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ORIGINAL PAPER

Circulating estrogens and postmenopausal ovarian and endometrial cancer risk among current hormone users in the Women's Health Initiative Observational Study

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Abstract

Purpose Menopausal hormone therapy (MHT) use induces alterations in circulating estrogens/estrogen metabolites, which may contribute to the altered risk of reproductive tract cancers among current users. Thus, the current study assessed associations between circulating estrogens/estrogen metabolites and ovarian and endometrial cancer risk among MHT users.

Methods We conducted a nested case–control study among postmenopausal women using MHT at baseline in the Women's Health Initiative Observational Study (179 ovarian cancers, 396 controls; 230 endometrial cancers, 253 controls). Multivariable logistic regression was utilized to estimate odds ratios and 95% confidence intervals overall and by subtype.

Results Estrogen/estrogen metabolite levels were not associated with overall or serous ovarian cancer risk, examined separately. However, unconjugated estradiol was positively associated with non-serous ovarian cancer risk [quintile 5 vs. quintile 1: 3.01 (1.17–7.73); p-trend = 0.03; p-het < 0.01]. Endometrial cancer risk was unrelated to estrogen/estrogen metabolite levels among women who took combined estrogen/progestin therapy (EPT).

Conclusions These findings provide novel evidence that may support a heterogeneous hormonal etiology across ovarian cancer subtypes. Circulating estrogens did not influence endometrial cancer risk among women with EPT-induced high-estrogen levels. Larger studies are needed to delineate the relationship between ovarian/endometrial cancer subtypes and estrogen levels in the context of MHT use.

Keywords Endogenous estrogens · Estrogen metabolites · Ovarian cancer · Endometrial cancer · Nested case–control study · Current hormone therapy users

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Introduction

It is well established that unopposed estrogen therapy (ET) increases endometrial cancer risk, leading to contraindication among women with an intact uterus. ET is also associated with increased risk of ovarian cancer among women with a hysterectomy [1, 2]. Additionally, estrogen plus progestin therapy (EPT) is associated with an increased risk of ovarian cancer, while the association between EPT and endometrial cancer risk depends on the number of days of progestin exposure per month: risk is increased for sequential progestin exposure (≤ 15 days per month) and decreased for continuous progestin exposure (> 15 days per month) [1, 3]. Among non-users of menopausal hormone therapy (MHT), elevated levels of endogenous estrogens have been associated with the risk of non-serous ovarian cancer [4] and were strongly associated with increased risk of endometrial cancer [5], even after adjustment for body mass index (BMI) in prior studies within the Women's Health Initiative (WHI) [4, 5] and other cohorts [6–9]. Associations with ovarian cancer overall have been largely null [10, 11] (Table 1). It is plausible that cancer risk associated with exogenous hormone use is due to altered levels of circulating estrogen and/or estrogen metabolite concentrations among women taking these medications [12]. Little is known, however, about whether circulating estrogen concentrations differentiate postmenopausal ovarian and/or endometrial cancer risks among women with already high-circulating hormone levels due to exogenous MHT use.

To address this question, we conducted a nested case–control study within the WHI Observational Study (OS), to evaluate the associations between 15 pre-diagnostic estrogens and estrogen metabolites and ovarian and endometrial cancer risks among postmenopausal women using MHT at blood draw. We evaluated whether associations varied by histologic subtype, given increasing evidence of etiologic heterogeneity of both tumors, and further evaluated whether associations for ovarian cancer varied by MHT formulation.

Materials and methods

Study population

Details of the WHI-OS [13, 14] have been described previously. Briefly, the WHI-OS is a prospective cohort that enrolled 93,676 postmenopausal women aged 50 to 79 years at 40 clinical centers through the United States between 1993 and 1998 [13, 14]. Women were excluded (n = 148) if they had medical conditions with a predicted survival of less than 3 years or if they were participating in a clinical trial. The present nested case–control study included incident invasive ovarian (including fallopian tube and peritoneal cancers) and endometrial cancer cases that were diagnosed between study initiation and May 2012 and a shared control group. Both cases and controls met the following criteria to be eligible: no history of cancer at baseline other than non-melanoma skin cancer (*n* excluded = 10,455); were current users of exogenous hormones (*n* excluded = 32,338); no history of bilateral oophorectomy (all controls) or hysterectomy (endometrial cancer controls only) at baseline (*n* excluded = 13,723) and at least 1.1 mL of available pre-diagnostic serum (*n* excluded = 397). Baseline serum samples were available for all participants. We excluded women from analysis if their unconjugated estrone concentration

Table 1 Summary of ovarian and endometrial cancer associations with exogenous menopausal hormone therapy (MHT) use and circulating levels of endogenous estrogens/estrogen metabolites among postmenopausal women

	Ovarian cancer	Endometrial cancer
Exogenous MHT use		
Unopposed estrogen therapy (ET)	Increased risk [1, 2]	Increased risk, no longer prescribed to women with intact uterus
Estrogen plus progestin therapy (EPT)	Increased risk, does not depend on number of days progestin per month [1, 2]	Risk depends on days per month of progestin: < 15 days/month progestin associated with increased risk; decreased risk with 15 or more days per month on progestin [3]
Circulating estrogens (among never/former MHT users)		
Estrone/estradiol (radioimmunoassay-based assays)	Mostly null associations reported [10, 11]	Increased risk [8, 9]
Estrogen/estrogen metabolites	Increased risk for non-serous tumors [4], null association when not evaluated by subtype [7]	Increased risk for both parent estrogens and metabolites; estradiol strongest association and independent of BMI [5]
Circulating estrogens (among women using MHT at blood draw)	Not evaluated	Not evaluated

was < 184 pmol/L (~ 50 pg/mL; $n = 196$) to mitigate misclassification of MHT use, and further limited analyses of endometrial cancer cases and controls to women reporting EPT use only, since ET use is contraindicated for women with an intact uterus (n excluded = 50 [35 cases/15 controls]).

