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Alcohol and oestrogen metabolites in postmenopausal women in the Women's Health Initiative Observational Study

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Background: Alcohol consumption is associated with an increased risk of several cancers. Potential mechanisms include altered oestrogen metabolism. Parent oestrogens metabolise into alternate pathways of oestrogen metabolites that may have variable effects on cancer pathogenesis. We examined associations of alcohol consumption with circulating oestrogen/oestrogen metabolites in postmenopausal women in the Women's Health Initiative (WHI)-Observational Study (OS).

Methods: We conducted a cross-sectional analysis of prediagnosis ovarian/endometrial cancer case-control data within WHI-OS ($N = 1864$). Alcohol consumption was measured by validated food frequency questionnaire. Fasting serum parent oestrogens/oestrogen metabolites were assayed using liquid chromatography tandem mass-spectrometry. Geometric mean analyte concentrations (GM, pmol l^{-1}) were calculated by alcohol category using inverse-probability weighted linear regression, adjusting for venepuncture age/year, race, smoking, body mass index, years since menopause, oral contraceptive duration, caffeine intake, and physical activity.

Results: There was evidence for a positive association between alcohol consumption and oestrone, oestradiol and 2-hydroxylation oestrogen metabolite concentrations among menopausal hormone therapy (MHT) users. We observed an association between liquor consumption and parent oestrogens among non-MHT users, who consumed larger doses of liquor than MHT users.

Conclusions: Among postmenopausal women, the association between alcohol intake and parent oestrogen, but not oestrogen metabolite concentrations, may be influenced by MHT and type of alcohol.

Alcohol consumption is ubiquitous across the globe, particularly among developed countries. There is convincing evidence that alcohol increases risk for breast cancer with a dose-response starting at low intake levels (World Cancer Research Fund/ American Institute for Cancer Research, 2010; Schutze *et al*, 2011; Jung *et al*, 2015). Alcohol may affect breast cancer risk through several potential mechanisms including acetaldehyde promotion of tumour initiation, ethanol metabolism-induced oxidative stress and tumour promotion, altered metabolism and clearance of carcinogens, and impaired immunity, as well as increased circulating sex steroid hormones, in part through aromatisation of androgens to oestrogens (IARC, 2010; Key *et al*, 2011).

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Evidence from large, pooled analyses of prospective studies (Key *et al*, 2011) and several randomised-controlled crossover feeding studies (Dorgan *et al*, 2001; Mahabir *et al*, 2004; Sierksma *et al*, 2004) that used 95% ethanol as the alcohol exposure has shown that alcohol consumption is associated with higher circulating parent oestrogens (oestradiol, oestrone), androstenedione, dehydroepiandrosterone sulphate (DHEAS), testosterone, and lower sex hormone binding globulin (SHBG). Parent oestrogens can be irreversibly hydroxylated at steroid ring carbon positions 2, 4, or 16 to yield 2-, 4- or 16-hydroxylated oestrogen metabolites (Jefcoate *et al*, 2000). These pathways are interrelated, with metabolite concentrations within each pathway dependent on the overall oestrogen substrate pool. The mitogenic and genotoxic properties of oestrogen metabolites that affect cancer risk have been shown to differ by oestrogen metabolic pathway, partly influenced by degree of hydroxylation and/or methylation (Cavalieri *et al*, 2006; Yager, 2015; Cavalieri and Rogan, 2016; Dallal *et al*, 2016; Sampson *et al*, 2016). For example, in prospective studies utilising liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure oestrogen/oestrogen metabolites, relative increase in levels of 2-hydroxylation pathway metabolites to total has been associated with reduced breast cancer risk (Sampson *et al*, 2016). Oestrogen metabolism is consequently a highly complex hormonal exposure characterised by competing metabolic pathways. To understand the potential effects of alcohol on the hormonal mechanisms that drive cancer risk, a better understanding of downstream metabolic influences is needed.

Studies that explore the relationship between alcohol intake and oestrogen metabolism that include oestrogen pathway metabolites are limited (Hartman *et al*, 2016), and none, to our knowledge, have been conducted among postmenopausal women. Our objective was to examine associations of circulating serum oestrogen/oestrogen metabolite concentrations (in conjugated and unconjugated forms) with alcohol consumption, stratified by menopausal hormone therapy (MHT) use, in postmenopausal women participating in a nested case-control study within the Women's Health Initiative (WHI) Observational Study (OS). We hypothesised that alcohol drinkers would have higher parent oestrogens and oestrogen metabolites, and lower 2-pathway metabolites, favouring a pro-oestrogenic effect of alcohol with metabolism of parent oestrogen into 4- and 16-pathway compared with the 2-pathway metabolites.

MATERIALS AND METHODS

Study population. The WHI-OS is a prospective cohort that enrolled 93 676 postmenopausal women aged 50–79 years at 40 clinical centres across the United States between 1993 and 1998 (The Women's Health Initiative Study Group, 1998). We conducted a cross-sectional analysis of baseline data from a nested ovarian and endometrial cancer case-control study within the WHI-OS ($N=1864$ women, $N=510$ cases and 473 controls among never/former MHT users; $N=458$ cases and 423 controls among current MHT users) (Trabert *et al*, 2015; Brinton *et al*, 2016). Both dietary information and blood samples were prospectively collected prior to cancer development. Cases and controls were enrolled into the prospective observational study based on the same selection criteria; thus cases and controls were pooled for cross-sectional analysis. These data were obtained an average of 6.9 ± 3.7 years prior to endometrial cancer diagnosis and 6.9 ± 3.8 years prior to ovarian cancer diagnosis among the cases. Cases were women diagnosed with incident invasive ovarian or endometrial cancer between study enrolment and May 2012, and controls were frequency matched to cases on age at venepuncture (50–54, 55–59, 60–64, 65–69, 70–74, 75–79 years), year at venepuncture (1993–1996, 1997–1998), race/ethnicity (white, black, Hispanic, other/unknown), and time since last MHT use ($\leqslant 1$, >1 year). Controls were alive and cancer-free at the date of diagnosis of their matched case. Eligible women had no history of cancer, besides non-melanoma skin cancer, no history of bilateral oophorectomy or hysterectomy (endometrial controls only), and had adequate prediagnosis serum sample volume available (1.1 ml). Demographic, medical and family history, and lifestyle information was collected via a baseline questionnaire. The study was approved by the institutional review board at the Fred Hutchinson Cancer Research Centre (WHI Clinical Coordinating Centre) and all participating clinical centres. All participants provided written informed consent to participate.

Exposure assessment. Alcohol intake was measured by 122-item self-administered food frequency questionnaire (FFQ) at the same clinic visit as the fasting venepuncture. The FFQ was previously calibrated against both 24-h dietary recalls and 4-day food records (Patterson *et al*, 1999). The FFQ asked questions about frequency of intake (never/<1 per month to $\geqslant 6$ servings per day) of specific portion sizes of alcohol (12 oz or 1 can beer, 6 oz or 1 medium glass wine, and 1.5 oz or 1 shot liquor). A personal habits questionnaire also inquired about ever alcohol use (12 alcoholic beverages ever- no, yes, missing), and current alcohol use (no, yes, missing). Total alcohol intake (beer, wine and liquor) was calculated, converted into number of standard drinks/week and categorised (non-drinker *versus* current drinker; drinking categories: <1, $1-<3$, $3-<7$, $\geqslant 7$ drinks/week). One serving of beer, wine, or liquor per week contained the same amount of alcohol (approximately 15 g, on average). Alcohol type was also categorised to reflect overall consumption patterns: (1) non-drinker, drinker but not liquor, $>0-1$, >1 drink/week for liquor; (2) non-drinker, drinker but not beer, $>0-1$, >1 drink/week for beer; and (3) non-drinker, drinker but not wine, $>0-1$, $>1-3$, >3 drinks/week for wine).

Laboratory assay. Fifteen fasting serum oestrogens and oestrogen metabolites were quantified using stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) (Leidos Biomedical Research, Inc., Frederick, MD, USA) (25). Quantified metabolites included: unconjugated and combined (unconjugated + conjugated) oestrone and oestradiol (parent oestrogens), and 13 oestrogen metabolites (2-hydroxyoestrone, 2-hydroxyoestradiol, 2-hydroxyoestrone-3-methyl ether, 4-hydroxyoestrone, 4-methoxyoestrone, 4-methoxyoestradiol, 16α-hydroxyoestrone, 16-ketoestradiol, 16-epioestriol, 17-epioestriol, as well as unconjugated and combined oestriol, 2-methoxyoestrone, and 2-methoxyoestradiol). Conjugated concentrations of oestrone, oestradiol, oestriol, 2-methoxyoestrone, and 2-methoxyoestradiol were calculated by subtracting unconjugated from total combined concentration. Assay reliability was assessed using masked technical replicates analysed across batches. Coefficients of variation (CV) were $<6\%$ for all analytes; median (range) intraclass correlation coefficients (ICC) across all analytes was 0.98 (0.93–0.996) (Trabert *et al*, 2015; Brinton *et al*, 2016).

