Research Article

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Serum Estrogens and Estrogen Metabolites and Endometrial Cancer Risk among Postmenopausal Women

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Abstract

Background: Although endometrial cancer is clearly influenced by hormonal factors, few epidemiologic studies have investigated the role of endogenous estrogens or especially estrogen metabolites.

Methods: We conducted a nested case–control study within the Women's Health Initiative Observational Study (WHI-OS), a cohort of 93,676 postmenopausal women recruited between 1993 and 1998. Using baseline serum samples from women who were non-current hormone users with intact uteri, we measured 15 estrogens/estrogen metabolites via HPLC/MS-MS among 313 incident endometrial cancer cases (271 type I, 42 type II) and 354 matched controls, deriving adjusted ORs and 95% confidence intervals (CI) for overall and subtype-specific endometrial cancer risk.

Results: Parent estrogens (estrone and estradiol) were positively related to endometrial cancer risk, with the highest risk observed for unconjugated estradiol (OR 5th vs. 1st quintile = 6.19; 95% CI, 2.95–13.03, $P_{trend} = 0.0001$). Nearly all metabolites were significantly associated with elevated risks, with some attenuation after adjustment for unconjugated estradiol (residual risks of 2- to 3-fold). Body mass index (kg/m^2, BMI) relations were somewhat reduced after adjustment for estrogen levels. The association with unconjugated estradiol was stronger for type I than type II tumors ($P_{het} = 0.01$).

Conclusions: Parent estrogens as well as individual metabolites appeared to exert generalized uterotropic activity, particularly for type I tumors. The effects of obesity on risk were only partially explained by estrogens.

Impact: These findings enhance our understanding of estrogen mechanisms involved in endometrial carcinogenesis but also highlight the need for studying additional markers that may underlie the effects on risk of certain risk factors, for example, obesity. *Cancer Epidemiol Biomarkers Prev; 25(7); 1081–9. ©2016 AACR.*

Introduction

Endometrial cancer is strongly influenced by hormonal factors, with established risk factors including obesity, menstrual and reproductive factors, and exogenous hormones (1). Despite acceptance of the hormonal etiology of endometrial cancer, relatively few studies have explored the role of endogenous estrogens (2). Although several epidemiologic investigations have shown strong associations with risk (3–6), results have generally been based on limited numbers of cases (e.g., 57–124 cases were involved with two of the studies). In addition, previous investigations have mainly relied on measurement of estrogens by radioimmunoassays, which have recognized limitations, particularly for measuring low concentrations of estrogens in postmenopausal women.

The use of mass spectrometry to measure endogenous hormones has opened up new research avenues (7). In addition to improving sensitivity, accuracy, and reproducibililty, these assays have enabled concurrent measurement of estrogen metabolites, which purportedly have divergent effects on cancer development. The metabolism of estradiol or estrone with irreversible hydroxylation at the C-2, C-4, or C-16 positions of the steroid ring results in metabolites with varying mitogenic and genotoxic properties. Two major hypotheses about estrogen metabolites have emerged from experimental research, namely, that (i) 16α-hydroxyestrone (16α-OHE$_1$) is carcinogenic because it can bind covalently to the estrogen receptor with strong mitogenic effects, and (ii) the 2- and 4-hydroxylation catechol estrogen metabolites are carcinogenic because they can be oxidized into mutagenic quinones that form DNA adducts and lead to oxidative DNA damage (7).

Estrogen metabolites have been most extensively evaluated with respect to breast cancer, where initial attention focused on

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potential mitogenic effects, with the hypotheses that high levels of 16α-OHE_1 would be directly associated with risk, whereas high ratios of 2-hydroxyestrone (2-OHE_1) to 16α-OHE_1 would be inversely related (8, 9). More recent studies have focused on genotoxicity (10), given that hydroxylation at the C-2 and C-4 positions produces catechol estrogens [notably 2-OHE_1, 2-hydroxyestradiol (2-OHE_2), and 4-hydroxyestrone (4-OHE_1)], which, unless methylated, can be metabolized to mutagenic catechol quinones. Several studies have in fact found decreased risks of postmenopausal breast cancer associated with elevated ratios of 2-pathway metabolites relative to parent estrogens (11, 12), and one study found increased risks for higher levels of catechols to methylated catechols (12).

For endometrial cancer, a cancer known to be influenced by epithelial cell proliferation (particularly in the absence of progesterone), metabolites that are mitogenic may be of particular interest. However, few investigations have explored the role of estrogen metabolites in endometrial carcinogenesis. One prospective study, involving 124 cases, that evaluated circulating 2-OHE_1 and 16α-OHE_1 levels using an enzyme immunoassay found some increases with high levels of both metabolites, but this relation did not persist after adjustment for estrone or estradiol levels (13). In another cohort study (66 cases), estradiol was significantly associated with risk, but there was no further discrimination of risk according to levels of metabolites or their ratios (14). Two endometrial cancer case–control studies have also evaluated estrogen metabolites, with suggestive relationships for certain metabolites (15, 16), although interpretation of the results was limited by small numbers and assessment of hormone levels after disease onset.

We assessed the relation of estrogen metabolites to endometrial cancer risk among participants in the Women's Health Initiative Observational Study (WHI-OS). Strengths of this study included relatively large numbers of endometrial cancers as well as availability of detailed information on risk factors, enabling a thorough assessment of confounding and interactive effects. Given recent interest in the etiologic heterogeneity of endometrial cancers, we evaluated whether the effects of estrogen metabolites differed across distinct tumor subtypes, including type I versus type II tumors, which are characterized by different hormonally related risk factor profiles (17, 18).