Cases were WHI-OS participants with incident primary epithelial ovarian ($n = 146$), fallopian tube ($n = 15$), or peritoneal cancer ($n = 18$), combined into one group as 'ovarian cancers' or incident primary endometrial cancer ($n = 230$). All cancers, other than cancers identified via the National Death Index, were medical record-confirmed and centrally coded according to SEER standards. The average time from baseline sample collection to cancer diagnosis was 6.5 years (standard deviation = 3.5 years; range = 252 days–14.7 years).

Controls were eligible WHI-OS cohort members selected from strata defined by age at baseline blood draw (50–54, 55–59, 60–64, 65–69, 70–74, 75–79), year at blood draw (1993–1996, 1997–1998), race/ethnicity (white, black, Hispanic, other/unknown) for both case groups. Controls were additionally matched on hysterectomy status at baseline or follow-up prior to event (yes/no) for ovarian cancer cases, while controls were alive and at risk of endometrial cancer (did not have hysterectomy) at the time of diagnosis of their matched endometrial case. Shared controls were drawn from the set of eligible cohort members in each stratum containing ovarian and endometrial cancer cases that were alive at the time of diagnosis of their matched case and were selected with a ratio of at least 2 controls per case for ovarian cancer and at least 1 control per case for endometrial cancer. The control:case ratio was established a priori based on estimated cancer incidence and study power. The present study included 179 epithelial ovarian cancer cases and 396 matched controls and 230 endometrial cancer cases and 253 matched controls.

Approval for conducting the study was obtained from human subjects' review at the Fred Hutchinson Cancer Research Center (WHI Clinical Coordinating Center), as well as at all 40 clinical centers. Written informed consent was obtained from study participants.

Laboratory assays

Details of the method have been published previously [4, 5, 15]. Briefly, stable isotope dilution LC–MS/MS was used to quantify 15 estrogens and estrogen metabolites including: estrone, estradiol, 2-pathway metabolites (2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether); 4-pathway metabolites (4-hydroxyestrone, 4-methoxyestrone, and 4-methoxyestradiol); and 16α -pathway metabolites (16α-hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, and 17-epiestriol). This method detects 15 estrogens and estrogen metabolites in serum which circulate, at least in part, as sulfated and/or glucuronidated conjugates to facilitate storage, transport, and excretion. Five of the estrogens (estrone, estradiol, estriol, 2-methoxyestrone and 2-methoxyestradiol) were also measured in unconjugated forms in circulation. For those metabolites with both combined and unconjugated measurements, the concentration of the conjugated form was calculated as the difference between the combined estrogen measurement and the unconjugated estrogen measurement; for estradiol that calculation was (conjugated E2 = combined E2 – unconjugated E2). The limit of detection for each estrogen and estrogen metabolite measured using this LC–MS/MS assay was 10 fg on column (approximately 0.33–0.37 pmol/L) [15, 16]. There were no samples in the current study with undetectable levels for any of the hormones measured. Laboratory coefficients of variation (CV) of masked technical replicates across batches were < 6.0% for all hormones measured. Intraclass correlation coefficients (ICCs) ranged from 0.93–0.996 with a median value of 0.98.

Statistical analysis

Estrogens and estrogen metabolites were analyzed individually and categorized into quintiles based on the distribution in their respective control groups. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of cancer risk conditioning on matching factors: age at blood draw, calendar year of blood draw, race/ethnicity, and hysterectomy status (for ovarian cancer analyses). Models were further adjusted for a priori potential-confounding factors: ever pregnant, body mass index (BMI), cigarette-smoking status, and duration of oral contraceptive use for ovarian cancer analyses and for education, cigarette-smoking status, BMI, years of oral contraceptive use, age at menarche, ever pregnant, age at menopause, and previous tubal ligation for endometrial cancer analyses. Adjustment factors were categorized as listed in Table 2. Additional adjustment for other variables (e.g., tubal ligation in ovarian cancer analyses) had minimal effects on the derived associations. Tests for trend were based on the Wald statistic modeling the intra-category quintile median as a continuous parameter.