Statistical analysis

Primary analysis. Since the serum samples for the nested case-control study of ovarian and endometrial cancer were collected prior to diagnosis, we included both cases and controls ($N=1864$) in this cross-sectional analysis. Geometric means (GM, pmol l^{-1}) of oestrogen/oestrogen metabolite concentrations were calculated by (1) overall alcohol exposure category and (2) alcohol type category (liquor, beer, wine) using inverse probability weighted linear regression. Analyses were re-weighted by inverse probability sampling weights to account for case-control selection criteria and

to represent the WHI-OS cohort (Li and Gail, 2012). Sampling weights were the inverse of sampling fractions for cases (one) and controls (adjusted depending on strata and matching factors). Serum concentrations of oestrogen/oestrogen metabolites were evaluated individually, and in unconjugated and combined forms for select analytes. Analytes were log-transformed to account for non-normality. All analyses were multivariable and adjusted for *a priori*-defined potential confounders (Key *et al*, 2003; Setiawan *et al*, 2006; Chan *et al*, 2007; Kotsopoulos *et al*, 2009; Friedenreich *et al*, 2010; Brand *et al*, 2011; Sisti *et al*, 2015): venepuncture age (<60, 60–<70, ⩾70 years), venepuncture year (1993–1996, 1997–1998), race (white, non-white), smoking status (never, past, current), body mass index (BMI) (kg m^{-2}, continuous), years since menopause (<10 years, 10–<20 years, ⩾20), oral contraceptive use duration (0, <5, ⩾5 years), caffeine intake (quartiles), sedentary/sitting time (<5, 5–<10, ⩾10 h per day), and metabolic equivalent (MET) hours of physical activity/week (none, >0–<7.5, 7.5–15 (National recommendations), ⩾15). We conducted Wald tests for trend among alcohol drinkers using the median values of the alcohol categories (servings/week). We ran restricted cubic spline regression on alcohol associations with unconjugated and conjugated parent oestrogens, creating 5-knot splines at the 5th, 25th, 50th, 75th, and 95th percentiles of number of alcohol servings/week (Durrleman and Simon, 1989). The main analyses were stratified by never/former MHT users *versus* current users given that oestrogen/oestrogen metabolite concentrations vary by MHT use.

Sensitivity analyses. Stratification and Effect Modification: In sensitivity analyses, we excluded: (1) cancer cases, (2) women with diabetes, (3) oestrogen metabolite outliers, (4) alcohol intake outliers. We tested for effect modification with the inclusion of an alcohol-modifier interaction term in the main analyses. Outliers were identified using an extreme studentised deviate many-outlier procedure (Rosner, 1983).

Confounding: We evaluated the role of MHT formulation (unopposed oestrogen use, both oestrogen and progesterone use, or a combination of one of these formulations with past use of the other formulation) by further adjusting analyses of total alcohol intake and oestrogen/oestrogen metabolite concentrations for MHT formulation among current MHT users. MHT dosage did not vary substantially among MHT users. We also further adjusted select metabolite findings for parent oestrogens to assess whether their associations with alcohol were independent. We additionally controlled alcohol type for total alcohol consumption. We also checked for residual confounding by smoking by adjusting for cigarette pack years.

Metabolite ratio: Finally, we evaluated associations between overall alcohol intake and oestrogen/oestrogen metabolite ratios, including: ratio of parent to total oestrogens; ratio of unconjugated to combined oestrone, oestradiol, oestriol, 2-methoxyoestrone, and 2-methoxyoestradiol; ratio of 2-hydroxyoestrone to 4-hydroxyoestrone; ratio of 2-hydroxyoestrone to 16-alpha-hydroxyoestrone, and ratio of 4-hydroxyoestrone to 16-alpha-hydroxyoestrone. We calculated *P*-value for trends of increasing/decreasing metabolite ratios across alcohol drinking categories.

All *P*-values were two-sided and considered statistically significant if less than 0.05. For descriptive purposes, we also calculated the false discovery rate (FDR), and set a threshold of less than 0.05 as a second, more stringent, threshold of statistical significance (Benjamini–Hochberg procedure) (Benjamini and Hochberg, 1995; Ganna *et al*, 2014). All analyses were conducted with appropriate sampling weights using SAS Survey Procedures (PROC SURVEYREG, SAS version 9.3, SAS Institute, Cary, NC, USA).

RESULTS

Study population. In this cross-sectional analysis among 1864 women participating in the WHI-OS, never/former MHT users were aged 64 ± 7 years and current MHT users were aged 63 ± 7 years at their baseline visit, on average (Table 1). Most women were Caucasian, non-smokers that met national physical activity guidelines (⩾7.5 MET hours per week), had an average BMI in the overweight range (25–<30 kg m^{-2}), and had been in menopause for >10 years. Thirty-nine percent of never/former MHT users and 55% of current MHT users had a history of oral contraceptive use.

Alcohol intake. The median (interdecile range) servings/week of alcohol was 0.4 (0, 8.3) among never/former MHT users and 0.7 (0, 8.7) among current MHT users. Among drinkers, median (interdecile range) servings/week alcohol was 2.6 (0.2, 16.0) among never/former MHT users and 2.7 (0.4, 10.9) among current MHT users. Twenty-nine percent of never/former MHT users reported not currently being alcohol drinkers ($N = 280$), compared with 22% of current MHT users ($N = 190$). Median (interdecile range) standard drinks/week of alcohol types were: (1) beer 0.42 (0.21, 7.00), liquor 1.00 (0.21, 7.88), wine 1.00 (0.21, 7.00) among never/former MHT users; (2) beer 0.42 (0.21, 2.72), liquor 0.49 (0.21, 7.00), wine 1.36 (0.21, 7.88) among current MHT users.

Among never/former MHT users, the highest category of drinkers had higher circulating oestrogens and 2-, 4-, and 16-pathway metabolite concentrations compared with no current alcohol intake, although associations were not statistically significant (Table 2). Among MHT users who were drinkers, increasing alcohol intake (GM for 7+ compared with <1 drink per week) was associated with higher concentrations of unconjugated oestrone (26% increase, p-trend = 0.01), unconjugated and conjugated oestradiol (26 and 29% increases, respectively, p-trend = 0.04), unconjugated 2-methoxyoestrone (37% increase, p-trend = 0.01), and unconjugated 2-methoxyoestradiol (17% increase, p-trend = 0.04) (Table 3). However, these trends did not meet FDR < 0.05. The trends for increasing unconjugated oestrone ($P = 0.02$) and oestradiol ($P = 0.03$) with increasing servings of alcohol/week among MHT users were linear based on cubic spline regression analysis (results not shown). Results were not materially changed with finer adjustment for cigarette pack years (analyte concentrations changed 1–12%), although nominal statistical significance was retained only for increasing trends of unconjugated oestrone, 2-methoxyestrone, and 2-methoxyestradiol among current MHT users.

Among never/former MHT users, liquor consumption was associated with 19–32% increases in GM of parent oestrogen and oestrogen metabolite concentration for highest level compared with no intake of that alcohol type (p-trend across current liquor drinkers = 0.001 to 0.04), including: combined and conjugated oestrone, unconjugated oestradiol, 2-hydroxyoestrone, and 2-hydroxyoestradiol, combined and conjugated 2-methoxyoestrone, unconjugated 2-methoxyoestradiol, 3-methyl ether-hydroxyoestrone, 4-hydroxyoestrone, 4-methoxyoestrone, 16-alpha-hydroxyoestrone, all three measures of oestriol, 16-ketoestradiol, 16-epioestriol, and 17-epioestriol. FDR was <0.05 for all associations (Table 4; see Supplementary Table 1 for current MHT users). These associations were not retained after further adjustment for parent oestrogens. Consumption of beer and wine were not associated with oestrogen/oestrogen metabolites. Findings were not materially altered with further adjustment for total alcohol intake (data not shown).

Since we observed alcohol associations primarily with the unconjugated component of oestrogen/oestrogen metabolites, we compared the proportion of unconjugated to combined

Table 1. Characteristics of study participants in the Women's Health Initiative (WHI) Observational Study (OS) by alcohol category, stratified by menopausal hormone therapy (MHT) use

	Never/former MHT users ($N=983$)[a]									Current MHT users ($N=881$)								
	Total			Non-drinker			Current drinker			Total			Non-drinker			Current drinker		
Characteristic	*N*	Wt *N*	(%)[b]	*N*	Wt *N*	(%)[b]	*N*	Wt *N*	(%)[b]	*N*	Wt *N*	(%)[b]	*N*	Wt *N*	(%)[b]	*N*	Wt *N*	(%)[b]
Age at blood draw (years)																		
<60	259	8029	(26)	106	3000	(10)	153	5029	(16)	297	10792	(43)	84	3471	(14)	213	7321	(29)
60–69	433	13635	(44)	170	5079	(16)	263	8556	(28)	397	10689	(42)	129	3765	(15)	268	6924	(27)
70–79	961	31002	(30)	114	3802	(12)	155	5536	(18)	187	3840	(15)	65	1607	(6)	122	2233	(9)
Year of blood draw																		
1993–1996	588	18988	(61)	236	7148	(23)	352	11841	(38)	546	15655	(62)	164	5473	(22)	382	10182	(40)
1997–1998	373	12014	(39)	154	4733	(15)	219	7280	(23)	335	9666	(38)	114	3370	(13)	221	6296	(25)
Race																		
White	844	27738	(89)	310	9684	(31)	534	18054	(58)	827	23616	(93)	244	7736	(31)	583	15880	(63)
Non-white	117	3264	(11)	80	2197	(7)	37	1067	(3)	54	1705	(7)	34	1108	(4)	20	597.5	(2)
Smoking status																		
Never	489	15517	(51)	232	7215	(24)	257	8302	(27)	429	11624	(46)	162	5104	(20)	267	6520	(26)
Former	397	12531	(41)	128	3497	(11)	269	9034	(29)	412	12271	(49)	103	3153	(12)	309	9118	(36)
Current	67	2630	(9)	25	1048	(3)	42	1582	(5)	35	1348	(5)	10	510	(2)	25	838	(3)
Body mass index (kg m^{-2})																		
<25	349	13671	(44)	112	4214	(14)	237	9457	(31)	447	11554	(46)	116	3317	(13)	331	8236	(33)
25–<30	295	9352	(30)	125	4180	(14)	170	5172	(17)	258	7903	(31)	85	2788	(11)	173	5115	(20)
30+	315	7894	(26)	151	3401	(11)	164	4493	(15)	175	5810	(23)	77	2738	(11)	98	3071	(12)
Years since menopause																		
<10	299	9267	(31)	105	3144	(11)	194	6123	(21)	351	11154	(44)	95	3204	(13)	256	7950	(31)
10–<20	361	11390	(39)	146	3953	(13)	215	7437	(25)	331	8838	(35)	111	3472	(14)	220	5367	(21)
20+	252	8888	(30)	114	3924	(13)	138	4964	(17)	199	5328	(21)	72	2167	(9)	127	3161	(12)
Duration of oral contraceptive use (years)																		
Never	618	18930	(61)	255	7722	(25)	363	11208	(36)	453	11445	(45)	155	4158	(16)	298	7287	(29)
<5	182	6476	(21)	79	2367	(8)	103	4109	(13)	212	6085	(24)	59	1833	(7)	153	4252	(17)
5+	160	5518	(18)	55	1715	(6)	105	3804	(12)	216	7791	(31)	64	2853	(11)	152	4938	(20)
Caffeine intake quartile (median mg per day)																		
1	235	7360	(24)	122	3638	(12)	113	3722	(12)	222	6354	(25)	96	3318	(13)	126	3036	(12)
2	232	7598	(25)	91	2798	(9)	141	4800	(15)	229	5850	(23)	69	2061	(8)	160	3789	(15)
3	224	7613	(25)	79	2838	(9)	145	4775	(15)	238	6992	(28)	48	1475	(6)	190	5517	(22)
4	270	8431	(27)	98	2607	(8)	172	5824	(19)	191	6069	(24)	65	1990	(8)	126	4079	(16)
Sedentary/sitting time (hours per day)																		
<5	332	11519	(37)	122	4258	(14)	210	7261	(24)	306	8285	(33)	98	3117	(12)	208	5169	(21)
5 to 9	398	12407	(40)	164	4664	(15)	234	7743	(25)	351	9994	(40)	107	3020	(12)	244	6974	(28)
10+	224	6809	(22)	102	2957	(10)	122	3853	(13)	220	6798	(27)	72	2662	(11)	148	4136	(16)
MET hours per week																		
None	206	6139	(20)	99	2814	(9)	107	3325	(11)	145	4642	(18)	67	2188	(9)	78	2454	(10)
>0–<7.5	216	6724	(22)	96	2724	(9)	120	4000	(13)	216	7180	(28)	69	2319	(9)	147	4861	(19)
7.5–15	224	7586	(25)	89	3111	(10)	135	4475	(15)	196	4671	(18)	73	2174	(9)	123	2497	(10)
⩾15	303	10407	(34)	101	3171	(10)	202	7236	(23)	319	8771	(35)	68	2109	(8)	251	6661	(26)