Materials and Methods

Study population

The WHI-OS is a prospective cohort involving 93,676 postmenopausal women, ages 50 to 79 years, enrolled at U.S. centers between 1993 and 1998 (19, 20). Excluded were 148 women who had medical conditions with a predicted survival <3 years or adherence/retention issues, or who were participating in a clinical trial. The current study, conducted as a case–control study nested in the cohort, included incident invasive endometrial cancers diagnosed between study initiation and May 2012. Study subjects were excluded if they had a history of cancer at baseline other than nonmelanoma skin cancer ($n = 11{,}674$); were current users of exogenous hormones (38,419); had a hysterectomy at baseline or during follow-up (13,862); and did not have at least 1.1 mL of available prediagnosis baseline serum (720).

Cases were identified via self-reports and medical records and centrally adjudicated. The mean time from sample collection to diagnosis was 6.9 years (SD = 3.7 years; range = 45 days–15.0 years). Controls were eligible WHI-OS cohort members selected from strata defined by age at blood draw (50–54, 55–59, 60–64, 65–69, 70–74, 75–79 years), year at blood draw (1993–1996, 1997–1998), race/ethnicity (white, black, Hispanic, other/unknown), and time since last menopausal hormone therapy (MHT) use (≤1, >1 year). Controls were alive and at risk of endometrial cancer (did not have hysterectomy) at the time of diagnosis of their matched case. Controls were shared with a nested case–control study of ovarian cancer (21) and drawn from strata containing endometrial cancer cases, with a ratio of at least 1 control per case, resulting in 313 endometrial cancer cases and 354 matched controls. Institutional review board clearance was received at the Fred Hutchinson Cancer Research Center (WHI Clinical Coordinating Center), as well as the clinical centers. All study participants provided written informed consent.

Laboratory assays

Stable isotope dilution HPLC/MS-MS was used to quantify 15 estrogens and estrogen metabolites (Fig. 1), as described previously (22). Six labeled internal standards were used: deuterated 2-hydroxyestradiol, 2-methoxyestradiol, and estriol (C/D/N Isotopes Inc); deuterated 16-epiestriol (Medical Isotopes Inc); and ^{13}C-labeled estrone and estradiol (Cambridge Isotope Laboratories).

The serum method detects 15 estrogens and estrogen metabolites which circulate primarily as sulfated and/or glucuronidated conjugates. Five estrogen metabolites (estrone, estradiol, estriol, 2-methoxyestrone, and 2-methoxyestradiol) were measured in unconjugated forms. The serum sample was split into two aliquots to measure the combined concentration of each of the 15 metabolites (sum of conjugated plus unconjugated forms) and the unconjugated forms. To measure the combined parent estrogen or estrogen metabolite level, an enzyme with sulfatase and glucuronidase activity was added to the samples to cleave any sulfate and glucoronide groups (22). To measure the unconjugated forms, the enzyme was not included in sample preparation. For metabolites with both combined and unconjugated measurements, the concentration of the conjugated form was calculated as the difference between the combined and unconjugated estrogen measurements. The limit of detection for each estrogen and estrogen metabolite measured was 10 fg on column (~0.33–0.37 pmol/L; refs. 22, 23). No samples had undetectable hormone levels. Laboratory coefficients of variation of masked technical replicates across batches were <6.0% for all hormones measured. Intraclass correlation coefficients ranged from 0.93 to 0.996 (median value 0.98).

Statistical analysis

We included self-reported never or former MHT pill/patch users and excluded individuals with unconjugated estrone concentrations ≥184 pmol/L (~50 pg/mL; $n = 9$), typically indicative of current exogenous hormone use. Individual estrogens and estrogen metabolites were categorized into quintiles based on control distributions.

Conditional logistic regression was used to estimate ORs and 95% confidence intervals (CI) of endometrial cancer risk while conditioning on matched case–control sets (determined by age and calendar year of blood draw, race/ethnicity, and time since last MHT use), and further adjusted for *a priori* potential confounding factors [education, body mass index (BMI), cigarette smoking status, years of oral contraception use, age at menarche,