We conducted the following analyses by histologic subtype: (1) among ovarian cancer cases, stratified by 116 serous tumors (102 high grade (tumor grade > 1), 4 low grade (grade = 1), and 10 missing grade) and 63 non-serous (13 endometrioid, 6 clear cell, 7 mucinous, and 37 other-epithelial subtypes) tumors; (2) among endometrial cancer cases, stratified by 208 type I (155 endometrioid, 45 adenocarcinomas, 8 mucinous) tumors and 22 type II (18 were serous, 4 clear cell) tumors. For analyses stratified by case characteristics (i.e., histologic subtype and time

Table 2 Demographic and health characteristics of controls, and ovarian and endometrial cancer cases, nested case–control study within the Women's Health Initiative Observational Study

	Ovarian cancer				Endometrial cancer			
	Cases $n=179$		Controls $n=396$		Cases $n=230$		Controls $n=253$	
Age in years, mean (SD)	62.7	(6.8)	62.8	(7.0)	62.5	(6.7)	61.7	(6.7)
	*n**	%	*n**	%	*n**	%	*n**	%
Year of blood draw								
1993–1996	108	60.3	244	61.6	150	65.2	171	67.6
1997–1998	71	39.7	152	38.4	80	34.8	82	32.4
Race								
White	168	93.9	381	96.2	218	94.8	237	93.7
Other	11	6.1	15	3.8	12	5.2	16	6.3
Hysterectomy‡								
No	115	64.2	255	64.4	230	100.0	253†	100.0
Yes	64	35.8	141	35.6	0	0.0	0	0.0
Smoking status								
Never	86	48.3	181	45.9	123	53.9	111	44.0
Former	88	49.4	192	48.7	96	42.1	128	50.8
Current	4	2.2	21	5.3	9	3.9	13	5.2
BMI (kg/m^2)								
< 25	88	49.2	174	44.1	141	61.3	125	49.4
25–29.9	57	31.8	129	32.7	48	20.9	73	28.9
30 +	34	19.0	92	23.3	41	17.8	55	21.7
Diabetic status								
Non-diabetic	173	96.6	380	96.0	225	97.8	247	97.6
Diabetic	6	3.4	16	4.0	5	2.2	6	2.4
Age at menarche (years)								
< 12	48	26.8	85	21.6	43	18.8	59	23.3
12–13	91	50.8	203	51.5	150	65.5	135	53.4
14 +	40	22.3	106	26.9	36	15.7	59	23.3
Ever pregnant								
No	17	9.6	34	8.6	30	13.1	25	9.9
Yes	161	90.4	362	91.4	199	86.9	228	90.1
Duration oral contraceptive use								
Never	105	58.7	198	50.0	106	46.1	120	47.4
< 5 years	35	19.6	90	22.7	69	30.0	55	21.7
5 to < 10 years	19	10.6	56	14.1	26	11.3	42	16.6
10 + years	20	11.2	52	13.1	29	12.6	36	14.2
Tubal ligation								
No	147	82.1	324	82.2	180	78.6	196	77.5
Yes	32	17.9	70	17.8	49	21.4	57	22.5
Age at menopause (years)								
< 45	28	15.6	74	18.7	20	8.7	23	9.1
45–49	45	25.1	114	28.8	51	22.2	67	26.5
50–54	78	43.6	146	36.9	116	50.4	113	44.7
55 +	28	15.6	62	15.7	43	18.7	50	19.8
MHT formulation								
ET	70	39.1	148	37.4	0	0.0	0	0.0
EPT	109	60.9	248	62.6	230	100.0	253	100.0
MHT duration								

Table 2 (continued)

	*n**	%	*n**	%	*n**	%	*n**	%
< 5 years	42	23.5	104	26.3	59	25.7	81	32.0
5 to < 10 years	62	34.6	117	29.5	70	30.4	82	32.4
10 to < 15 years	27	15.1	78	19.7	53	23.0	58	22.9
15 + years	48	26.8	97	24.5	48	20.9	32	12.6

SD standard deviation, *BMI* body mass index, *MHT* menopausal hormone therapy, *ET* unopposed estrogen therapy, *EPT* estrogen plus progestin therapy

*Values may not sum to total because of missing data

†All endometrial cancer controls are included in ovarian cancer control group, $n = 141$ ovarian controls excluded from endometrial cancer control group due to hysterectomy, $n = 2$ ovarian controls did not have a relevant endometrial cancer case

‡At baseline or during follow-up prior to the event

between blood draw and diagnosis) we used multinomial logistic regression models, with the controls as the reference group and adjustment for matching factors and a priori selected potential-confounding factors (listed above for ovarian and endometrial analyses). Differences in risk estimates across subgroups were assessed using Chi-square p values (reported as p-heterogeneity) from unconditional logistic regression models that treated the largest subgroup as the reference category and excluded non-cases.

We also evaluated associations for parent unconjugated estrogens stratified by age at blood draw (< 65 years old, ≥ 65 years old), BMI, and MHT formulation for ovarian cancer only (ET [$n = 218$], EPT [$n = 357$]). Lastly, the following sensitivity analyses were conducted: (1) excluding potential outliers (greater than five standard deviations above the median; median excluded subjects per hormone measure $n = 7$ [min–max: 3–10]), and (2) excluding women who reported a history of diabetes at baseline (ovarian cancer analysis $n = 22$; endometrial cancer analysis $n = 11$) given that biologic crosstalk between insulin and sex steroid hormones and/or diabetes medication may influence circulating estrogen levels. All p values were two-sided; nominal p values < 0.05 were considered statistically significant. All analyses were completed using SAS version 9.4.