Abbreviations: kg = kilograms; MET = metabolic equivalent; MHT = menopausal hormone therapy; Wt = weighted.
[a]Frequencies for never drinkers and past drinkers were similar.
[b]Percentages reflect weighted counts and refer to the study cohort.

concentration for these hormones across drinking categories (Supplementary Table 2). Trends for decreasing ratio of unconjugated to combined oestrone (18% *versus* 20% for 7+ compared with <1 drink per week) and 2-methoxyoestradiol (16% *versus* 18% for 7+ compared with <1 drink per week) were evident among never/former MHT users, while a trend for increasing unconjugated to combined 2-methoxyoestrone (40 *vs* 33% for 7+ compared with <1 drink per week) was evident for increasing categories of alcohol intake among current MHT users. For liquor, highest category compared with no liquor consumption was inversely associated with ratio of unconjugated to combined oestrone (17 *vs* 21% for >1 compared with no liquor intake). These trends did not meet FDR<0.05.

We further excluded cases ($N=510$ never/former MHT users; $N=458$ current MHT users; Supplementary Table 3) and women with diabetes ($N=42$ never/former MHT users; $N=28$ current MHT users; results not shown) from the overall alcohol and liquor analyses. There were no differences in the association between overall alcohol consumption and oestrogen/oestrogen metabolites between cases and controls, or between women with and without diabetes. There was no evidence for alcohol-case status effect modification (all *P*-values for interaction >0.05). Similarly, excluding cases and women with diabetes did not alter the patterns of association for liquor consumption. When we removed oestrogen metabolite outliers or alcohol outliers (results not shown), unconjugated oestrone and 2-methoxyoestrone remained nominally associated with overall alcohol consumption among MHT users. When we additionally adjusted for MHT formulation (Supplementary Table 4), we observed similar trends among MHT users for the main effects of alcohol consumption on oestrogen and 2-hydroxylation pathway catechol oestrogen metabolites.

Table 2. Geometric means (pmol l^{-1}) and 95% confidence intervals (CI) of serum oestrogens and oestrogen metabolites by total alcohol consumption category among postmenopausal women not using menopausal hormone therapy in the Women's Health Initiative (WHI) Observational Study (OS)

Alcoholic drinks per week	Non-drinker	Current drinker		<1	1 to <3	3 to <7	7+		
Median	0	2.6		0.4	1.8	4	11.5		
N	398	565		198	138	101	148		
Weighted *N*[a]	11 881	19 121		6560	4195	3322	5044		
	Geometric means (95% CI)[b]		*P*-diff[c]	Geometric means (95% CI)[b]				%Δ[d]	*p*-trend[e]
Oestrone	313.8 (266.1, 370.0)	332.6 (278.3, 397.6)	0.50	314.3 (255.2, 387.1)	290.7 (223.2, 378.6)	403.3 (301.2, 540.0)	340.4 (268.7, 431.3)	8%	0.66
Conjugated	245.2 (204.4, 294.1)	266.8 (218.9, 325.3)	0.37	249.2 (197.6, 314.3)	229.8 (171.1, 308.6)	320.5 (231.6, 443.7)	279.3 (216.0, 361.1)	12%	0.50
Unconjugated	60.6 (53.7, 68.5)	58.9 (51.8, 66.9)	0.66	58.9 (50.5, 68.6)	52.9 (44.3, 63.1)	73.9 (60.0, 90.9)	55.5 (46.6, 66.3)	−6%	0.51
Oestradiol	60.0 (50.3, 71.6)	57.7 (47.6, 70.0)	0.67	53.8 (43.1, 67.3)	53.6 (42.4, 67.9)	72.0 (52.2, 99.3)	57.3 (44.6, 73.7)	6%	0.82
Conjugated	38.6 (32.0, 46.7)	39.7 (32.1, 49.1)	0.77	36.5 (28.4, 46.9)	39.5 (30.5, 51.1)	47.6 (33.9, 66.8)	39.3 (29.7, 52.2)	8%	0.88
Unconjugated	15.9 (13.1, 19.3)	13.5 (11.2, 16.1)	0.09	13.2 (10.6, 16.4)	10.7 (8.4, 13.7)	17.6 (12.8, 24.3)	13.4 (10.8, 16.7)	2%	0.67
2-Hydroxyoestrone	68.7 (59.4, 79.5)	68.1 (58.2, 79.8)	0.91	65.8 (54.6, 79.2)	59 (47.3, 73.7)	80.4 (61.2, 105.6)	69.6 (57.7, 84.1)	6%	0.52
2-Hydroxyoestradiol	16.9 (14.6, 19.5)	16.9 (14.6, 19.7)	0.97	16.5 (13.8, 19.9)	14.6 (11.7, 18.1)	19.7 (15.2, 25.5)	17.3 (14.4, 20.9)	5%	0.52
2-Methoxyoestrone	43.5 (38.6, 48.9)	43.3 (38.2, 49.1)	0.95	42.1 (35.9, 49.4)	38.2 (31.9, 45.8)	49.0 (39.2, 61.3)	44.4 (37.6, 52.6)	6%	0.38
Conjugated	31.9 (28.0, 36.3)	31.9 (27.9, 36.5)	0.98	30.8 (25.8, 36.8)	27.8 (22.9, 33.7)	35.0 (27.6, 44.5)	33.7 (28.2, 40.2)	9%	0.23
Unconjugated	10.3 (9.0, 11.9)	10.0 (8.6, 11.6)	0.65	9.9 (8.2, 12.1)	9.4 (7.5, 11.7)	12.1 (9.3, 15.7)	9.5 (7.7, 11.6)	−5%	0.63
2-Methoxyoestradiol	13.9 (12.0, 16.2)	14.1 (11.9, 16.8)	0.81	13.8 (11.5, 16.6)	12.1 (9.7, 15.2)	17 (12.7, 22.8)	14.3 (11.5, 17.7)	4%	0.56
Conjugated	11.2 (9.5, 13.3)	11.5 (9.5, 13.8)	0.75	11.1 (9.1, 13.6)	9.3 (7.2, 12.2)	14.0 (10.3, 19.2)	11.9 (9.5, 14.9)	7%	0.32
Unconjugated	2.2 (1.9, 2.5)	2.1 (1.8, 2.4)	0.56	2.1 (1.7, 2.5)	2.0 (1.7, 2.5)	2.3 (1.8, 3.0)	2.0 (1.7, 2.4)	−2%	0.48
3-Methyl ether-hydroxyoestrone	7.7 (6.8, 8.7)	7.8 (6.8, 9)	0.76	7.7 (6.5, 9.0)	7.0 (5.8, 8.4)	8.2 (6.4, 10.4)	8.2 (6.9, 9.8)	7%	0.51
4-Hydroxyoestrone	8.4 (7.3, 9.7)	8.5 (7.3, 10.0)	0.85	8.3 (6.9, 10.0)	7.4 (5.9, 9.2)	9.9 (7.5, 13.1)	8.6 (7.1, 10.4)	4%	0.61
4-Methoxyoestrone	4.6 (4.1, 5.2)	4.5 (3.9, 5.1)	0.71	4.6 (3.9, 5.4)	3.9 (3.2, 4.6)	4.9 (3.8, 6.2)	4.6 (3.9, 5.4)	−1%	0.57
4-Methoxyoestradiol	2.0 (1.7, 2.3)	2.0 (1.7, 2.4)	0.65	2.0 (1.7, 2.5)	1.8 (1.5, 2.3)	2.4 (1.8, 3.2)	2.0 (1.6, 2.4)	−2%	0.95
16-Alphahydroxyoestrone	34.3 (29.5, 40.0)	34.0 (28.7, 40.2)	0.90	32.6 (26.8, 39.6)	29.5 (23.3, 37.4)	40.2 (29.7, 54.4)	34.9 (28.7, 42.5)	7%	0.51
Oestriol	147.1 (127.1, 170.2)	144.5 (122.5, 170.5)	0.82	142.0 (116.6, 172.9)	126.7 (99.4, 161.6)	171.0 (127.2, 229.9)	144.3 (118.5, 175.7)	2%	0.88
Conjugated	115.6 (97.8, 136.7)	114.3 (95, 137.6)	0.90	111.9 (89.5, 139.8)	101.4 (77.6, 132.6)	137.3 (99.8, 188.9)	113.1 (90.4, 141.5)	1%	0.99
Unconjugated	27.8 (24.7, 31.3)	26.0 (22.8, 29.8)	0.38	25.7 (21.8, 30.3)	21.8 (17.6, 27)	29.5 (22.7, 38.4)	27.1 (23, 31.8)	5%	0.25
16-Ketooestradiol	36.9 (31.6, 43.1)	37.3 (31.5, 44.3)	0.88	36.3 (29.7, 44.5)	32.2 (25.1, 41.3)	43.0 (31.7, 58.5)	38.4 (31.3, 47.1)	6%	0.64
16-Epioestriol	15.8 (13.7, 18.1)	15.5 (13.3, 18.1)	0.80	14.2 (11.9, 17.1)	13.8 (11.1, 17.1)	18.5 (14.3, 24.1)	16.2 (13.5, 19.5)	14%	0.26
17-Epioestriol	12.4 (10.9, 14.2)	12.7 (11, 14.6)	0.76	11.9 (10.0, 14.2)	11.1 (9.1, 13.6)	14.6 (11.6, 18.4)	13.4 (11.1, 16.1)	12%	0.28

