women[a]		
Total testosterone	8.8 (3.7–13.8)	.0006
Free testosterone	11.1 (5.5–16.8)	.0001
SHBG	−10.5 (−16.2 to −4.9)	.0003
One sample MR—postmenopausal women[a]		
Total testosterone	3.0 (−0.3 to 6.3)	.07
Free testosterone	4.8 (1.2–8.3)	.008
SHBG	−6.2 (−9.8 to −2.7)	.0006

Abbreviations: MR, Mendelian randomization; SHBG, sex hormone binding globulin.

[a]$p_{\text{heterogeneity by menopausal status}}$ = .6 for total and free testosterone and .2 for SHBG.

TABLE 3 Posterior probabilities from colocalization analyses for rs1229984.

	PP0	PP1	PP2	PP3	PP4
Total testosterone	1.68E-58	0.910	1.08E-60	0.006	0.084
Free testosterone	3.46E-59	0.188	7.34E-61	0.004	0.808
SHBG	5.57E-60	0.030	2.56E-61	0.001	0.968

Abbreviations: PP0, posterior probability for hypothesis 0 (H0): no association with either trait (alcohol intake or testosterone/SHBG concentration); PP1, posterior probability for H1: association with trait 1 (alcohol intake), not with trait 2 (testosterone/SHBG concentration); PP2, posterior probability for H2: association with trait 2 (testosterone/SHBG concentration), not with trait 1 (alcohol intake); PP3, posterior probability for H3: association with both traits (alcohol intake and testosterone/SHBG concentration), two distinct SNPs; PP4, posterior probability for H4: association with both traits (alcohol intake and testosterone/SHBG concentration), one shared SNP; SHBG, sex hormone binding globulin.

DISCUSSION

In this meta-analysis involving more than 45,000 premenopausal and 173,000 postmenopausal women, we found positive associations of alcohol intake with concentrations of sex hormones. We also found an inverse association with SHBG in postmenopausal women and some evidence of a small positive association in premenopausal women. The genetic analyses supported potential causal associations of alcohol intake with higher free testosterone and lower SHBG.

Estrogens

Alcohol may influence estrogen concentrations by altering its metabolism and clearance,[5] or by affecting aromatization of androgens to estrogens.[42] Earlier intervention studies reported an increase in concentrations of estradiol and/or estrone after alcohol intake in both pre-[5,15] and postmenopausal women,[16,17] although some found a positive association only in those on hormone therapy,[10,11] or no clear effect (possibly because of small sample sizes).[6,7,14,18]

Our observational analyses showed positive associations of alcohol with estrone in both pre- and postmenopausal women and with estradiol in postmenopausal women. Although the overall association with estradiol in premenopausal women was not significant, we found a weak inverse association in the follicular phase and a weak positive association in the luteal phase. In an earlier crossover trial, daily alcohol intake for three consecutive menstrual cycles significantly increased plasma concentrations of ovulatory estradiol but not follicular or luteal estradiol.[15]

The less conclusive findings observed for estradiol in premenopausal women may be related to the challenges in measuring this hormone reliably; measurement based on a single serum sample may not reflect its long-term average because the hormone level varies substantially across the menstrual cycle. We standardized estradiol concentrations for cycle phase in the observational analyses, but this may not be sufficient to account for all the variations.[43] Moreover, the studies included in the meta-analysis variably used forward or backward dating to define the cycle phase when blood was collected. The positive association of alcohol with estradiol in postmenopausal women was also of small magnitude, possibly because the estradiol concentration is low in this group and could be below or close to the lower limit of detection of some of the assays used, which is likely to have reduced statistical power; however, we found no differences in the association by assay type.

Progesterone

Alcohol might influence progesterone concentration by altering its metabolism in the liver,[6,7] but the results from previous intervention studies have been mixed.[6,7,15] We found no association in premenopausal women overall as well as across three cycle phases, although our ability to detect any association may have been limited because

of measurement errors associated with the large variations in the hormone level throughout the menstrual cycle.