Results

On average, participants were 63 years old at baseline blood draw, and were predominantly white (greater than 90% of cases and controls) (Table 2). Geometric means of estrogens and estrogen metabolites did not differ between ovarian cancer cases and controls or endometrial cancer cases and controls (Table 3).

Among current MHT users, estrogens and estrogen metabolites were not associated with overall ovarian cancer risk (Table 4). Similar (null) results were found when assessing risk for predominantly high-grade serous ovarian cancers. However, women with increased levels of unconjugated estradiol were at increased risk of developing non-serous ovarian cancer [quintile (Q)5 vs. Q1: 3.01 (1.17–7.73); p-trend = 0.03; p-het < 0.01].

Among women taking EPT at blood draw, circulating estrogen/estrogen metabolite levels were not associated with risk of endometrial cancer overall or by dualistic endometrial cancer subtype (Type I/II) (Table 5).

Associations between unconjugated estrone or unconjugated estradiol and risk of serous and non-serous ovarian cancer and endometrial cancer did not differ significantly when stratified by the time between blood draw and diagnosis, age at blood draw, BMI, or MHT formulation (for ovarian cancers) (Table 6). For unconjugated estradiol, there was at least a three-fold increased risk of non-serous ovarian cancer across all strata of the potential effect modifiers evaluated, except for women aged 65 years or older at blood draw (Table 6). In sensitivity analyses, results were not quantitatively different after excluding women with a history of diabetes at baseline or excluding outliers (results not shown).

Discussion

We previously reported that among women not using menopausal hormones at blood draw that higher levels of circulating estrogens were strongly associated with increased risk of non-serous ovarian cancer and endometrial cancer [4, 5]. However, little is known about these associations among women using MHT at blood draw, when circulating estrogen levels are substantially higher. In the current study, no associations were observed between circulating estrogens or estrogen metabolites and total or serous ovarian cancers, while unconjugated estradiol was strongly associated with risk of developing non-serous ovarian cancer. Among users of EPT, associations with endometrial cancer, overall and by subtype, were null.

Table 3 Average estrogen and estrogen metabolite concentrations among controls and ovarian and endometrial cancer cases, Women's Health Initiative Observational Study

	Ovarian cancer				Endometrial cancer			
	Cases		Controls		Cases		Controls	
	$n=179$		$n=396$		$n=230$		$n=253$	
	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI
Parent estrogens								
Estrone	3231	(2808, 3717)	3183	(2876, 3522)	3105	(2748, 3508)	2880	(2552, 3251)
Unconjugated estrone	266	(235, 301)	252	(232, 273)	234	(212, 259)	225	(204, 249)
Conjugated estrone	2930	(2536, 3384)	2919	(2629, 3242)	2847	(2510, 3231)	2673	(2361, 3028)
Estradiol	425	(369, 490)	424	(385, 466)	406	(360, 459)	369	(329, 414)
Unconjugated estradiol	50.2	(44.3, 57.0)	49.6	(45.6, 54.0)	44.1	(39.8, 49.0)	45.0	(40.4, 50.1)
Conjugated estradiol	346	(295, 407)	352	(316, 391)	337	(294, 387)	309	(273, 351)
2-Hydroxylation pathway								
2-Hydroxyestrone	447	(396, 504)	467	(431, 506)	461	(416, 510)	442	(400, 489)
2-Hydroxyestradiol	105	(93.2, 118)	108	(100, 117)	107	(97.5, 118)	103	(93.2, 113)
2-Methoxyestrone	276	(247, 309)	264	(243, 286)	256	(232, 281)	248	(225, 274)
Unconjugated 2-methoxyestrone	85.7	(73.2, 100)	74.6	(66.7, 83.4)	70.5	(61.6, 80.6)	68.1	(59.5, 78.0)
Conjugated 2-methoxyestrone	167	(150, 186)	167	(154, 182)	165	(150, 183)	159	(144, 177)
2-Methoxyestradiol	86.4	(77.0, 96.9)	86.3	(79.7, 93.6)	86.4	(77.8, 95.8)	82.6	(74.8, 91.4)
Unconjugated 2-methoxyestradiol	10.7	(9.41, 12.07)	9.44	(8.71, 10.22)	9.61	(8.63, 10.70)	9.0	(8.12, 9.93)
Conjugated 2-methoxyestradiol	72.5	(64.2, 81.9)	73.9	(67.8, 80.6)	73.5	(65.6, 82.3)	70.7	(63.5, 78.7)
2-Hydroxyestrone-3-methyl ether	42.2	(37.9, 47.0)	41.7	(38.7, 44.9)	41.0	(37.4, 44.9)	40.4	(37.0, 44.2)
4-Hydroxylation pathway								
4-Hydroxyestrone	59.4	(52.6, 67.1)	62.4	(57.5, 67.7)	62.0	(56.1, 68.6)	59.2	(53.4, 65.5)
4-Methoxyestrone	30.0	(26.8, 33.6)	29.5	(27.3, 31.9)	29.5	(26.8, 32.3)	28.0	(25.4, 30.8)
4-Methoxyestradiol	12.1	(10.7, 13.7)	12.3	(11.3, 13.5)	12.3	(11.0, 13.8)	11.7	(10.4, 13.0)
16α-Hydroxylation pathway								
16α-Hydroxyestrone	234	(208, 265)	247	(227, 268)	242	(218, 269)	234	(211, 260)
Estriol	1131	(994, 1287)	1177	(1076, 1288)	1157	(1036, 1291)	1127	(1008, 1260)
Unconjugated estriol	143	(127, 161)	145	(134, 157)	138	(125, 152)	132	(120, 146)
Conjugated estriol	964	(842, 1104)	1012	(920, 1112)	1003	(895, 1125)	987	(877, 1110)
16-Ketoestradiol	270	(239, 305)	290	(266, 316)	284	(256, 315)	276	(248, 308)
16-Epiestriol	77.5	(68.9, 87.1)	84.6	(77.9, 91.8)	81.3	(73.3, 90.2)	81.4	(73.4, 90.3)
17-Epiestriol	56.7	(50.2, 64.1)	58.5	(53.6, 63.8)	58.3	(52.4, 64.7)	57.3	(51.3, 64.0)