[a]Weighted *N*: frequency counts weighted to whole WHI-OS cohort.
[b]Adjusted for age at blood draw (<60, 60–<70, ⩾70 years), year of blood draw (1993–1996, 1997–1998), race (white, non-white), smoking status (never smoked, past smoker, current smoker), BMI (kg m^{-2}), years since menopause (<10 years, 10–<20 years, ⩾20 years), duration of oral contraceptive use (0, <5, ⩾5 years), caffeine intake (quartiles), sedentary/sitting time (<5, 5–<10, ⩾10 h per day), and metabolic equivalent (MET) hours of physical activity per week (none, >0–<7.5, 7.5–15 (National recommendations), ⩾15).
[c]*P*-diff refers to the *P*-value comparing the geometric mean concentration of parent oestrogens and oestrogen metabolites for current compared with non-alcohol drinkers. Calculated using the Wald test.
[d]%Δ is the percentage difference in oestrogen concentrations comparing 7+ and <1 standard alcoholic drink/week.
[e]*P*-trend is the *P*-value for trend in association between alcohol consumption and parent oestrogens and oestrogen metabolite concentration among current drinkers. Calculated using Wald test for a continuous drinking variable based on the median number of standard servings of alcohol consumed per week according to the categories presented in the table.

DISCUSSION

In this population of 1864 postmenopausal women from a nested case-control study within the WHI-OS cohort, we observed nominal associations for increasing concentrations of parent oestrogens with increasing alcohol intake among both never/former and current MHT users, consistent with prior studies. We also observed trends of increased 2-hydroxylation pathway oestrogen metabolites with increasing alcohol intake, although trends did not meet the FDR threshold. Liquor was associated with increased concentrations of circulating parent oestrogens and 2-, 4-, and 16-pathway oestrogen metabolites among never/former MHT users across frequency of use categories; oestrogen metabolite associations with liquor intake did not remain with further adjustment for parent oestrogens.

Prior randomised dietary interventions and prospective studies have supported that alcohol intake is associated with postmenopausal hormone levels. The Women's Alcohol study, a placebo controlled 8-week crossover feeding study among 51 healthy postmenopausal women not using MHT, showed that 15–30 g per day alcohol intake increased serum oestrone sulphate by 7.5–10.7% and DHEAS by 5.1–7.5% (Dorgan *et al*, 2001). In a pooled meta-analysis of 13 prospective studies ($N = 6291$) from across the globe, sex hormone concentrations were ~10–25% higher among postmenopausal women consuming ⩾20 g alcohol per day (~20% women) compared with non-drinkers, including oestradiol, oestrone, androstenedione, DHEAS, and testosterone; SHBG concentrations were reduced by 10% (Key *et al*, 2011). Fifteen to 30 g alcohol per day approximates one to two standard drinks/day, in contrast to median intakes of ~0.4 standard drinks/day among drinkers in our population. The effect sizes we observed in our analysis for parent oestrogens were consistent with these prior

Table 3. Geometric means (pmol l^{-1}) and 95% confidence intervals (CI) of serum oestrogens and oestrogen metabolites by total alcohol consumption category among postmenopausal women currently using menopausal hormone therapy in the Women's Health Initiative (WHI) Observational Study (OS)

Alcoholic drinks per week	Nondrinker	Current drinker		<1	1 to <3	3 to <7	7+		
Median	0	2.7		0.4	1.9	4.5	10.5		
N	278	603		199	136	125	142		
Weighted *N*[a]	8843	16 478		5310	3639	3694	3779		
	Geometric means (95% CI)[b]		*P*-diff[c]	Geometric means (95% CI)[b]				%Δ[d]	*p*-trend[e]
Oestrone	2835.3 (2147.4, 3743.5)	3468.8 (2606.5, 4616.4)	0.10	3411.3 (2476.0, 4700.0)	3171.6 (2051.0, 4904.4)	3386.1 (2320.6, 4940.9)	4013.1 (2808.6, 5734.1)	18%	0.10
Conjugated	2601.9 (1955.2, 3462.6)	3167.2 (2361.9, 4247.1)	0.12	3196.5 (2301.7, 4439.3)	2806.4 (1785.0, 4412.4)	3091.4 (2094.2, 4563.5)	3614.9 (2499.4, 5228.2)	13%	0.15
Unconjugated	205.5 (167.9, 251.5)	221.3 (178.6, 274.2)	0.45	218.8 (171.0, 280.0)	174.4 (127.0, 239.6)	231.6 (170.2, 315.3)	276.2 (209.9, 363.3)	26%	0.01[f]
Oestradiol	403.7 (306.3, 532.2)	438.5 (333.7, 576.2)	0.48	393.9 (286.2, 542.3)	435.5 (289.3, 655.7)	445.8 (312.0, 637.0)	525.4 (376.5, 733.2)	33%	0.02[f]
Conjugated	342.8 (251.1, 468.1)	360.4 (267.5, 485.4)	0.70	338.1 (239.8, 476.8)	344.7 (217.4, 546.3)	350.0 (237.7, 515.4)	436.8 (302.8, 630.0)	29%	0.04[f]
Unconjugated	40.4 (32.0, 51.0)	45.8 (36.2, 57.8)	0.21	41.7 (31.3, 55.7)	41.2 (30.1, 56.5)	52.6 (36.8, 75.1)	52.6 (38.8, 71.3)	26%	0.04[f]
2-Hydroxyoestrone	408.2 (329.3, 506.1)	441.9 (356.4, 547.9)	0.42	447.5 (345.6, 579.4)	409.0 (299.1, 559.3)	437.9 (321.8, 595.8)	472.2 (358.2, 622.4)	6%	0.25
2-Hydroxyoestradiol	100.0 (82.1, 121.8)	101.3 (82.5, 124.4)	0.89	105.6 (82.4, 135.4)	89.1 (66.2, 119.9)	99.7 (73.9, 134.7)	109.1 (83.5, 142.4)	3%	0.25
2-Methoxyoestrone	250.8 (209.8, 299.9)	248.4 (206.2, 299.1)	0.92	248.6 (195.3, 316.5)	202.1 (152.8, 267.4)	270.2 (208.2, 350.6)	281.7 (218.6, 363.1)	13%	0.05
Conjugated	155.4 (124.4, 194.0)	149.3 (120.3, 185.2)	0.69	157.7 (121.6, 204.6)	126.1 (92.3, 172.1)	153.3 (115.2, 204.1)	156.5 (116.7, 209.9)	−1%	0.46
Unconjugated	68.9 (53.1, 89.4)	72.0 (55.8, 92.9)	0.73	67.4 (48.7, 93.3)	54.6 (38.4, 77.5)	84.4 (58.6, 121.6)	92.4 (65.8, 129.6)	37%	0.01[f]
2-Methoxyoestradiol	81.5 (63.2, 105.2)	80.3 (63.1, 102.2)	0.88	77.6 (58.8, 102.4)	69.5 (49.4, 97.9)	84.0 (61.6, 114.6)	94.6 (70.6, 126.8)	22%	0.02[f]
Conjugated	70.8 (53.7, 93.3)	66.4 (51.1, 86.1)	0.54	66.8 (49.6, 90.1)	56.0 (38.5, 81.5)	67.2 (48.2, 93.6)	77.2 (56.2, 106.1)	16%	0.06
Unconjugated	8.0 (6.6, 9.7)	8.9 (7.5, 10.7)	0.23	8.7 (6.9, 11.1)	7.7 (5.9, 10.2)	9.5 (7.1, 12.7)	10.2 (8.0, 13.0)	17%	0.04[f]
3-Methyl ether-hydroxyoestrone	39.4 (32.1, 48.4)	39.4 (32.1, 48.3)	0.99	40.8 (32.1, 51.9)	30.5 (23.0, 40.6)	43.5 (33.4, 56.7)	43.7 (33.7, 56.6)	7%	0.09
4-Hydroxyoestrone	54.1 (43.6, 67)	58.9 (47.3, 73.4)	0.39	59.9 (46.1, 77.8)	54.4 (39.7, 74.7)	59.2 (43.4, 80.7)	61.9 (46.8, 81.9)	3%	0.33
4-Methoxyoestrone	24.9 (20.6, 30.1)	25.7 (21.4, 30.9)	0.75	25.9 (20.4, 32.9)	21.2 (16.3, 27.7)	28.5 (22.1, 36.6)	27.7 (21.9, 35.1)	7%	0.16
4-Methoxyoestradiol	10.6 (8.0, 13.9)	10.7 (8.2, 13.9)	0.91	10.6 (7.8, 14.4)	9.0 (6.3, 13.0)	11.1 (7.9, 15.6)	12.5 (9.2, 17.0)	18%	0.05
16-Alphahydroxyoestrone	216.0 (172.0, 271.3)	235.0 (187.9, 294.0)	0.40	239.9 (183.9, 312.9)	213.8 (155.4, 293.9)	237.8 (172.8, 327.3)	247.1 (185.1, 329.7)	3%	0.30
Oestriol	983.7 (770.7, 1255.5)	1134.9 (886.5, 1453)	0.19	1105.7 (833.1, 1467.6)	1110.2 (776.9, 1586.4)	1210.8 (850.2, 1724.4)	1140.0 (830.3, 1565.1)	3%	0.47
Conjugated	849.8 (659.8, 1094.5)	975.5 (754.7, 1261.1)	0.23	967.7 (719.1, 1302.3)	946.7 (654.7, 1369.0)	1049.9 (724.8, 1520.9)	949.5 (675.6, 1334.5)	−2%	0.69
Unconjugated	117.9 (96.3, 144.4)	122.3 (98.5, 151.9)	0.68	129.3 (101.6, 164.6)	95.1 (69.9, 129.3)	133.0 (98.8, 179.1)	132.0 (101.4, 171.8)	2%	0.20
16-Ketooestradiol	245.2 (195.0, 308.5)	275.3 (219.9, 344.8)	0.27	277.7 (212.4, 363.0)	251.2 (180.7, 349.2)	286.1 (205.6, 398.0)	287.0 (213.4, 385.9)	3%	0.34
16-Epioestriol	77.0 (62.6, 94.8)	79.6 (64.4, 98.3)	0.74	83.8 (65.2, 107.7)	68.6 (50.5, 93.3)	84.6 (64.1, 111.7)	79.6 (60.2, 105.3)	−5%	0.56
17-Epioestriol	54.2 (43.8, 67.0)	54.8 (44.0, 68.3)	0.91	56.4 (43.6, 73.0)	48.4 (35.3, 66.5)	59.6 (44.6, 79.7)	54.4 (40.3, 73.5)	−4%	0.58