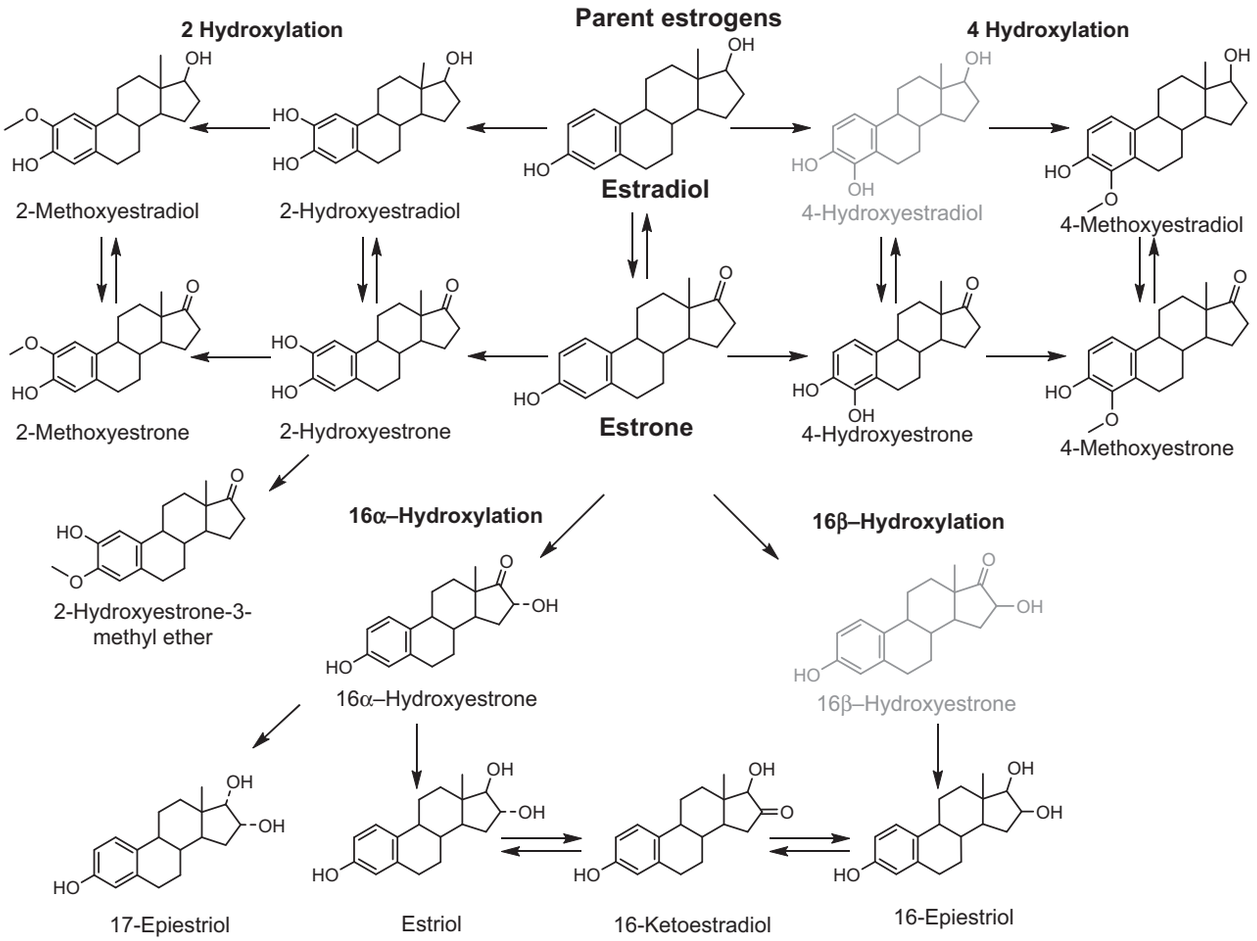

Figure 1.
Formation of 2-, 4-, and 16-hydroxylation pathway estrogen metabolites from parent estrogens. The current serum estrogen metabolite assay measures 15 of 17 metabolites pictured. 4-Hydroxyestradiol and 16β-hydroxyestrone (in light gray) are not measured with the current assay due to very low abundance in circulation.

gravidity, age at menopause, never/former MHT use, and previous tubal ligation]. Additional adjustment for other variables (e.g., parity) or finer adjustment for some factors (e.g., BMI) had minimal effects on derived associations. Tests for trend were based on the Wald statistic modeling the intracategory quintile median as a continuous parameter.

For analyses stratified by clinical characteristics of the cases, we used baseline category polytomous logistic regression models, with the controls as the reference group. Analyses stratified by BMI, age at blood draw, or duration of oral contraception use were evaluated using unconditional logistic regression models. The baseline category and unconditional logistic regression models were adjusted for matching factors and *a priori* selected potential confounding factors (listed above), with one exception: ever and time since last MHT use were combined (never, $\leq$1 year, >1 year). Likelihood ratio tests for the interaction across levels of BMI, age at blood draw, or duration of oral contraception use were computed on the basis of cross-product terms with hormones categorized as the ordinal variable.

We evaluated associations stratified by histologic subtype [type I (66 adenocarcinomas, 194 endometrioid carcinomas, 11 mucinous) vs. type II (34 serous, 6 clear cell, 2 other tumors)], grade of the tumor among the endometrioid tumors and adenocarcinomas [low-grade (grades 1–2) vs. high-grade (3–4)], and time between blood collection and diagnosis (<5 year, $\geq$ 5 years). Differences in risk estimates across subgroups were assessed using P values (reported as $P_{\text{heterogeneity}}$ or P_{het}) from unconditional logistic regression models that treated the largest subgroup as the reference category and excluded non-cases. We also conducted the following sensitivity analyses excluding: (i) individuals diagnosed within 2 years of blood draw (n = 27), (ii) potential outliers [greater than five SDs above the median; median excluded subjects per hormone n = 10 (min–max: 7–17)], (iii) diabetics (n = 38), and (iv) former MHT users (n = 220).

Q values which reflect FDR were calculated to account for multiple comparisons in the main analyses; all other analyses were considered exploratory and therefore not corrected for multiple comparisons. All P values were two-sided; P values <0.05 were considered statistically significant for uncorrected tests.

Results

The endometrial cancer cases and controls were comparably aged, being 64.5 and 64.0 years, respectively (Table 1). The

Table 1. Demographic and health characteristics of endometrial cancer cases and matched controls

	Endometrial cancer cases $n = 313$ Mean (SD)	Controls $n = 354$ Mean (SD)
Age at baseline blood draw, years	64.5 (7.0)	64.0 (7.0)
Year of blood draw	*n* (%)	*n* (%)
1993–1996	190 (60.7)	218 (61.6)
1997–1998	123 (39.3)	138 (39.0)
Race/ethnicity		
White	284 (90.7)	319 (90.1)
Black	15 (4.8)	17 (4.8)
Hispanic	2 (0.6)	3 (0.8)
Other	12 (3.8)	15 (4.2)
Time since last menopausal hormone therapy use, years		
>1	300 (95.8)	340 (96.0)
≤1	13 (4.2)	14 (4.0)
Family income		
<$35,000	110 (35.1)	133 (37.6)
$35,000–$74,999	119 (38.0)	137 (38.7)
≥$75,000	60 (19.2)	57 (16.1)
Education		
High school or less	56 (17.9)	74 (20.9)
Some post high school	111 (35.5)	114 (32.2)
College graduate	144 (46.0)	163 (46.0)
Alcohol use, drinks/week		
Never	33 (10.5)	32 (9.0)
Former	52 (16.6)	59 (16.7)
Current, <1	110 (35.1)	107 (30.2)
Current, 1–<7	68 (21.7)	94 (26.6)
Current, ≥7	48 (15.3)	62 (17.5)
Smoking status		
Never	163 (52.1)	177 (50.0)
Former	134 (42.8)	142 (40.1)
Current	13 (4.2)	32 (9.0)
BMI, kg/m^2		
<25	74 (23.6)	161 (45.5)
25–29.9	90 (28.8)	104 (29.4)
≥30	148 (47.3)	88 (24.9)
Ever pregnant		
No	43 (13.7)	55 (15.5)
Yes	270 (86.3)	299 (84.5)
Duration of oral contraceptive use, years		
Never	221 (70.6)	214 (60.5)
<5	50 (16.0)	72 (20.3)
5–<10	23 (7.3)	36 (10.2)
≥10	19 (6.1)	32 (9.0)
Age at menopause, years		
<40	5 (1.6)	11 (3.1)
40–44	15 (4.8)	22 (6.2)
45–49	67 (21.4)	82 (23.2)
50–54	146 (46.6)	170 (48.0)
≥55	68 (21.7)	59 (16.7)
Menopausal hormone therapy use		
Never	237 (75.7)	210 (59.3)
Former	76 (24.3)	144 (40.7)
Tubal ligation		
No	268 (85.6)	289 (81.6)
Yes	43 (13.7)	64 (18.1)