Androgens

Alcohol may influence androgen concentrations by altering their secretion from the ovaries and/or adrenal glands, or their metabolism in the liver.[8] Previous intervention studies reported an acute elevation in concentrations of one or more androgens after alcohol intake in both pre-[9,12,13] and postmenopausal women,[17,18] although others found no clear effect in premenopausal women possibly because of small sample sizes.[6,7,14,15]

In this meta-analysis, we found positive associations of alcohol with testosterone, androstenedione, and DHEAS in both pre- and postmenopausal women. The association with testosterone seemed to be of greater magnitude in premenopausal women even after restricting to those with intake of <15 g/day, which might be due to biological differences or possibly from differences in the accuracy of self-reported alcohol intake by menopausal status; this difference, however, was observed in the UK Biobank only. The associations with androstenedione and DHEAS did not differ by menopausal status.

In the MR analyses, genetically predicted alcohol intake was positively associated with testosterone concentrations, with a larger effect on free testosterone compared with total testosterone. We observed strong colocalization for alcohol intake at the *ADH1B* locus with free but not total testosterone. This raises the question as to whether or not alcohol has a direct causal effect on testosterone concentration because the strong association with free testosterone could be related to the inverse association of alcohol intake with SHBG concentrations.

SHBG

Alcohol may influence SHBG concentrations by affecting hormonal balance,[44] cytokine levels,[45] hepatic synthesis/release, or blood clearance.[46,47] An earlier intervention study in premenopausal women showed a slight increase in SHBG concentration, particularly in the mid-luteal phase,[15] whereas another study of postmenopausal women found a decrease in concentration after 8 to 12 weeks of daily alcohol intake[16]; however, the results in both studies were not significant possibly because of small sample sizes.

Similarly in this meta-analysis, we found an inverse association of alcohol intake with SHBG in postmenopausal women and some evidence of a small positive association in premenopausal women; the latter was driven by the results from the UK Biobank with no association in the other data sets, therefore this observation should be interpreted cautiously. The MR and colocalization analyses at the *ADH1B* locus identified an inverse association regardless of menopausal status. Because SHBG binds testosterone to a greater degree than estradiol, any reduction in SHBG caused by alcohol would be expected to have a bigger effect in increasing the bioavailable fraction of testosterone than estradiol, as observed in our analyses.

Hormones and alcohol-induced breast carcinogenesis

Alcohol has been associated with an increased risk of several cancers, including female breast cancer.[48,49] In the Million Women Study, with more than 68,000 cases, there was a 12% increase in risk per 10 g/day increment in alcohol intake.[49] Our findings confirming the positive associations of alcohol intake with sex hormones, particularly their bioavailable fractions, support a probable role of sex hormones in alcohol-induced breast carcinogenesis. However, given that there is some evidence supporting the effects of alcohol on both estrogen receptor –positive and estrogen receptor–negative breast cancer,[50] it is possible that alcohol may also increase breast cancer risk through other intermediates such as acetaldehyde.[51–53]

Strengths and limitations

To our knowledge, this is the largest study to date on this topic. Our pooled analysis of individual participant data involved more than 45,000 premenopausal and 173,000 postmenopausal women, enabling us to reliably assess associations between alcohol and sex hormones while taking account of relevant potential confounders, and to undertake important subgroup analyses by menopausal status, cycle phase in premenopausal women, and assay type. We additionally conducted MR and colocalization analyses to support the observational results where possible.

Our main exposure, alcohol intake, was self-reported. Although self-reported measures of alcohol intake have been shown to have reasonable levels of reliability and validity,[54] underreporting is common particularly among those with very high intake,[55] which may lead to overestimation of the magnitude of associations of reported alcohol intake with circulating hormones. However, this may not be substantial because the questionnaires used in most studies included in this analysis were highly standardized and validated. Hormone concentrations were measured using direct immunoassays in some studies, which are typically less accurate and specific than immunoassays preceded by an extraction procedure, or mass spectrometry; we found no significant differences in the associations by assay type (as also observed in our previous pooled analysis of sex hormones and breast cancer risk in postmenopausal women[28]), but future studies should use the most accurate and specific assays possible. The potential limitations related to estradiol measurement have been discussed previously; we have therefore not undertaken genetic analyses for this hormone. We have also not undertaken genetic analyses for progesterone, DHEAS, and androstenedione as the genetic instruments for these hormones were not publicly available. Finally, whereas we have been able to analyze the available data from 14 studies, the generalizability of the results may be limited as the study

samples comprising mainly women of White European ancestry (e.g., approximately 95% in the UK Biobank).