[a]Weighted *N*: frequency counts weighted to whole WHI-OS Cohort.
[b]Adjusted for age at blood draw (<60, 60–<70, ⩾70 years), year of blood draw (1993–1996, 1997–1998), race (white, non-white), smoking status (never smoked, past smoker, current smoker), BMI (kg m^{-2}), years since menopause (<10 years, 10–<20 years, ⩾20 years), duration of oral contraceptive use (0, <5, ⩾5 years), caffeine intake (quartiles), sedentary/sitting time (<5, 5–<10, ⩾10 h per day), and metabolic equivalent (MET) hours of physical activity per week (none, >0–<7.5, 7.5–15 (National recommendations), ⩾15).
[c]*P*-diff refers to the *P*-value comparing the geometric mean concentration of parent oestrogens and oestrogen metabolites for current compared with non-alcohol drinkers. Calculated using the Wald test.
[d]%Δ is the percentage difference in oestrogen concentrations comparing 7+ and <1 standard alcoholic drink/week.
[e]*P*-trend is the *P*-value for trend in association between alcohol consumption and parent oestrogens and oestrogen metabolite concentration among current drinkers. Calculated using Wald test for a continuous drinking variable based on the median number of standard servings of alcohol consumed per week according to the categories presented in the table.
[f]Nominal *P*-value<0.05.

studies, but did not reach statistical significance, possibly due to sample size.

Few prior studies have evaluated associations of alcohol consumption with circulating sex steroid hormone metabolites, which may provide insight into alcohol's potential role in modifying oestrogen metabolic pathways. Understanding how alcohol influences endogenous metabolic pathways is of high importance given that the biological mechanisms underlying alcohol's strong association with breast and other cancers, which include sex steroid hormone metabolism, remain poorly defined. Alcohol may influence oestrogen metabolism through increased aromatase activity in the liver and other tissues, stimulating conversion of androgens to oestrogens (Rinaldi *et al*, 2006), decreased catabolism of sex hormones by the liver through accumulation of hepatic nicotinamide-adenine dinucleotide (NADH) – leading to oxidation and inhibition of oestradiol conversion to oestrone (Ginsburg *et al*, 1996), and direct/indirect adrenal gland cell signalling promotion for DHEAS production (a precursor of oestradiol) (Onland-Moret *et al*, 2005; Shafrir *et al*, 2014). How alcohol associates with different oestrogen hydroxylation pathways among postmenopausal women, however, is unknown. A recent analysis evaluated associations of alcohol intake with parent oestrogen/oestrogen metabolites, although women were pre-menopausal and metabolites were measured in urine (Hartman *et al*, 2016). Alcohol intake was only associated with oestradiol, but not other parent oestrogens/metabolites. Although menopausal status has not been shown to be a significant modifier of the alcohol-breast cancer association (Trentham-Dietz *et al*, 2014), the majority of breast cancer cases are diagnosed after menopause, with elevated circulating oestrogens being a strong risk factor (Key *et al*, 2002), highlighting the importance of evaluating alcohol's effects on oestrogen metabolism in this population.

Evaluating ratios of unconjugated to combined oestrogens provides insight into how the pattern of metabolism might vary between never/former and current MHT users. We saw that circulating oestrogen metabolites among MHT users were predominantly conjugated in comparison with non-users. Relative

Table 4. Geometric means (pmol l^{-1}) and 95% confidence intervals (CI) of serum oestrogens and oestrogen metabolites by liquor, beer and wine consumption category among postmenopausal alcohol drinkers who are never/former menopausal hormone therapy users in the Women's Health Initiative (WHI) Observational Study (OS)