majority of the subjects provided blood samples during 1993–1996 and were Caucasian. Approximately 4% of the subjects reported last MHT use within the year prior to study interview.

Expected associations were seen with respect to endometrial cancer risk factors, with cases generally having higher incomes, more education, higher BMIs, earlier ages at menarche and later ages at menopause, and less often reporting current smoking, long-term oral contraceptive usage, and prior tubal ligation than controls.

Median and interdecile ranges of serum estrogen metabolites for cases versus controls are shown in Table 2. The most abundant estrogen was conjugated estrone, followed by estriol. Several metabolites were detected at quite low levels, including metabolites in the 4-hydroxylation pathway, as well as the 2-methoxy metabolites. Cases showed higher levels of all parent estrogens and individual metabolites than controls.

Adjusted ORs associated with the 5th versus 1st quintile of the parent estrogens were 3.19 (95% CI, 1.69–6.04) for estrone and 1.41 (0.75–2.67) for estradiol (Table 3). There was little difference in the association between unconjugated and conjugated estrone, but for estradiol the difference in risk was striking, being 6.19 (2.95–13.03) for unconjugated estradiol and 0.95 (0.51–1.77) for conjugated estradiol. There were significant trends across quintiles of estrone, regardless of conjugation, and for unconjugated estradiol (individual quintile ORs are shown in Supplementary Table S1).

Nearly all of the individual metabolites also showed significant linear trends and associations with risk (the one exception being conjugated 2-methoxyestrone), with the magnitude of the risks ranging from 2.1 to 4.0. We cautiously (given high correlations of estrogens and metabolites, see Supplementary Table S2) also examined ratios of different estrogens and metabolites; however, we observed no convincing or statistically significant patterns of risk associated with these quantities (results not shown).

When we adjusted all other estrogens and metabolites for the strongest predictor of risk, namely, unconjugated estradiol (as a continuous variable; Table 3), we generally saw some attenuation in the observed risks, and several metabolite associations became nonsignificant (2-hydroxyestradiol, 2-methoxyestrone, estriol, 17-epiestriol). However, a number of the metabolites remained associated with risk, showing significant associations for the highest quintiles (magnitude of association 2.0–3.0) and significant linear trends.

As endogenous estrogens are known to be correlated with BMI, we assessed relations of the various estrogens and metabolites across different BMI categories. This demonstrated that estrogens were generally associated with increased risks across all BMI categories (parent estrogens shown in Table 4 and estrogen metabolites in Supplementary Table S3). Similar analyses were pursued according to different categories of age at diagnosis and years of use of oral contraceptives. While there was no evidence of interactive effects of estrogens with oral contraceptive usage, some of the estrogens showed stronger relations for older subjects, with interactions being statistically significant at $P < 0.05$ for conjugated 2-methoxyestradiol, 4-methoxyestrone, and 17-epiestriol.

We also assessed the extent to which estrogens might explain the effects of obesity on endometrial cancer risk, comparing BMI estimates in models unadjusted for estrogens with estrogen-adjusted estimates. Without adjustment for estrogens and compared with a referent group of BMI < 25, the ORs were 2.17 (95% CI, 1.40–3.34) for BMI 25–29.9 and 3.97 (2.54–6.21) for BMI ≥ 30. Additional adjustment for unconjugated estradiol resulted in an attenuation of these risks to 1.55 (0.97–2.48) and 2.25 (1.33–3.81), respectively.

Given interest in the etiologic heterogeneity of endometrial cancer, we also examined relations of the estrogens and metabolites according to several clinical characteristics (Table 5). A total of 271 (86.6%) cases were classified as type I and 42 (13.4%) as

Table 2. Median and interdecile range (IDR) of estrogen metabolites among endometrial cancer cases and controls