GM geometric means, *CI* confidence interval

We observed a wide range of hormone concentrations among women using MHT at blood collection. Combined estrogen concentrations (e.g., conjugated + unconjugated estrone) and, when measured, conjugated estrogen concentrations among current MHT users in this study were at least ten times higher than previously measured concentrations among former/never MHT users. While, unconjugated estrogen concentrations were almost five times higher among current MHT users. Estrogen metabolite concentrations were shown to vary by MHT formulation in a previous analysis of these data, in which it was found that women using ET had higher levels of all estrogen metabolites, including conjugated and unconjugated levels [12]. The ratios of 2- and 4-pathway estrogen metabolites did not differ by formulation, while EPT users had more extensive metabolism along the 16-pathway as reflected by higher levels compared to ET users [12]. Although we were able to detect variations in the concentration of estrogen metabolites by MHT formulation in a prior analysis [12], the estrogen metabolites were not associated with ovarian or endometrial cancer risk in the current study. Further, in ovarian cancer analyses stratified by MHT formulation, we observed elevated risk of non-serous ovarian cancer with unconjugated estradiol among both ET and EPT users.

Our results of an increased risk of non-serous ovarian cancer in association with relatively high levels of

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) for the risk of epithelial ovarian cancer overall and by serous/non-serous subtype comparing the 5th quintile to the 1st quintile for individual estrogens and estrogen metabolites, nested case–control study within the Women's Health Initiative Observational Study

	All ovarian cancer cases			Serous			Non-serous			p_{het}§
	$n=179$			$n=116$			$n=63$			
	OR*	(95% CI)	p-trend‡	OR†	(95% CI)	p-trend‡	OR	(95% CI)	p-trend‡	
Parent estrogens										
Estrone	0.90	(0.48, 1.70)	0.39	0.86	(0.40, 1.85)	0.21	0.97	(0.41, 2.32)	0.99	0.77
Unconjugated estrone	0.87	(0.48, 1.58)	0.99	0.83	(0.41, 1.70)	0.76	0.99	(0.43, 2.31)	0.50	0.66
Conjugated estrone	0.74	(0.39, 1.40)	0.17	0.80	(0.37, 1.74)	0.12	0.70	(0.29, 1.68)	0.60	0.99
Estradiol	0.82	(0.44, 1.53)	0.59	0.64	(0.29, 1.38)	0.29	1.20	(0.52, 2.79)	0.62	0.44
Unconjugated estradiol	1.08	(0.59, 1.97)	0.78	0.60	(0.29, 1.25)	0.08	3.01	(1.17, 7.73)	0.03	< 0.01
Conjugated estradiol	0.87	(0.48, 1.58)	0.61	0.69	(0.33, 1.46)	0.38	1.19	(0.53, 2.68)	0.81	0.63
2-Hydroxylation pathway										
2-Hydroxyestrone	0.83	(0.44, 1.55)	0.29	0.90	(0.44, 1.86)	0.23	0.56	(0.21, 1.51)	0.53	0.73
2-Hydroxyestradiol	0.81	(0.44, 1.47)	0.27	0.85	(0.42, 1.72)	0.19	0.66	(0.26, 1.66)	0.65	0.89
2-Methoxyestrone	0.92	(0.52, 1.63)	0.91	0.87	(0.44, 1.72)	0.90	0.90	(0.40, 2.03)	0.89	0.98
Unconjugated 2-methoxyestrone	1.31	(0.71, 2.41)	0.64	1.56	(0.75, 3.25)	0.60	0.96	(0.39, 2.31)	0.85	0.70
Conjugated 2-methoxyestrone	0.95	(0.53, 1.72)	0.62	0.91	(0.46, 1.78)	0.64	0.81	(0.32, 2.06)	0.57	0.64
2-Methoxyestradiol	0.81	(0.45, 1.48)	0.74	0.73	(0.36, 1.48)	0.67	0.91	(0.39, 2.13)	0.84	0.78
Unconjugated 2-methoxyestradiol	1.33	(0.75, 2.36)	0.18	1.18	(0.61, 2.29)	0.39	1.74	(0.74, 4.09)	0.13	0.56
Conjugated 2-methoxyestradiol	0.67	(0.35, 1.25)	0.54	0.66	(0.32, 1.37)	0.61	0.64	(0.26, 1.63)	0.58	0.51
2-Hydroxyestrone-3-methyl ether	0.76	(0.41, 1.40)	0.85	0.64	(0.30, 1.34)	0.52	0.97	(0.42, 2.21)	0.78	0.81
4-Hydroxylation pathway										
4-Hydroxyestrone	0.91	(0.48, 1.71)	0.25	0.97	(0.47, 2.02)	0.28	0.60	(0.22, 1.66)	0.26	0.31
4-Methoxyestrone	1.00	(0.56, 1.80)	0.87	0.78	(0.40, 1.53)	0.59	1.24	(0.51, 3.02)	0.84	0.93
4-Methoxyestradiol	0.81	(0.45, 1.48)	0.46	0.88	(0.45, 1.73)	0.81	0.75	(0.30, 1.91)	0.31	0.18
16α-Hydroxylation pathway										
16α-Hydroxyestrone	0.75	(0.40, 1.39)	0.11	0.86	(0.42, 1.76)	0.17	0.45	(0.16, 1.27)	0.16	0.38
Estriol	0.96	(0.52, 1.76)	0.53	1.08	(0.52, 2.24)	0.71	0.71	(0.29, 1.73)	0.31	0.20
Unconjugated estriol	0.97	(0.54, 1.74)	0.70	0.76	(0.38, 1.53)	0.31	1.23	(0.54, 2.84)	0.63	0.45
Conjugated estriol	0.98	(0.52, 1.82)	0.43	1.10	(0.52, 2.33)	0.59	0.68	(0.28, 1.68)	0.27	0.20
16-Ketoestradiol	0.76	(0.41, 1.41)	0.15	0.78	(0.37, 1.61)	0.16	0.66	(0.26, 1.67)	0.30	0.68
16-Epiestriol	0.67	(0.35, 1.28)	0.06	0.61	(0.28, 1.30)	0.05	0.60	(0.23, 1.58)	0.29	0.99
17-Epiestriol	0.88	(0.46, 1.68)	0.53	0.72	(0.34, 1.54)	0.20	0.94	(0.37, 2.41)	0.98	0.57