	Liquor					Beer					Wine				
Alcoholic drinks/week	Non-liquor drinker	>0-1	>1			Non-beer drinker	>0-1	>1			Non-wine drinker	>0-1	>1-3	>3	
Median	0	0.42	5.25			0	0.422	2.73			0	0.42	2.73	7	
N	299	166	120			391	137	57			91	262	113	119	
Weighted N[d]	9977	5277	3867			13114	3910	2097			2638	8938	3205	4341	
	Geometric means (95% CI)[a]			%Δ[b]	p-diff[c]	Geometric means (95% CI)[a]			%Δ[b]	p-diff[c]	Geometric means (95% CI)[a]				%Δ[b]	p-diff[c]
Oestrone	293.1 (242.4, 354.3)	343.9 (266.9, 443)	411.3 (321.3, 526.5)	29%	0.007[e]	334.0 (279.4, 399.3)	329.9 (241.8, 450.3)	331.0 (243.0, 451.0)	-1%	0.96	332.0 (252.1, 437.2)	316.8 (254.6, 394.4)	350.5 (257.6, 476.9)	355.1 (274.6, 459.0)	7%	0.68
Conjugated	230.5 (186.4, 285.1)	278.2 (211.1, 366.7)	340.2 (260.1, 444.9)	32%	0.004[e]	270.2 (221.7, 329.4)	258.5 (183.1, 365.1)	265.6 (188.8, 373.6)	-2%	0.92	273.7 (202.5, 369.9)	249.6 (195.8, 318.2)	277.7 (197.9, 389.8)	292.6 (221.8, 386.0)	7%	0.70
Unconjugated	55.4 (48.4, 63.4)	59.2 (48.7, 72.0)	65.8 (54.4, 79.5)	16%	0.07	58.2 (51.0, 66.6)	61.7 (49.8, 76.4)	57.8 (46.7, 71.5)	-1%	0.94	54.5 (44.4, 66.9)	59.6 (51.2, 69.4)	63.9 (51.9, 78.7)	57.1 (47.0, 69.5)	5%	0.71
Oestradiol	53.3 (43.3, 65.6)	61.8 (47.1, 81.1)	63.4 (48.7, 82.6)	16%	0.19	57 (47.4, 68.6)	62.3 (44.8, 86.6)	55.3 (40.5, 75.6)	-3%	0.85	56.1 (42.5, 74.1)	54.4 (43.6, 67.8)	63.9 (46.0, 88.8)	62.2 (48.0, 80.6)	11%	0.52
Conjugated	37.1 (29.6, 46.6)	44.5 (33.1, 59.7)	40.9 (30.6, 54.7)	9%	0.50	38.5 (31.4, 47.3)	44.3 (30.8, 63.7)	38.7 (27.2, 55.0)	0%	0.98	39.7 (29.5, 53.6)	36.8 (29.1, 46.7)	44.3 (30.6, 64.3)	42.5 (31.7, 57.2)	7%	0.70
Unconjugated	12.0 (9.8, 14.7)	13.3 (10.4, 16.9)	17.0 (12.9, 22.5)	30%	0.014[e]	13.8 (11.4, 16.9)	13.2 (10.1, 17.1)	12.6 (9.6, 16.6)	-10%	0.50	13.1 (9.8, 17.4)	13.2 (10.6, 16.6)	13.5 (10.3, 17.7)	14.4 (11.4, 18.2)	10%	0.54
2-Hydroxyoestrone	62.5 (52.8, 73.9)	66.7 (53.0, 84.0)	82.0 (67.1, 100.2)	24%	0.009[e]	68.5 (58.2, 80.7)	69.6 (53.6, 90.3)	65.5 (50.7, 84.8)	-4%	0.75	68.0 (53.4, 86.6)	66.2 (55.3, 79.3)	69.9 (53.4, 91.5)	71.3 (56.3, 90.4)	5%	0.76
2-Hydroxyoestradiol	15.6 (13.2, 18.4)	16.7 (13.4, 20.8)	20.1 (16.5, 24.5)	22%	0.016[e]	17.1 (14.6, 20.0)	17.4 (13.5, 22.4)	16.1 (12.6, 20.5)	-6%	0.65	16.8 (13.2, 21.5)	16.6 (13.9, 19.8)	17.2 (13.3, 22.2)	17.7 (14.0, 22.5)	5%	0.74
2-Methoxyoestrone	39.7 (34.6, 45.5)	43.9 (36.1, 53.2)	50.6 (42.2, 60.6)	22%	0.012[e]	44.3 (39.0, 50.4)	43.7 (34.4, 55.5)	39.8 (32.2, 49.2)	-11%	0.36	40.1 (32.9, 48.9)	42.9 (36.7, 50.0)	46.3 (36.3, 59.2)	44.9 (37.3, 54.1)	12%	0.37
Conjugated	29.4 (25.3, 34.3)	32.3 (26.3, 39.6)	36.9 (30.5, 44.8)	20%	0.029[e]	33.1 (28.9, 38.1)	31.5 (24.2, 40.9)	28.8 (23.0, 36.0)	-15%	0.26	29.0 (23.6, 35.8)	31.5 (26.8, 37.2)	33.9 (26.1, 44.0)	34.1 (28.1, 41.4)	18%	0.21
Unconjugated	9.0 (7.7, 10.6)	10.6 (8.4, 13.2)	11.5 (9.2, 14.5)	22%	0.039[e]	9.7 (8.3, 11.4)	10.9 (8.6, 13.8)	9.9 (7.5, 13.0)	1%	0.94	9.9 (7.6, 12.8)	9.9 (8.2, 12.0)	10.8 (8.3, 14.1)	9.6 (7.7, 12.1)	-2%	0.88
2-Methoxyoestradiol	13.6 (11.4, 16.2)	13.4 (10.5, 17.1)	16.1 (12.8, 20.3)	16%	0.13	14.7 (12.6, 17.2)	13.6 (10.2, 18.0)	13.1 (9.7, 17.8)	-12%	0.43	12.1 (9.6, 15.3)	13.6 (11.4, 16.3)	17.1 (12.2, 23.8)	15.0 (12.2, 18.6)	24%	0.12
Conjugated	11.0 (9.0, 13.4)	10.9 (8.4, 14.1)	13.1 (10.3, 16.8)	16%	0.15	11.9 (10.0, 14.2)	11.0 (8.1, 15.0)	10.7 (7.7, 14.8)	-11%	0.49	9.9 (7.8, 12.7)	11.0 (9.0, 13.3)	13.5 (9.3, 19.8)	12.7 (10.1, 16)	28%	0.09
Unconjugated	1.9 (1.6, 2.2)	2.2 (1.8, 2.7)	2.5 (2.1, 3.0)	24%	0.01[e]	2.2 (1.9, 2.5)	2.0 (1.6, 2.5)	2.0 (1.6, 2.5)	-8%	0.50	1.9 (1.5, 2.4)	2.1 (1.8, 2.5)	2.5 (1.9, 3.1)	1.9 (1.6, 2.3)	-1%	0.93
3-Methyl ether-hydroxyoestrone	7.2 (6.2, 8.3)	7.6 (6.3, 9.2)	9.5 (7.8, 11.4)	24%	0.004[e]	7.8 (6.8, 9.0)	8.0 (6.4, 9.9)	7.7 (6.1, 9.5)	-2%	0.85	7.9 (6.6, 9.5)	7.9 (6.8, 9.3)	7.8 (6.1, 10.0)	7.5 (6.2, 9.1)	-5%	0.65
4-Hydroxyoestrone	7.8 (6.6, 9.3)	8.1 (6.4, 10.3)	10.4 (8.5, 12.7)	25%	0.008[e]	8.6 (7.3, 10.2)	8.5 (6.6, 11.1)	8 (6.2, 10.4)	-8%	0.59	8.4 (6.5, 10.8)	8.3 (7.0, 10.0)	8.8 (6.7, 11.6)	8.7 (6.8, 11.1)	4%	0.82
4-Methoxyoestrone	4.2 (3.7, 4.9)	4.3 (3.5, 5.3)	5.2 (4.4, 6.2)	19%	0.026[e]	4.7 (4.1, 5.4)	4.3 (3.4, 5.5)	4.1 (3.3, 5.1)	-15%	0.21	4.2 (3.5, 5.1)	4.5 (3.9, 5.2)	4.7 (3.6, 6.1)	4.6 (3.8, 5.7)	9%	0.50
4-Methoxyoestradiol	2 (1.6, 2.4)	2 (1.6, 2.6)	2.1 (1.7, 2.7)	8%	0.43	2.1 (1.8, 2.5)	1.9 (1.4, 2.5)	1.9 (1.4, 2.5)	-12%	0.39	1.8 (1.4, 2.3)	2.0 (1.7, 2.4)	2.5 (1.8, 3.5)	2.0 (1.7, 2.5)	15%	0.32
16-Alpha-hydroxyoestrone	31.1 (26.0, 37.1)	33.1 (25.9, 42.4)	41.3 (33.5, 50.9)	25%	0.009[e]	34.3 (28.9, 40.8)	34.5 (26.2, 45.7)	32.4 (24.7, 42.6)	-6%	0.70	33.4 (26.0, 42.9)	33.0 (27.3, 39.9)	34.9 (26, 46.9)	36.0 (28, 46.3)	8%	0.64
Oestriol	132.3 (110.4, 158.6)	140.8 (110.8, 178.9)	175.0 (141.7, 216.2)	24%	0.013[e]	146.2 (123.0, 173.8)	144.9 (110.6, 189.8)	138.9 (104.2, 185.2)	-5%	0.74	141.9 (111.5, 180.5)	143.3 (118.4, 173.5)	150.4 (112.6, 201.0)	144.6 (113.4, 184.4)	2%	0.90
Conjugated	103.0 (84.0, 126.4)	114.0 (87.9, 147.9)	140.0 (110.6, 176.7)	26%	0.014[e]	115.9 (95.4, 140.8)	116.0 (86.3, 156.1)	107.8 (77.7, 149.6)	-8%	0.68	112.4 (86.0, 146.9)	113.4 (91.5, 140.6)	119.0 (87.3, 162.3)	114.1 (86.9, 149.8)	2%	0.93
Unconjugated	25.0 (21.5, 29.1)	22.8 (18.5, 28.2)	31.6 (26.2, 38.1)	21%	0.029[e]	26.3 (22.8, 30.4)	25.5 (20.6, 31.7)	25.7 (20.5, 32.2)	-2%	0.86	25.7 (20.8, 31.8)	25.5 (21.7, 29.8)	27.2 (20.7, 35.8)	26.7 (22.0, 32.4)	4%	0.78
16-Ketooestradiol	34.1 (28.4, 41)	35.6 (27.7, 45.8)	46.4 (37.3, 57.7)	26%	0.009[e]	37.7 (31.6, 45)	38.1 (28.7, 50.6)	35.5 (26.6, 47.5)	-6%	0.71	37.5 (28.8, 48.9)	36.5 (30.0, 44.4)	38.3 (28.3, 51.9)	38.4 (29.9, 49.3)	2%	0.89
16-Epioestriol	13.7 (11.6, 16.1)	16.2 (13.0, 20.2)	18.9 (15.6, 23.0)	28%	0.001[e]	15.3 (13.0, 17.9)	16.1 (12.7, 20.4)	15.5 (11.9, 20.2)	2%	0.89	16.5 (12.9, 21.2)	14.6 (12.2, 17.5)	15.3 (11.9, 19.7)	16.8 (13.5, 20.9)	2%	0.91
17-Epioestriol	11.5 (9.9, 13.4)	12.5 (10.3, 15.3)	15.4 (12.8, 18.7)	25%	0.003[e]	12.5 (10.8, 14.6)	13.0 (10.4, 16.3)	12.8 (10.1, 16.3)	2%	0.87	12.9 (10.4, 15.9)	12.3 (10.3, 14.6)	12.4 (9.9, 15.7)	13.7 (11.1, 17)	7%	0.64

[a] Weighted *N* reflects frequency counts weighted to the whole WHI-OS Cohort.

[b] Adjusted for age at blood draw (<60, 60–<70, ⩾70 years), year of blood draw (1993–1996, 1997–1998), race (white, non-white), smoking status (never smoked, past smoker, current smoker), BMI (kg m^{-2}), years since menopause (<10 years, 10–<20 years, ⩾20 years), duration of oral contraceptive use (0, <5, ⩾5 years), caffeine intake (quartiles), sedentary/sitting time (<5, 5–<10, ⩾10 h per day), and metabolic equivalent (MET) hours of physical activity per week (none, >0–<7.5, 7.5–15 (National recommendations), ⩾15).

[c] %Δ is the percentage difference in oestrogen concentrations comparing highest with non-alcohol type (liquor/beer/wine) drinkers.

[d] *P*-diff refers to the *P*-value comparing the geometric mean concentration of parent oestrogens and oestrogen metabolites for highest category compared with non-alcohol type (liquor/beer/wine) drinkers. Calculated using the Wald test.

[e] FDR q-value < 0.05.

concentration of parent to total oestrogens was also higher, suggesting a potential mechanism for enhanced breast cancer risk with alcohol intake in MHT users. Similar to our observation of an overall alcohol association with parent oestrogens among MHT users, moderate alcohol consumption increased circulating oestradiol among postmenopausal women using MHT but not those who were not on MHT in a randomised, double-blind, placebo-controlled, crossover feeding study (Ginsburg *et al*, 1996). Furthermore, among postmenopausal women in a prospective cohort ($N = 5035$), an interaction between intake of two or more alcoholic drinks per day and MHT use was observed for risk of developing breast cancer; drinkers that also used MHT had an increased risk of breast cancer compared with non-MHT users that abstained from alcohol (HR = 1.27, 95% CI = 1.09–1.49 per 1 drink/day increase, p-trend = 0.004 among MHT users; HR = 0.98, 95% CI = 0.82–1.78 per 1 drink/day increase, p-trend = 0.79 among non MHT users) (Nielsen and Gronbaek, 2008). An alcohol-MHT synergistic effect on breast cancer has been supported by analyses within four other large, prospective cohorts (Gapstur *et al*, 1992; Chen *et al*, 2002; Suzuki *et al*, 2005; Zhang *et al*, 2007). A biological rationale for these findings has been suggested, including decreased conversion of oestrogen to its metabolites, a shift in metabolism to different hydroxylation pathways (Ginsburg *et al*, 1996), and altered rate of ethinyloestradiol clearance (Ginsburg *et al*, 1995) among MHT users. In our analysis, drinkers who were taking MHT consumed more total alcohol, on average, than never/former MHT users. It is also possible that dose of alcohol consumption among women in our study may have influenced the differences in magnitudes of association we observed between MHT groups.