Estrogens and estrogen metabolites (pmol/L)	Cases Median (IDR)	Controls Median (IDR)
Parent estrogens		
Estrone	441.4 (176.3–1239.8)	293.9 (137.5–880.9)
Unconjugated estrone	73.7 (37.3–155.8)	55.4 (30.1–115.9)
Conjugated estrone	372.2 (127.6–1096.8)	238.8 (98.1–792.1)
Estradiol	68.2 (29.4–172.6)	49.3 (21.9–154.7)
Unconjugated estradiol	20.0 (6.3–55.0)	11.6 (4–38.8)
Conjugated estradiol	44.2 (18.9–120)	37.1 (14.9–110.1)
2-Hydroxylation pathway		
2-Hydroxyestrone	80.9 (43.5–178.2)	64.9 (32.8–164)
2-Hydroxyestradiol	19.0 (11.0–43.0)	15.9 (8.3–40.6)
2-Methoxyestrone	50.8 (28.2–104.5)	41.7 (24.8–89.5)
Unconjugated 2-methoxyestrone	12.8 (6.3–26.4)	10.4 (4.4–23.3)
Conjugated 2-methoxyestrone	36.2 (18.4–85.8)	31.8 (16.9–73.5)
2-Methoxyestradiol	15.9 (8.0–36.8)	12.4 (6.6–32.9)
Unconjugated 2-methoxyestradiol	2.7 (1.4–6.5)	2.0 (1.1–4.8)
Conjugated 2-methoxyestradiol	12.8 (6.3–34.4)	10.5 (5.1–28.1)
2-Hydroxyestrone-3-methyl ether	9.1 (5.1–19.0)	7.6 (4.2–17.1)
4-Hydroxylation pathway		
4-Hydroxyestrone	9.5 (5.1–24.0)	7.6 (4.1–21.1)
4-Methoxyestrone	4.6 (3.2–14.1)	3.9 (2.8–10.5)
4-Methoxyestradiol	2.1 (1.2–5.6)	1.7 (0.9–4.1)
16α-Hydroxylation pathway		
16α-Hydroxyestrone	40.9 (21.3–90.9)	32.3 (15.8–86.8)
Estriol	181.3 (87.8–421.9)	140.4 (63.5–391.5)
Unconjugated estriol	34.1 (15.4–74.4)	25.7 (12.2–63.7)
Conjugated estriol	146.9 (66.1–352.6)	109.7 (49.3–332)
16-Ketoestradiol	45.1 (22.3–109.5)	34.2 (16.6–101.3)
16-Epiestriol	18.5 (9.6–40.9)	15.0 (7.5–38.3)
17-Epiestriol	15.6 (7.9–37.3)	12.8 (6.2–31.7)

type II tumors. Significant heterogeneity in the associations of several estrogens were seen between the two tumor types, including unconjugated estradiol [OR for highest vs. lowest quintile for type I tumors was 6.97 (95% CI, 3.20–15.20) vs. 1.80 (0.43–7.49) for type II tumors, $P_{het} = 0.01$]. Other significant differences were seen for unconjugated 2-methoxyestrone (3.47 vs. 0.95), 4-methoxyestrone (3.54 vs. 0.84), and unconjugated estriol (3.34 vs. 1.83). When we further limited the analyses to endometrioid tumors and adenocarcinomas and assessed whether there were differences in estrogen associations according to grade of the tumors, we observed stronger associations of unconjugated estradiol with risks of low-grade versus high-grade tumors (OR 8.92 vs. 3.27), but the difference was not statistically significant ($P_{het} = 0.14$).

Associations were not significantly different by time since blood draw (<5/≥5 years). In sensitivity analyses, ORs excluding cases diagnosed within 2 years of blood draw or diabetics remained unchanged. We also excluded individuals with outlier estrogen measurements, but given the small numbers involved this had minimal effects on derived associations. Finally, we restricted analyses to never users of MHT (excluding former users) and did not observe large alterations from previously derived risks.

Discussion

In this largest study to date addressing the relation of endogenous estrogens and estrogen metabolites to endometrial cancer risk, we found that endometrial cancer risk was directly associated with serum levels of endogenous estrogens, with the highest risks associated with levels of unconjugated estradiol. Using a highly sensitive, accurate, and reproducible assay, we found that the association with unconjugated estradiol was stronger than generally has been seen with less robust assays, with subjects in the highest quintile showing a 6-fold increased risk compared with those in the lowest quintile. After adjustment for unconjugated estradiol, the associations with the other estrogens and metabolites were attenuated, but most continued to show risk elevations on the order to 2- to 3-fold for women in the highest versus lowest quintiles.

Our findings provided little support for varying effects of different estrogen metabolites on endometrial cancer risk, in line with two prior smaller prospective studies (13, 14). Dissimilar to our hypothesis that endometrial cancer might be affected by metabolites that have unique mitogenic properties, and in contrast to finding from several breast cancer studies (9, 11), we did not observe distinctive associations with high ratios of the 2- to 16-pathway estrogens. We also found no support for mutagenicity given that endometrial cancer risk was not related to increased levels of catechols to methylated catechols. Divergent findings for breast and endometrial cancer are further supported by experimental data, which fail to support unique antineoplastic activity of 2-hydroxy metabolites in endometrial tissue (24, 25) and instead are consistent with all three pathways (2, 4, and 16) having uterotropic activity that can result in endometrial proliferation (26) and endometrial tumor progression (27). Findings that unopposed estrogens are inversely associated with breast cancer risk (28), but show strong positive relations for endometrial cancer (28, 29), provide additional support for differing etiologic roles of estrogens on the two cancer sites.

Table 3. Risk of endometrial cancer comparing the 5th to the 1st quintile for each estrogen metabolite, before and after adjustment for unconjugated estradiol