*OR from model conditioned on matching factors (age at baseline, year of blood draw, race/ethnicity, and hysterectomy status) and adjusted for body mass index, smoking status, gravidity, and duration of oral contraceptive use

†OR from model adjusted for age at baseline, year of blood draw, race/ethnicity, hysterectomy status, body mass index, smoking status, gravidity, and duration of oral contraceptive use

‡p value for trend across quintile (median value of category)

§p-het Chi-square p value for heterogeneity across subgroup association estimated from logistic regression model that treated serous tumors as the reference and excluded controls

unconjugated estradiol among women using MHT at blood draw is consistent with our prior analysis in WHI evaluating estrogens/estrogen metabolites among women who were not using MHT at blood draw [4]. The heterogenous ovarian cancer association is also consistent with positive associations between BMI and non-serous tumors, and null associations between BMI and serous tumors, reported in prospective cohort studies [17–19], further supporting a role of estrogens in non-serous ovarian cancers. The group of non-serous tumors included endometrioid and clear cell tumors as well as mucinous tumors, the former have also been associated with other potentially pro-estrogenic exposures (e.g., endometriosis) [20, 21]. For endometrial cancer, circulating estrogens were strongly associated with risk among women not using EPT or other exogenous hormones at blood collection [5], where the

Table 5 Odds ratios (OR) and 95% confidence intervals (CI) for the risk of endometrial cancer overall and by dualistic subtype (Type I/II) comparing the 5th quintile to the 1st quintile for individual estrogens and estrogen metabolites, nested case–control study within the Women's Health Initiative Observational Study