We found that highest compared with lowest category of liquor intake among drinkers was associated with 20–30% increases in both parent oestrogens and 2-, 4-, and 16-hydroxylation pathway metabolites among never/former MHT users. In contrast to total alcohol consumption, never/former MHT users consumed more liquor, on average, than current MHT users. The liquor associations remained with further adjustment for total alcohol intake. When we additionally adjusted for parent oestrogens, the pathway associations were not retained, suggesting that they were not independent of parent oestrogens. This might suggest increased oestrogen formation/reduced catabolism as opposed to hydroxylation pathway effects. The ratio of unconjugated to combined oestrone was nominally reduced with higher liquor consumption, which suggests increased oestrone sulphation or glucuronidation (conversion to oestrone sulphate/glucuronide). Sulphated oestrone is stored as an oestrogen substrate reservoir, whereas glucuronidated oestrone is excreted (Hong and Chen, 2011). Few studies have evaluated associations of sex hormones by types of alcohol, in part due to limitations in sample size (London *et al*, 1991; Hankinson *et al*, 1995; Newcomb *et al*, 1995; Hartman *et al*, 2016). Feeding studies have generally utilised 95% ethanol as the alcohol exposure thus have not evaluated wine or beer intake (Dorgan *et al*, 2001; Mahabir *et al*, 2004). Further research on differences in sex steroid hormone metabolism by alcohol type is warranted. Polyphenols in red wine have been shown to be a cancer chemopreventive agent (Scalbert *et al*, 2005). Nonetheless, updated dose-response meta-analyses support that all alcohol types, including red wine intake, are associated with increased risk for colorectal and breast cancers (World Cancer Research Fund/American Institute for Cancer Research, 2010; World Cancer Research Fund/American Institute for Cancer Research, 2017). While we did not observe an association of wine or beer intake with oestrogen metabolism, it is possible that these alcohol types increase cancer risk through other mechanisms such as formation of acetaldehyde, oxidative stress, altered carcinogen clearance, and impaired immunity.

In the context of cancer prevention, understanding hormonal mechanisms influenced by lifestyle exposures like alcohol consumption may provide targets for cancer prevention intervention in high risk populations. In the same population of women to the current study, parent oestrogens were found to be positively related to endometrial cancer risk (particularly unconjugated oestradiol) (Brinton *et al*, 2016). Higher oestrone, and 2- and 4- and 16-pathway metabolites were also associated with non-serous ovarian cancer risk (Trabert *et al*, 2016). Our data from the current analysis support current recommendations to limit alcohol consumption for cancer prevention.

Strengths of our study include the high reliability, sensitivity and specificity of the LC-MS/MS assay, comprehensive evaluation of oestrogen/oestrogen metabolites by exogenous hormone use, and the ability to evaluate associations by alcohol type. However, several limitations exist. The median dose of alcohol consumption in our study was modest and may have been inadequate to enable us to detect associations with oestrogen metabolism. Additionally, sample size may have been limited to detect statistically significant associations, given adjustment for multiple comparisons. We were unable to evaluate earlier life alcohol exposure, which may have been higher than postmenopausal consumption. Additionally, the current analysis was cross-sectional at the time of entry into the study. We did not evaluate how alcohol intake changed, or how alcohol intake was associated with oestrogen metabolism over time, which may be relevant in terms of future disease risk. Alcohol was evaluated using a self-reported questionnaire and thus misreporting could lead to alcohol usage misclassification. However, misreporting of intake is not likely to be associated with endogenous hormone concentrations, so associations would likely be attenuated. The analysis was cross-sectional so we were unable to make inference about causality. We evaluated usual (12-month) frequency of alcohol consumption, but were unable to compare acute *versus* chronic alcohol exposure, or alcohol exposure across the life course. We evaluated average weekly alcohol consumption, which may not be the relevant exposure to evaluate effects on oestrogen metabolism since the timing of last alcohol ingestion could influence oestrogen concentration due to the short half-life of oestrogens (Ginsburg *et al*, 1998). Women enrolled in the WHI-OS included a subset that declined participation in WHI trials, which could have introduced selection bias in terms of alcohol exposure, although participants of the WHI clinical trials (Jackson *et al*, 2003; Ritenbaugh *et al*, 2003; Stefanick *et al*, 2003) had a similar distribution of alcohol consumption to the observational study (Langer *et al*, 2003). Future studies would also benefit from evaluating adrenal steroid precursors to oestrogens in addition to the analytes evaluated in the current study. We did not have participant genotype data; evaluating gene–alcohol interaction in relation to oestrogen hormone metabolism could contribute to the literature in this area.

In conclusion, this study strengthens the evidence that alcohol consumption increases circulating oestrogen among postmenopausal women. Our observations suggest that alcohol, specifically liquor, influences parent oestrogen concentrations, potentially through increased oestrogen production, as opposed to modifying oestrogen metabolism. Future large, prospective studies with larger average dose of alcohol consumption are needed to further explore alcohol's differential effects on oestrogen metabolism in the 2-, 4-, and 16-hydroxylation pathways.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

BT designed the study, oversaw analyses and has primary responsibility for the final content. MCP conducted statistical analysis and wrote the paper. SBC, RP and RTF contributed to the statistical analysis. LAB, NW, and GA contributed to the design and conduct of the original nested case-control studies. GA, LAB, SBC, RTF, SCM, RP, RW, NW and XX provided critical intellectual content to revise the manuscript. All authors reviewed and approved the final manuscript.

REFERENCES

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc B* **57**: 289–300.

Brand JS, Chan MF, Dowsett M, Folkerd E, Wareham NJ, Luben RN, van der Schouw YT, Khaw KT (2011) Cigarette smoking and endogenous sex hormones in postmenopausal women. *J Clin Endocrinol Metab* **96**(10): 3184–3192.

Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, Gass ML, Kuller LH, Pfeiffer RM, Rohan TE, Strickler HD, Xu X, Wentzensen N (2016) Serum estrogens and estrogen metabolites and endometrial cancer risk among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **25**(7): 1081–1089.

Cavalieri E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, Sutter T (2006) Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochim Biophys Acta* **1766**(1): 63–78.

Cavalieri EL, Rogan EG (2016) Depurinating estrogen-DNA adducts, generators of cancer initiation: their minimization leads to cancer prevention. *Clin Transl Med* **5**(1): 12.

Chan MF, Dowsett M, Folkerd E, Bingham S, Wareham N, Luben R, Welch A, Khaw KT (2007) Usual physical activity and endogenous sex hormones in postmenopausal women: the European prospective investigation into cancer-norfolk population study. *Cancer Epidemiol Biomarkers Prev* **16**(5): 900–905.

Chen WY, Colditz G, Rosner B, Hankinson SE, Hunter DJ, Manson JE, Stampfer MJ, Willett WC, Speizer FE (2002) Use of postmenopausal hormones, alcohol, and risk for invasive breast cancer. *Ann Intern Med* **137**(10): 798–804.

Dallal CM, Lacey Jr. JV, Pfeiffer RM, Bauer DC, Falk RT, Buist DS, Cauley JA, Hue TF, LaCroix AZ, Tice JA, Veenstra TD, Xu X, Brinton LA, Group BaFR (2016) Estrogen metabolism and risk of postmenopausal endometrial and ovarian cancer: the B approximately FIT cohort. *Horm Cancer* **7**(1): 49–64.

Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK, Campbell WS, Hartman TJ, Tejpar AA, Clevidence BA, Giffen CA, Chandler DW, Stanczyk FZ, Taylor PR (2001) Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J Natl Cancer Inst* **93**(9): 710–715.

Durrleman S, Simon R (1989) Flexible regression models with cubic splines. *Stat Med* **8**: 551–561.

Friedenreich CM, Woolcott CG, McTiernan A, Ballard-Barbash R, Brant RF, Stanczyk FZ, Terry T, Boyd NF, Yaffe MJ, Irwin ML, Jones CA, Yasui Y, Campbell KL, McNeely ML, Karvinen KH, Wang Q, Courneya KS (2010) Alberta physical activity and breast cancer prevention trial: sex hormone changes in a year-long exercise intervention among postmenopausal women. *J Clin Oncol* **28**(9): 1458–1466.

Ganna A, Lee D, Ingelsson E, Pawitan Y (2014) Rediscovery rate estimation for assessing the validation of significant findings in high-throughput studies. *Brief Bioinform* **16**(4): 563–575.

Gapstur SM, Potter J, Sellers TA, Folsom AR (1992) Increased risk of breast cancer with alcohol consumption in postmenopausal women. *Am J Epidemiol* **136**(10): 1221–1231.

Ginsburg ES, Gao X, Shea BF, Barbieri RL (1998) Half-life of estradiol in postmenopausal women. *Gynecol Obstet Invest* **45**(1): 45–48.

Ginsburg ES, Mello NK, Mendelson JH, Barbieri RL, Teoh SK, Rothman M, Gao X, Sholar JW (1996) Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA* **276**(21): 1747–1751.

Ginsburg ES, Walsh BW, Shea BF, Gao X, Gleason RE, Barbieri RL (1995) The effects of ethanol on the clearance of estradiol in postmenopausal women. *Fertil Steril* **63**(6): 1227–1230.

Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, Longcope C, Speizer FE (1995) Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *J Natl Cancer Inst* **87**(17): 1297–1302.

Hartman TJ, Sisti JS, Hankinson SE, Xu X, Eliassen AH, Ziegler R (2016) Alcohol consumption and urinary estrogens and estrogen metabolites in premenopausal women. *Horm Cancer* **7**(1): 65–74.

Hong Y, Chen S (2011) Aromatase, oestrone sulfatase, and 17beta-hydroxysteroid dehydrogenase: structure-function studies and inhibitor development. *Mol Cell Endocrinol* **340**(2): 120–126.

IARC (2010) *IARC monographs on the evaluation of carcinogenic risks to humans. Volume 96. Alcoholic beverage consumption and ethyl carbamate (urethane)*. IARC: Lyon.

Jackson RD, LaCroix AZ, Cauley JA, McGowan J (2003) The Women's Health Initiative calcium-vitamin D trial: overview and baseline characteristics of participants. *Ann Epidemiol* **13**(9 Suppl): S98–106.