	OR[a] (95% CI)	P_{trend}[b]	FDR	OR[c] (95% CI)	P_{trend}[b]
Parent estrogens					
Estrone	3.19 (1.69–6.04)	0.0001	0.0004	2.70 (1.34–5.45)	0.003
Unconjugated estrone	2.70 (1.48–4.92)	0.0002	0.0005	2.23 (1.10–4.51)	0.017
Conjugated estrone	2.98 (1.59–5.58)	0.0002	0.0005	2.46 (1.25–4.85)	0.010
Estradiol	1.41 (0.75–2.67)	0.4531	0.4720		
Unconjugated estradiol	6.19 (2.95–13.03)	0.0001	0.0004		
Conjugated estradiol	0.95 (0.51–1.77)	0.6747	0.6747		
2-Hydroxylation pathway					
2-Hydroxyestrone	3.00 (1.56–5.78)	0.0033	0.0055	2.41 (1.21–4.82)	0.048
2-Hydroxyestradiol	2.31 (1.22–4.37)	0.0328	0.0373	1.82 (0.93–3.55)	0.237
2-Methoxyestrone	2.12 (1.16–3.88)	0.0022	0.0042	1.77 (0.94–3.33)	0.025
Unconjugated 2-methoxyestrone	2.69 (1.46–4.96)	0.0049	0.0074	2.00 (1.03–3.89)	0.099
Conjugated 2-methoxyestrone	1.60 (0.90–2.86)	0.0246	0.0293	2.86 (1.45–5.64)	0.001
2-Methoxyestradiol	3.35 (1.76–6.37)	0.0001	0.0004	2.45 (1.23–4.85)	0.018
Unconjugated 2-methoxyestradiol	3.06 (1.63–5.73)	0.0006	0.0014	2.73 (1.39–5.37)	0.003
Conjugated 2-methoxyestradiol	3.26 (1.70–6.25)	0.0002	0.0005	2.25 (1.13–4.48)	0.233
2-Hydroxyestrone-3-methyl ether	2.83 (1.45–5.51)	0.0361	0.0392	2.45 (1.24–4.84)	0.025
4-Hydroxylation pathway					
4-Hydroxyestrone	3.04 (1.59–5.80)	0.0019	0.0040	2.46 (1.31–4.60)	0.001
4-Methoxyestrone	2.89 (1.57–5.31)	0.0001	0.0004	3.01 (1.51–6.03)	0.001
4-Methoxyestradiol	3.52 (1.82–6.80)	0.0001	0.0004	2.19 (1.12–4.29)	0.063
16α-Hydroxylation pathway					
16α-Hydroxyestrone	2.73 (1.44–5.16)	0.0050	0.0074	3.29 (1.66–6.51)	0.004
Estriol	4.00 (2.09–7.67)	0.0001	0.0004	1.37 (0.76–2.48)	0.101
Unconjugated estriol	3.25 (1.71–6.19)	0.0002	0.0005	2.69 (1.36–5.33)	0.005
Conjugated estriol	3.37 (1.75–6.49)	0.0032	0.0055	2.54 (1.28–5.04)	0.058
16-Ketoestradiol	3.13 (1.63–6.03)	0.0053	0.0074	2.77 (1.40–5.46)	0.034
16-Epiestriol	2.88 (1.49–5.54)	0.0060	0.0079	2.30 (1.17–4.56)	0.086
17-Epiestriol	2.30 (1.23–4.31)	0.0146	0.0183	1.78 (0.92–3.44)	0.181

[a]OR (quintile 5 vs. quintile 1) from model conditioned on matching factors [age at baseline, calendar year of blood draw, race/ethnicity, and recency of MHT use (≤1/>1 year)] and adjusted for education, BMI, cigarette smoking status, years of oral contraceptive use, age at menarche, gravidity, age at menopause, never/former MHT use, and previous tubal ligation.
[b]*P* values for trend across quintile (median value of category).
[c]Same model as 1, also adjusted for unconjugated estradiol.

Table 4. Risk of endometrial cancer comparing the 5th to the 1st quintile for parent estrogens across categories of BMI, age, and years of oral contraceptive use

BMI	OR[a] (95% CI)	P_{trend}	$P_{interaction}$	Age	OR[a] (95% CI)	P_{trend}	$P_{interaction}$	Years of OC use	OR[a] (95% CI)	P_{trend}	$P_{interaction}$
Parent estrogens											
Estrone											
<25	3.62 (1.00–13.11)	0.02	0.41	<60	2.44 (0.63–9.44)	0.25	0.36	Never	2.12 (1.00–4.49)	0.01	0.10
25–29.9	1.08 (0.33–3.54)	0.29		60–69	2.60 (1.03–6.54)	0.003		<5 years	14.52 (2.25–93.77)	0.01	
≥30	8.06 (1.82–35.77)	0.004		≥70	10.60 (2.80–40.10)	0.001		≥5 years	15.15 (0.79–292.14)	0.02	
Unconjugated estrone											
<25	3.35 (1.16–9.69)	0.01	0.50	<60	2.90 (0.81–10.42)	0.17	0.23	Never	1.71 (0.85–3.44)	0.04	0.12
25–29.9	1.16 (0.39–3.43)	0.48		60–69	2.46 (1.02–5.92)	0.05		<5 years	29.90 (4.08–219.16)	0.003	
≥30	6.00 (1.51–23.94)	0.02		≥70	4.45 (1.29–15.29)	0.001		≥5 years	7.12 (0.50–100.97)	0.008	
Conjugated estrone											
<25	3.43 (1.05–11.26)	0.03	0.53	<60	2.53 (0.67–9.56)	0.22	0.10	Never	2.01 (0.95–4.26)	0.04	0.15
25–29.9	1.19 (0.35–4.02)	0.32		60–69	2.45 (0.99–6.09)	0.009		<5 years	10.60 (2.12–53.14)	0.005	
≥30	7.89 (2.02–30.77)	0.01		≥70	8.10 (2.23–29.46)	0.003		≥5 years	7.43 (0.39–142.68)	0.06	
Estradiol											
<25	0.88 (0.25–3.03)	0.86	0.30	<60	2.76 (0.70–10.94)	0.59	0.08	Never	1.22 (0.57–2.60)	0.33	0.36
25–29.9	0.52 (0.16–1.67)	0.37		60–69	0.92 (0.33–2.52)	0.76		<5 years	3.75 (0.54–26.15)	0.69	
≥30	6.56 (1.06–40.51)	0.05		≥70	1.53 (0.48–4.87)	0.51		≥5 years	6.23 (0.45–85.47)	0.52	
Unconjugated estradiol											
<25	7.84 (2.1–29.22)	0.01	0.48	<60	8.74 (1.47–51.92)	0.17	0.50	Never	4.19 (1.84–9.56)	0.01	0.25
25–29.9	2.39 (0.64–8.99)	0.06		60–69	4.82 (1.60–14.52)	0.04		<5 years	[b]	0.002	
≥30	6.60 (1.02–42.75)	0.05		≥70	12.61 (3.17–50.13)	0.001		≥5 years	4.62 (0.29–74.7)	0.05	
Conjugated estradiol											
<25	0.79 (0.23–2.74)	0.79	0.16	<60	1.85 (0.55–6.25)	0.64	0.24	Never	0.78 (0.37–1.66)	0.66	0.10
25–29.9	0.29 (0.09–0.94)	0.08		60–69	0.68 (0.25–1.83)	0.41		<5 years	1.26 (0.22–7.16)	0.99	
≥30	5.31 (0.87–32.4)	0.21		≥70	0.68 (0.20–2.29)	0.86		≥5 years	7.41 (0.54–100.96)	0.19	