	All endometrial cancer cases $n=230$			Type I $n=208$			Type II $n=22$			p_{het}[§]
	OR*	(95% CI)	p-trend[‡]	OR[†]	(95% CI)	p-trend[‡]	OR[†]	(95% CI)	p-trend[‡]	
Parent estrogens										
Estrone	0.79	(0.41, 1.51)	0.24	0.86	(0.46, 1.63)	0.42	1.16	(0.28, 4.78)	0.80	0.97
Unconjugated estrone	1.06	(0.54, 2.08)	0.40	1.07	(0.56, 2.06)	0.61	1.16	(0.24, 5.67)	0.46	0.66
Conjugated estrone	0.83	(0.44, 1.57)	0.31	0.87	(0.47, 1.64)	0.50	1.18	(0.29, 4.88)	0.80	0.97
Estradiol	1.40	(0.73, 2.70)	0.79	1.40	(0.73, 2.69)	0.69	1.97	(0.52, 7.51)	0.21	0.25
Unconjugated estradiol	0.83	(0.43, 1.59)	0.51	0.96	(0.50, 1.82)	0.71	0.35	(0.08, 1.52)	0.23	0.25
Conjugated estradiol	1.28	(0.67, 2.44)	0.65	1.24	(0.66, 2.33)	0.60	2.58	(0.60, 11.15)	0.17	0.22
2-Hydroxylation pathway										
2-Hydroxyestrone	1.02	(0.54, 1.92)	0.64	0.98	(0.53, 1.82)	0.72	1.49	(0.33, 6.86)	0.76	0.97
2-Hydroxyestradiol	0.84	(0.44, 1.60)	0.43	0.80	(0.42, 1.52)	0.55	1.32	(0.27, 6.41)	0.63	0.89
2-Methoxyestrone	0.87	(0.47, 1.64)	0.39	0.85	(0.46, 1.57)	0.50	1.07	(0.24, 4.81)	0.39	0.54
Unconjugated 2-methoxyestrone	1.01	(0.52, 1.97)	0.48	1.20	(0.63, 2.31)	0.76	0.28	(0.05, 1.50)	0.16	0.18
Conjugated 2-methoxyestrone	0.83	(0.44, 1.58)	0.36	0.79	(0.43, 1.48)	0.40	3.13	(0.55, 17.95)	0.88	0.63
2-Methoxyestradiol	0.85	(0.45, 1.62)	0.42	0.78	(0.42, 1.46)	0.37	–	–	0.43	0.19
Unconjugated 2-methoxyestradiol	1.23	(0.67, 2.25)	0.34	1.13	(0.62, 2.05)	0.44	0.97	(0.25, 3.74)	0.89	0.94
Conjugated 2-methoxyestradiol	0.83	(0.44, 1.57)	0.41	0.78	(0.42, 1.45)	0.43	–	–	0.61	0.29
2-Hydroxyestrone-3-methyl ether	0.83	(0.44, 1.55)	0.53	0.86	(0.47, 1.59)	0.56	0.82	(0.18, 3.73)	0.94	0.96
4-Hydroxylation pathway										
4-Hydroxyestrone	0.99	(0.52, 1.89)	0.42	0.93	(0.49, 1.74)	0.47	2.24	(0.40, 12.55)	0.93	0.77
4-Methoxyestrone	1.02	(0.54, 1.92)	0.46	1.02	(0.55, 1.88)	0.52	0.76	(0.15, 3.72)	0.53	0.69
4-Methoxyestradiol	0.77	(0.41, 1.46)	0.38	0.74	(0.40, 1.36)	0.30	4.54	(0.44, 46.77)	0.84	0.37
16α-Hydroxylation pathway										
16α-Hydroxyestrone	0.99	(0.53, 1.86)	0.52	0.97	(0.53, 1.81)	0.59	1.67	(0.37, 7.67)	0.80	0.53
Estriol	1.19	(0.63, 2.24)	0.64	1.12	(0.60, 2.09)	0.65	1.82	(0.39, 8.46)	0.92	0.67
Unconjugated estriol	1.03	(0.54, 1.98)	0.57	1.06	(0.56, 2.00)	0.89	0.68	(0.14, 3.40)	0.30	0.43
Conjugated estriol	1.24	(0.66, 2.35)	0.67	1.14	(0.61, 2.13)	0.66	2.75	(0.49, 15.59)	0.84	0.56
16-Ketoestradiol	1.08	(0.57, 2.05)	0.43	0.97	(0.52, 1.83)	0.39	2.40	(0.43, 13.47)	0.85	0.55
16-Epiestriol	1.38	(0.72, 2.64)	0.55	1.22	(0.65, 2.28)	0.46	2.95	(0.52, 16.89)	0.80	0.61
17-Epiestriol	0.90	(0.47, 1.75)	0.34	0.82	(0.43, 1.56)	0.30	2.01	(0.43, 9.49)	0.56	0.32

*OR from model conditioned on matching factors (age at baseline, year of blood draw, race/ethnicity) and adjusted for body mass index, smoking status, education, gravidity, duration of oral contraceptive use, tubal ligation status, age at menarche and age at menopause

[†]OR from model adjusted for age at baseline, year of blood draw, race/ethnicity, body mass index, smoking status, education, gravidity, duration of oral contraceptive use, tubal ligation status, age at menarche and age at menopause

[‡]p value for trend across quintile (median value of category)

[§]p het Chi-square p-value for heterogeneity across subgroup association estimated from logistic regression model that treated Type I tumors as the reference and excluded controls

primary contribution to circulating estrogen levels in postmenopausal women is from the aromatization of androgen precursors in adipose tissue. In contrast, in the current study of women taking EPT at blood draw, we did not observe similar increased risks. Exogenous hormone use at blood draw was associated with elevated circulating estrogen concentrations by at least an order of magnitude in the current study compared with non-users from our previously published study in the WHI-OS [5], and likely strongly outweighed the contribution of obesity to circulating estrogen levels, and thus risk. This result is consistent with studies reporting that BMI is more strongly associated with endometrial cancer risk among non-EPT users than among EPT users [3, 22–24]; supporting the notion that there may be a threshold at which high-circulating estrogen levels do not further stratify endometrial cancer risk. We cannot, however, rule out the possibility that concomitant progestin exposure in these women counteracted

Table 6 Risk of serous/non-serous ovarian cancer and endometrial cancer comparing the 5th to 1st quintile for parent unconjugated estrogens across categories of time between blood draw and diagnosis, age at blood draw, BMI, and MHT formulation, nested case–control study within the Women's Health Initiative Observational Study