Jefcoate CR, Liehr JG, Santen RJ, Sutter TR, Yager JD, Yue W, Santner SJ, Tekmal R, Demers L, Pauley R, Naftolin F, Mor G, Berstein L (2000) Tissue-specific synthesis and oxidative metabolism of estrogens. *J Nat Cancer Inst Monogr* **27**: 95–112.

Jung S, Wang M, Anderson K, Baglietto L, Bergkvist L, Bernstein L, van den Brandt PA, Brinton L, Buring JE, Heather Eliassen A, Falk R, Gapstur SM, Giles GG, Goodman G, Hoffman-Bolton J, Horn-Ross PL, Inoue M, Kolonel LN, Krogh V, Lof M, Maas P, Miller AB, Neuhouser ML, Park Y, Robien K, Rohan TE, Scarmo S, Schouten LJ, Sieri S, Stevens VL, Tsugane S, Visvanathan K, Wilkens LR, Wolk A, Weiderpass E, Willett WC, Zeleniuch-Jacquotte A, Zhang SM, Zhang X, Ziegler RG, Smith-Warner SA (2015) Alcohol consumption and breast cancer risk by estrogen receptor status: in a pooled analysis of 20 studies. *Int j epidemiol* **45**(3): 916–928.

Key T, Appleby P, Barnes I, Reeves G, Endogenous H, Breast Cancer Collaborative G (2002) Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* **94**(8): 606–616.

Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson Jr HE, Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Key TJ, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB, Miller SR, Longcope C, Endogenous Hormones Breast Cancer Collaborative G

(2003) Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst* **95**(16): 1218–1226.

Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, Alberg AJ, Rollison DE, Dorgan JF, Brinton LA, Overvad K, Kaaks R, Trichopoulou A, Clavel-Chapelon F, Panico S, Duell EJ, Peeters PH, Rinaldi S, Fentiman IS, Dowsett M, Manjer J, Lenner P, Hallmans G, Baglietto L, English DR, Giles GG, Hopper JL, Severi G, Morris HA, Hankinson SE, Tworoger SS, Koenig K, Zeleniuch-Jacquotte A, Arslan AA, Toniolo P, Shore RE, Krogh V, Micheli A, Berrino F, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Lui LY, Cummings SR, Gunter MJ, Rohan TE, Strickler HD (2011) Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br j cancer* **105**(5): 709–722.

Kotsopoulos J, Eliassen AH, Missmer SA, Hankinson SE, Tworoger SS (2009) Relationship between caffeine intake and plasma sex hormone concentrations in premenopausal and postmenopausal women. *Cancer* **115**(12): 2765–2774.

Langer RD, White E, Lewis CE, Kotchen JM, Hendrix SL, Trevisan M (2003) The Women's Health Initiative Observational Study: baseline characteristics of participants and reliability of baseline measures. *Ann Epidemiol* **13**(9 Suppl): S107–S121.

Li HL, Gail MH (2012) Efficient adaptively weighted analysis of secondary phenotypes in case-control genome-wide association studies. *Hum Hered* **73**(3): 159–173.

London S, Willett W, Longcope C, McKinlay S (1991) Alcohol and other dietary factors in relation to serum hormone concentrations in women at climacteric. *Am J Clin Nutr* **53**(1): 166–171.

Mahabir S, Baer DJ, Johnson LL, Dorgan JF, Campbell W, Brown E, Hartman TJ, Clevidence B, Albanes D, Judd JT, Taylor PR (2004) The effects of moderate alcohol supplementation on oestrone sulfate and DHEAS in postmenopausal women in a controlled feeding study. *Nutr J* **3**: 11.

Newcomb PA, Klein R, Klein BE, Haffner S, Mares-Perlman J, Cruickshanks KJ, Marcus PM (1995) Association of dietary and life-style factors with sex hormones in postmenopausal women. *Epidemiology* **6**(3): 318–321.

Nielsen NR, Gronbaek M (2008) Interactions between intakes of alcohol and postmenopausal hormones on risk of breast cancer. *Int J Cancer* **122**(5): 1109–1113.

Onland-Moret NC, Peeters PH, van der Schouw YT, Grobbee DE, van Gils CH (2005) Alcohol and endogenous sex steroid levels in postmenopausal women: a cross-sectional study. *J Clin Endocrinol Metab* **90**(3): 1414–1419.

Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T (1999) Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* **9**(3): 178–187.

Rinaldi S, Peeters PH, Bezemer ID, Dossus L, Biessy C, Sacerdote C, Berrino F, Panico S, Palli D, Tumino R, Khaw KT, Bingham S, Allen NE, Key T, Jensen MK, Overvad K, Olsen A, Tjonneland A, Amiano P, Ardanaz E, Agudo A, Martinez-Garcia C, Quiros JR, Tormo MJ, Nagel G, Linseisen J, Boeing H, Schulz M, Grobbee DE, Bueno-de-Mesquita HB, Koliva M, Kyriazi G, Thrichopoulou A, Boutron-Ruault MC, Clavel-Chapelon F, Ferrari P, Slimani N, Saracci R, Riboli E, Kaaks R (2006) Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition. *Cancer Causes Control* **17**(8): 1033–1043.

Ritenbaugh C, Patterson RE, Chlebowski RT, Caan B, Fels-Tinker L, Howard B, Ockene J (2003) The Women's Health Initiative Dietary Modification trial: overview and baseline characteristics of participants. *Ann Epidemiol* **13**(9 Suppl): S87–S97.

Rosner B (1983) Percentage points for a generalized Esd many-outlier procedure. *Technometrics* **25**(2): 165–172.

Sampson JN, Falk RT, Schairer C, Moore SC, Fuhrman BJ, Dallal CM, Bauer DC, Dorgan JF, Shu XO, Zheng W, Brinton LA, Gail MH, Ziegler RG, Xu X, Hoover RN, Gierach GL (2016) Association of estrogen metabolism with breast cancer risk in different cohorts of postmenopausal women. *Cancer Res* **77**(4): 918–925.

Scalbert A, Manach C, Morand C, Remesy C, Jimenez L (2005) Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* **45**(4): 287–306.

Schutze M, Boeing H, Pischon T, Rehm J, Kehoe T, Gmel G, Olsen A, Tjonneland AM, Dahm CC, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Trichopoulou A, Benetou V, Zylis D, Kaaks R, Rohrmann S, Palli D, Berrino F, Tumino R, Vineis P, Rodriguez L, Agudo A, Sanchez MJ, Dorronsoro M, Chirlaque MD, Barricarte A, Peeters PH, van Gils CH, Khaw KT, Wareham N, Allen NE, Key TJ, Boffetta P, Slimani N, Jenab M, Romaguera D, Wark PA, Riboli E, Bergmann MM (2011) Alcohol attributable burden of incidence of cancer in eight European countries based on results from prospective cohort study. *Bmj* **342**: d1584.

Setiawan VW, Haiman CA, Stanczyk FZ, Le Marchand L, Henderson BE (2006) Racial/ethnic differences in postmenopausal endogenous hormones: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* **15**(10): 1849–1855.

Shafrir AL, Zhang X, Poole EM, Hankinson SE, Tworoger SS (2014) The association of reproductive and lifestyle factors with a score of multiple endogenous hormones. *Horm Cancer* **5**(5): 324–335.

Sierksma A, Sarkola T, Eriksson CJ, van der Gaag MS, Grobbee DE, Hendriks HF (2004) Effect of moderate alcohol consumption on plasma dehydroepiandrosterone sulfate, testosterone, and estradiol levels in middle-aged men and postmenopausal women: a diet-controlled intervention study. *Alcohol Clin Exp Res* **28**(5): 780–785.

Sisti JS, Hankinson SE, Caporaso NE, Gu F, Tamimi RM, Rosner B, Xu X, Ziegler R, Eliassen AH (2015) Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. *Cancer Epidemiol Biomarkers Prev* **24**(8): 1174–1183.

Stefanick ML, Cochrane BB, Hsia J, Barad DH, Liu JH, Johnson SR (2003) The Women's Health Initiative postmenopausal hormone trials: overview and baseline characteristics of participants. *Ann Epidemiol* **13**(9 Suppl): S78–S86.

Suzuki R, Ye W, Rylander-Rudqvist T, Saji S, Colditz G, Wolk A (2005) Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. *J Natl Cancer Inst* **97**(21): 1601–1608.

The Women's Health Initiative Study Group (1998) Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* **19**(1): 61–109.

Trabert B, Brinton LA, Anderson GL, Pfeiffer R, Falk RT, Strickler HD, Sliesoraitis S, Kuller LH, Gass ML, Fuhrman BJ, Xu X, Wentzensen N (2015) Circulating estrogens and postmenopausal ovarian cancer risk in the Women's Health Initiative Observational Study. *Cancer Epidemiol Biomarkers Prev* **25**(4): 648–656.

Trabert B, Brinton LA, Anderson GL, Pfeiffer RM, Falk RT, Strickler HD, Sliesoraitis S, Kuller LH, Gass ML, Fuhrman BJ, Xu X, Wentzensen N (2016) Circulating estrogens and postmenopausal ovarian cancer risk in the Women's Health Initiative Observational Study. *Cancer Epidemiol Biomarkers Prev* **25**(4): 648–656.

Trentham-Dietz A, Sprague BL, Hampton JM, Miglioretti DL, Nelson HD, Titus LJ, Egan KM, Remington PL, Newcomb PA (2014) Modification of breast cancer risk according to age and menopausal status: a combined analysis of five population-based case-control studies. *Breast Cancer Res Treat* **145**(1): 165–175.

World Cancer Research Fund/American Institute for Cancer Research (2010) *Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Breast Cancer.*

World Cancer Research Fund/American Institute for Cancer Research (2017) *Continuous Update Project Report: Diet, Nutrition, Physical Activity and Colorectal Cancer.*

Yager JD (2015) Mechanisms of estrogen carcinogenesis: the role of E2/E1-quinone metabolites suggests new approaches to preventive intervention–A review. *Steroids* **99**(Pt A): 56–60.

Zhang SM, Lee I, 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**(6): 667–676.

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