[a]OR (quintile 5 vs. quintile 1) from model adjusted for matching factors [age at baseline, calendar year of blood draw, race/ethnicity, and recency of MHT use (never, ≤1/>1 year)] and education, cigarette smoking status, age at menarche, gravidity, age at menopause, and previous tubal ligation. Additional adjustment was made for BMI and years of oral contraceptive use, if relevant.
[b]Could not be estimated; too few cases in reference category.

Table 5. Risk of endometrial cancer comparing the 5th to the 1st quintile for each estrogen metabolite: subtype analyses according to tumor type (I vs. II) and grade (low- vs. high-grade)

	Type I ($n = 271$)		Type II ($n = 42$)			Low grade ($n = 189$)		High grade ($n = 65$)		
Parent estrogens	**OR[a] (95% CI)**	**P_{trend}[b]**	**OR[a] (95% CI)**	**P_{trend}[b]**	**P_{het}**	**OR[a] (95% CI)**	**P_{trend}[b]**	**OR[a] (95% CI)**	**P_{trend}[b]**	**P_{het}**
Estrone	3.41 (1.79–6.50)	<0.001	3.39 (0.90–12.73)	0.14	0.48	3.38 (1.65–6.96)	<0.001	3.16 (1.09–9.13)	0.01	0.63
Unconjugated estrone	2.91 (1.58–5.37)	<0.001	1.50 (0.46–4.87)	0.69	0.07	2.65 (1.34–5.21)	<0.001	2.51 (0.89–7.08)	0.11	0.27
Conjugated estrone	3.04 (1.62–5.70)	<0.001	4.22 (1.02–17.54)	0.15	0.60	3.00 (1.49–6.06)	<0.001	2.67 (0.98–7.29)	0.03	0.68
Estradiol	1.70 (0.89–3.25)	0.13	0.76 (0.22–2.62)	0.67	0.29	1.48 (0.72–3.04)	0.16	1.89 (0.54–6.62)	0.98	0.35
Unconjugated estradiol	6.97 (3.20–15.20)	<0.001	1.80 (0.43–7.49)	0.83	0.01	8.92 (3.38–23.54)	<0.001	3.27 (1.03–10.36)	0.09	0.14
Conjugated estradiol	1.16 (0.62–2.19)	0.64	0.48 (0.13–1.77)	0.28	0.26	1.05 (0.52–2.13)	0.50	1.26 (0.39–4.08)	0.57	0.45
2-Hydroxylation pathway										
2-Hydroxyestrone	2.99 (1.55–5.78)	<0.001	3.38 (0.58–19.67)	0.78	0.16	3.56 (1.65–7.69)	0.001	2.30 (0.81–6.48)	0.06	0.62
2-Hydroxyestradiol	2.43 (1.28–4.63)	0.008	2.56 (0.56–11.72)	0.71	0.40	3.16 (1.47–6.82)	0.006	1.63 (0.62–4.29)	0.16	0.62
2-Methoxyestrone	2.51 (1.36–4.65)	<0.001	0.90 (0.28–2.90)	0.81	0.16	2.27 (1.16–4.45)	0.001	3.94 (1.30–11.92)	0.01	0.93
Unconjugated 2-methoxyestrone	3.47 (1.82–6.62)	<0.001	0.95 (0.30–3.05)	0.74	0.02	3.40 (1.63–7.11)	0.003	3.45 (1.16–10.23)	0.03	0.72
Conjugated 2-methoxyestrone	1.71 (0.95–3.08)	0.007	1.19 (0.37–3.84)	0.53	0.67	1.62 (0.85–3.09)	0.01	1.99 (0.77–5.13)	0.07	0.97
2-Methoxyestradiol	4.19 (2.18–8.07)	<0.001	1.48 (0.43–5.08)	0.66	0.08	3.50 (1.68–7.28)	<0.001	5.14 (1.76–14.99)	0.00	0.42
Unconjugated 2-methoxyestradiol	3.85 (1.99–7.45)	<0.001	1.68 (0.52–5.43)	0.55	0.20	3.43 (1.63–7.25)	<0.001	4.07 (1.33–12.45)	0.01	0.76
Conjugated 2-methoxyestradiol	3.79 (1.97–7.29)	<0.001	1.97 (0.52–7.41)	0.51	0.15	3.35 (1.61–6.94)	<0.001	4.03 (1.38–11.78)	0.01	0.87
2-Hydroxyestrone-3-methyl ether	3.58 (1.80–7.11)	0.004	1.08 (0.25–4.60)	0.81	0.17	3.93 (1.80–8.58)	0.003	2.33 (0.73–7.43)	0.43	0.18
4-Hydroxylation pathway										
4-Hydroxyestrone	3.19 (1.67–6.11)	<0.001	2.73 (0.59–12.63)	0.82	0.12	4.17 (1.93–9.03)	<0.001	1.91 (0.72–5.08)	0.07	0.55
4-Methoxyestrone	3.54 (1.91–6.59)	<0.001	0.84 (0.23–3.06)	0.96	0.03	3.20 (1.61–6.37)	<0.001	4.13 (1.6–10.66)	0.00	0.30
4-Methoxyestradiol	4.00 (2.06–7.79)	<0.001	2.23 (0.59–8.45)	0.44	0.12	3.70 (1.75–7.83)	<0.001	3.60 (1.31–9.90)	0.00	0.80
16α-Hydroxylation pathway										
16α-Hydroxyestrone	2.84 (1.49–5.41)	0.001	2.23 (0.47–10.61)	0.91	0.14	3.10 (1.48–6.50)	0.002	2.50 (0.90–6.96)	0.05	0.83
Estriol	4.14 (2.14–8.00)	<0.001	3.38 (0.75–15.20)	0.34	0.21	4.85 (2.20–10.66)	<0.001	3.53 (1.32–9.46)	0.00	0.40
Unconjugated estriol	3.34 (1.75–6.35)	<0.001	1.83 (0.43–7.84)	0.88	0.03	3.02 (1.47–6.19)	<0.001	4.05 (1.39–11.77)	0.01	0.84
Conjugated estriol	3.35 (1.73–6.45)	<0.001	4.60 (0.85–25.08)	0.40	0.38	3.69 (1.71–7.96)	0.003	3.23 (1.17–8.93)	0.01	0.54
16-Ketoestradiol	3.23 (1.67–6.27)	0.002	3.27 (0.74–14.51)	0.52	0.32	3.59 (1.65–7.79)	0.004	2.65 (0.95–7.40)	0.03	0.90
16-Epiestriol	3.33 (1.70–6.54)	0.001	1.35 (0.32–5.66)	0.99	0.11	4.43 (1.96–9.98)	<0.001	2.21 (0.76–6.46)	0.19	0.29
17-Epiestriol	2.57 (1.36–4.85)	0.005	1.60 (0.40–6.39)	0.60	0.40	3.62 (1.66–7.92)	0.006	1.33 (0.53–3.35)	0.17	0.53