CONCLUSIONS

Our meta-analysis confirmed positive associations of alcohol intake with sex hormones, including the more bioavailable fractions. There was also an inverse association with SHBG in postmenopausal women. Genetic analyses supported potential causal associations with higher free testosterone and lower SHBG. These associations are likely to contribute to the effect of alcohol on breast cancer risk.

AUTHOR CONTRIBUTIONS

Sandar Tin Tin: Formal analysis; writing - original draft; methodology; and funding acquisition. **Karl Smith-Byrne**: Methodology; writing - review & editing; and formal analysis. **Pietro Ferrari**: Conceptualization; writing - review & editing; methodology; and data curation. **Sabina Rinaldi**: Conceptualization; methodology; writing - review & editing; and data curation. **Marjorie L. McCullough**: Data curation and writing - review & editing. **Lauren R. Teras**: Data curation and writing - review & editing. **Jonas Manjer**: Data curation and writing - review & editing. **Graham Giles**: Data curation and writing - review & editing. **Loic Le Marchand**: Data curation and writing - review & editing. **Christopher A. Haiman**: Data curation and writing - review & editing. **Lynne R. Wilkens**: Data curation and writing - review & editing. **Yu Chen**: Data curation and writing - review & editing. **Sue Hankinson**: Data curation and writing - review & editing. **Shelley Tworoger**: Data curation and writing - review & editing. **A. Heather Eliassen**: Data curation and writing - review & editing. **Walter C. Willett**: Data curation and writing - review & editing. **Regina G. Ziegler**: Data curation and writing - review & editing. **Barbara J. Fuhrman**: Data curation and writing - review & editing. **Sabina Sieri**: Data curation and writing - review & editing. **Claudia Agnoli**: Data curation and writing - review & editing. **Jane Cauley**: Data curation and writing - review & editing. **Usha Menon**: Data curation and writing - review & editing. **Evangelia Ourania Fourkala**: Data curation and writing - review & editing. **Thomas E. Rohan**: Data curation and writing - review & editing. **Rudolf Kaaks**: Data curation and writing - review & editing. **Gillian K. Reeves**: Conceptualization; funding acquisition; writing - review & editing; methodology; and supervision. **Timothy J. Key**: Conceptualization; funding acquisition; writing - review & editing; methodology; supervision; and data curation.

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CONFLICT OF INTEREST STATEMENT

None.

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DATA AVAILABILITY STATEMENT

UK Biobank is an open access resource, and the study website https://www.ukbiobank.ac.uk/ has information on available data and access procedures. For EPIC, details of data access are at https://epic.iarc.fr/access/index.php. For EHBCCG, the principal investigators of each contributing study are responsible for access to the data. Full GWAS summary statistics from GSCAN are available at https://conservancy.umn.edu/handle/11299/201564. The instruments for concentrations of testosterone and SHBG were extracted from the OpenGWAS platform https://gwas.mrcieu.ac.uk/.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

UK Biobank is an open access resource and has been approved by the North West Multi-Centre Research Ethics Committee (approval number 16/NW/0274). This research was undertaken under project reference number 3248 (approved August 2013). EPIC has been approved by the Ethical Committee of the International Agency for Research on Cancer (IARC) and local ethical committees pertaining to EPIC Centres. Studies involved in EHBCCG have been approved

by relevant ethical committees. The current analyses did not need further ethics approval. Informed consent was obtained from all individual participants included in each study.

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SUPPORTING INFORMATION

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