	Serous ovarian cancer			Non-serous ovarian cancer			Endometrial cancer		
	OR*	(95% CI)	*p*-intx†	OR*	(95% CI)	*p*-intx†	OR‡	(95% CI)	*p*-intx†
Unconjugated estrone									
Time from blood draw to diagnosis									
< 5 years	1.36	(0.43–4.29)	0.14	0.71	(0.22–2.23)	0.73	1.89	(0.79, 4.50)	0.09
≥ 5 years	0.64	(0.27–1.53)		1.52	(0.46–4.97)		0.71	(0.33, 1.53)	
Unconjugated estradiol									
Time from blood draw to diagnosis									0.74
< 5 years	1.05	(0.32–3.51)	0.38	3.01	(0.88–10.26)	0.59	0.83	(0.35, 1.94)	
≥ 5 years	0.46	(0.19–1.12)		3.24	(0.82–12.74)		0.86	(0.42, 1.76)	
Unconjugated estrone									
Age at blood draw									
< 65 years old	0.76	(0.30–1.95)	0.52	0.96	(0.31–2.97)	0.74	0.80	(0.35, 1.79)	0.23
≥ 65 years old	0.93	(0.30–2.86)		1.07	(0.27–4.27)		2.34	(0.76, 7.16)	
Unconjugated estradiol									
Age at blood draw									
< 65 years old	0.60	(0.24–1.51)	0.63	7.48	(1.48–37.74)	0.22	0.64	(0.28, 1.44)	0.81
≥ 65 years old	0.65	(0.18–2.32)		1.31	(0.37–4.62)		1.41	(0.50, 3.97)	
Unconjugated estrone									
BMI									
< 25	1.57	(0.54–4.53)	0.59	2.37	(0.54–10.36)	0.84	0.71	(0.31, 1.61)	0.16
25–29.9	0.24	(0.04–1.35)		0.49	(0.11–2.20)		3.86	(0.74, 20.19)	
30 +	1.61	(0.19–13.48)		0.46	(0.04–5.01)		0.39	(0.03, 4.83)	
Unconjugated estradiol									
BMI									
< 25	0.46	(0.16–1.36)	0.16	4.72	(0.81–27.51)	0.80	0.72	(0.32, 1.62)	0.89
25–29.9	0.25	(0.04–1.37)		3.85	(0.82–18.17)		1.26	(0.29, 5.43)	
30 +	Insufficient numbers			4.05	(0.36–46.05)		0.63	(0.10, 4.16)	
Unconjugated estrone									
MHT formulation									
ET	0.48	(0.16–1.48)	0.21	0.71	(0.18–2.79)	0.83	NA	NA	
EPT	1.13	(0.44–2.92)		1.26	(0.39–4.00)		1.06	(0.54, 2.08)	
Unconjugated estradiol									
MHT formulation									
ET	0.37	(0.10–1.35)	0.29	3.18	(0.59–17.09)	0.78	NA	NA	
EPT	0.73	(0.29–1.85)		3.01	(0.90–10.09)		0.83	(0.43, 1.59)	

OR odds ratio, *CI* confidence interval, *BMI* body mass index, *MHT* menopausal hormone therapy, *ET* estrogen therapy, *EPT* estrogen plus progestin therapy, *NA* not applicable

*OR from model adjusted for age at baseline, year of blood draw, race/ethnicity, hysterectomy status, body mass index, smoking status, gravidity, and duration of oral contraceptive use

†*p*-interaction Chi-square *p* value from the cross-product interaction term between the modifier of interest and unconjugated estrone or estradiol (modeled as ordinal quintile variable)

‡OR from model adjusted for age at baseline, year of blood draw, race/ethnicity, body mass index, smoking status, education, gravidity, duration of oral contraceptive use, tubal ligation status, age at menarche and age at menopause

the uterotropic effect [25] of high-estrogen levels among EPT users.

The current study has several important strengths. The WHI-OS cohort is a large prospective study with standardized pre-diagnostic specimen collection and storage. The five estrogens and estrogen metabolites found in circulation in both unconjugated and combined forms and the ten measured in combined form provide a novel phenotypic

characterization of individual patterns of estrogen metabolism. We measured estrogens among all postmenopausal women in our study population using an LC–MS/MS assay with high sensitivity [15, 16]. This study also has limitations. Although we included all available cases, the study was still limited in power, which affected our ability to evaluate specific subtypes of non-serous ovarian tumors. Moreover, our analyses were not adjusted for multiple comparisons; however, the noted association between unconjugated estradiol and non-serous ovarian cancer risk is consistent with our previous research demonstrating increased non-serous ovarian cancer risk with estrogens among non-current MHT users at blood collection [4].

This is the first prospective epidemiologic study of circulating estrogen/estrogen metabolite levels and ovarian and endometrial cancer risks among women using MHT at blood collection. Our study provides novel molecular data that supports a potential role for unconjugated estradiol in non-serous ovarian cancer etiology among MHT users, further supporting the notion of a heterogeneous hormonal etiology across histologic subtypes of ovarian cancer [4, 26]. Additional investigation in a large prospective study is needed to replicate our finding and clarify the risk of individual non-serous ovarian cancer subtypes. Circulating estrogens did not differentiate risk of endometrial cancer among women with high-circulating estrogen levels due to EPT use; whether this was due to a threshold beyond which additional estrogenic exposures were not associated with further risk stratification or via a protective role of the progestin on endometrial tissue requires further study.

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Compliance with ethical standards

Conflicts of interest All authors declare they have no conflicts of interest.

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