NOTE: Type I (66 adenocarcinomas, 194 endometrioid carcinomas, 11 mucinous) vs. type II (34 serous, 6 clear cell, 2 other); grade of tumor (low = grade 1, 2, high = grade 3, 4) evaluated among endometrioid carcinomas and adenocarcinomas only.

[a]OR (quintile 5 vs. quintile 1) from model adjusted for matching factors [age at baseline, calendar year of blood draw, race/ethnicity, and recency of MHT use (never, ≤1/>1 year)] and education, BMI, cigarette smoking status, years of oral contracteptive use, age at menarche, gravidity, age at menopause, and previous tubal ligation.

[b]*P* values for trend across quintile (median value of category).

Especially high risks of endometrial cancer have been related to obesity (particularly for type I cancers), and it is widely accepted that this is partly due to higher levels of estrogens among obese postmenopausal women given peripheral conversion of androgens to estrogens in adipose tissue (30). Several investigations have explored the extent to which estrogens explain the effects of obesity on breast cancer risk (31, 32), with findings that the relations with obesity were attenuated and became nonsignificant after adjustment for estrogens, most notably estradiol. However, effects of estrogens on the obesity–endometrial cancer relation have been less well explored. We observed similar results as have been seen for breast cancer, namely, an attenuation of risks associated with obesity after adjustment for unconjugated estradiol levels. However, for endometrial cancer, where obesity is more strongly related to risk than it is for breast cancer, we were unable to completely eliminate the obesity-associated risks, suggesting that there may be other mediators of the association. Thus, further attention seems warranted on such factors as insulin, insulin-like growth factors, androgens, and inflammatory factors, which are also related to endometrial cancer risk as well as BMI (3, 4).

Much recent interest has focused on the etiologic heterogeneity of endometrial cancer, specifically whether there are differences between type I and II tumors. It has long been speculated that the numerically predominant type I tumors have hormonally driven etiologies (excess estrogen exposure) that develop from endometrial hyperplasias; in contrast, type II tumors are considered unrelated to typical endometrial cancer risk factors and endometrial hyperplasia (33). Risk factor differences, however, have only recently been robustly assessed in epidemiologic investigations. It has thus been of interest that these studies support risk factor differences, with accepted endometrial cancer risk factors (e.g., obesity, nulliparity, absence of cigarette smoking) being stronger for type I than II tumors (17, 18, 34–36). As an extension, it would be expected that the effects of endogenous estrogens would also be differential; however, apart from one small investigation (37), these relations have not been explored.

Although estrogens appeared to influence both type I and II tumors, our findings showed that the association of unconjugated estradiol was stronger for the type I tumors, consistent with a central role for estrogens in influencing the progression of endometrial hyperplasias to these malignancies (38). Although we did not observe the difference to be statistically significant, we also observed unconjugated estradiol more strongly linked to low-grade than high-grade tumors. This finding supports recent epidemiologic (17) as well as clinical (39) data suggesting that the high-grade endometrioid tumors may be etiologically distinct from other type I cancers and more resemble type II cancers.

This study had some notable strengths, as well as a few limitations. Strengths included the relatively large number of cases identified through a well-defined population for whom

prediagnostic estrogen levels could be measured and adjusted for other important endometrial cancer risk factors. The assay used to measure estrogens and their metabolites has extremely high sensitivity and specificity. Nonetheless, many of the metabolites were highly correlated with each other, presenting problems for disentangling effects. In addition, despite the relatively large number of endometrial cases, few were diagnosed with type II tumors, limiting our ability to explore etiologic heterogeneity across tumor subtypes. We also included only postmenopausal women, conducted assays on a single serum sample collected at baseline, and did not measure sex hormone blinding globulin levels. Furthermore, estrogens were circulating measures, whereas more biologically meaningful assessments may derive from tissue assays, which are methodologically challenging (40).

In summary, our results support a central role for estrogens in endometrial cancer. Although the strongest effect was for unconjugated estradiol, other estrogens and estrogen metabolites may also contribute to risk. The association with unconjugated estradiol predominated for type I cancers, consistent with an important role for estrogenic influences in the progression of endometrial hyperplasia to cancer. Additional attention focused on estrogen metabolism in relation to other endometrial cancer biomarkers, and as measured in tissue, may further our understanding of endometrial carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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