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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 1993–1998. 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 odds ratios (OR) and 95% confidence intervals (CIs) 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, $p_{\text{trend}}=0.0001$). Nearly all metabolites were significantly associated with

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elevated risks, with some attenuation after adjustment for unconjugated estradiol (residual risks of 2-3-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 II tumors ($p_{\text{het}}=0.01$).

Conclusions—Parent estrogens as well as individual metabolites appeared to exert generalized uterotrophic 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, e.g., obesity.

Keywords

Endometrial cancer; estrogen metabolites; postmenopausal women

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 reproducibility, 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, -4, or -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 1) 16 α -hydroxyestrone is carcinogenic because it can bind covalently to the estrogen receptor with strong mitogenic effects, and 2) 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 potential mitogenic effects, with the hypotheses that high levels of 16 α -hydroxyestrone (16 α -OHE₁) would be directly associated with risk, whereas high ratios of 2-hydroxyestrone (2-OHE₁) to 16 α -OHE₁ 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-OHE₁, 2-hydroxyestradiol (2-

OHE₂) and 4-hydroxyestrone (4-OHE₁)], 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-OHE₁ and 16 α -OHE₁ 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 vs. 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 50–79 years, enrolled at U.S. centers between 1993–1998 (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 present 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 non-melanoma 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 pre-diagnosis 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 (standard deviation=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 (Figure 1), as previously detailed (22). Six labeled internal standards were used: deuterated 2-hydroxyestradiol, 2-methoxyestradiol and estriol (C/D/N Isotopes Inc, Pointe-Claire, QC, Canada); deuterated 16-epiestriol (Medical Isotopes Inc, Pelham, NH, USA); and ^{13}C -labeled estrone and estradiol (Cambridge Isotope Laboratories, Andover, MA, USA).

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 glucuronide 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 (approximately 0.33–0.37 pmol/L) (22, 23). No samples had undetectable hormone levels. 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 (median value 0.98).

Statistical analysis

We included self-reported never or former MHT pill/patch users and excluded individuals with unconjugated estradiol 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 odds ratios (ORs) and 95% confidence intervals (CIs) 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,

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 intra-category 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, 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 based on 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 and adenocarcinomas [low-grade (grades 1–2) vs. high-grade (3–4)], and time between blood collection and diagnosis (<5 year, 5 years). Differences in risk estimates across subgroups were assessed using 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 conducted the following sensitivity analyses excluding: 1) individuals diagnosed within 2 years of blood draw (n=27), 2) potential outliers [greater than five standard deviations above the median; median excluded subjects per hormone n=10 (min-max: 7–17)], 3) diabetics (n=38), and 4) former MHT users (n=220).

Q-values which reflect false discovery rates (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 majority of the subjects provided blood samples during 1993–1996 and were Caucasian. Approximately 4 percent 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 inter-decile ranges of serum estrogen metabolites for cases vs. controls are shown in Table 2. The most abundant estrogen was conjugated estrone, followed by estrinol. 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 vs. 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 Supplemental Table 1).

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 Supplemental Table 2) 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 non-significant (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.

Since 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 Supplemental Table 3). 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 to 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 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 vs. 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 to 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 2–3 fold for women in the highest vs. lowest quintiles.

Our findings provided little support for varying effects of different estrogen metabolites on endometrial cancer risk, in line with two prior small 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 uterotrophic 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.

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 non-significant 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 in 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- 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. Additionally, 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 binding 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 was restricted to 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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For a list of all the investigators who have contributed to WHI science, please visit: <https://www.whi.org/researchers/Documents%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf>

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References

1. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research*, cosponsored by the American Society of Preventive Oncology. 2002; 11:1531–43.
2. Brown SB, Hankinson SE. Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers. *Steroids*. 2015; 99:8–10. [PubMed: 25555473]

3. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocrine-related cancer*. 2008; 15:485–97. [PubMed: 18509001]
4. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Manson JE, Li J, et al. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2008; 17:921–9.
5. Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, et al. Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. *International journal of cancer Journal international du cancer*. 2004; 108:425–32. [PubMed: 14648710]
6. Zeleniuch-Jacquotte A, Akhmedkhanov A, Kato I, Koenig KL, Shore RE, Kim MY, et al. Postmenopausal endogenous oestrogens and risk of endometrial cancer: results of a prospective study. *British journal of cancer*. 2001; 84:975–81. [PubMed: 11286480]
7. Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE. Epidemiologic studies of estrogen metabolism and breast cancer. *Steroids*. 2015; 99:67–75. [PubMed: 25725255]
8. Bradlow HL, Sepkovic DW, Klug T, Osborne MP. Application of an improved ELISA assay to the analysis of urinary estrogen metabolites. *Steroids*. 1998; 63:406–13. [PubMed: 9654647]
9. Fishman J, Schneider J, Hershcopf RJ, Bradlow HL. Increased estrogen-16 alpha-hydroxylase activity in women with breast and endometrial cancer. *Journal of steroid biochemistry*. 1984; 20:1077–81. [PubMed: 6727352]
10. Cavaliere E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, et al. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochimica et biophysica acta*. 2006; 1766:63–78. [PubMed: 16675129]
11. Dallal CM, Tice JA, Buist DS, Bauer DC, Lacey JV Jr, Cauley JA, et al. Estrogen metabolism and breast cancer risk among postmenopausal women: a case-cohort study within B~FIT. *Carcinogenesis*. 2014; 35:346–55. [PubMed: 24213602]
12. Fuhrman BJ, Schairer C, Gail MH, Boyd-Morin J, Xu X, Sue LY, et al. Estrogen metabolism and risk of breast cancer in postmenopausal women. *Journal of the National Cancer Institute*. 2012; 104:326–39. [PubMed: 22232133]
13. Zeleniuch-Jacquotte A, Shore RE, Afanasyeva Y, Lukanova A, Sieri S, Koenig KL, et al. Postmenopausal circulating levels of 2- and 16alpha-hydroxyestrone and risk of endometrial cancer. *British journal of cancer*. 2011; 105:1458–64. [PubMed: 21952628]
14. Dallal CM, Lacey JV Jr, Pfeiffer RM, Bauer DC, Falk RT, Buist DS, et al. Estrogen Metabolism and Risk of Postmenopausal Endometrial and Ovarian Cancer: the B approximately FIT Cohort. *Horm Cancer*. 2016; 7:49–64. [PubMed: 26728471]
15. Audet-Walsh E, Lepine J, Gregoire J, Plante M, Caron P, Tetu B, et al. Profiling of endogenous estrogens, their precursors, and metabolites in endometrial cancer patients: association with risk and relationship to clinical characteristics. *The Journal of clinical endocrinology and metabolism*. 2011; 96:E330–9. [PubMed: 21147881]
16. Zhao H, Jiang Y, Liu Y, Yun C, Li L. Endogenous estrogen metabolites as biomarkers for endometrial cancer via a novel method of liquid chromatography-mass spectrometry with hollow fiber liquid-phase microextraction. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. 2015; 47:158–64. [PubMed: 24722971]
17. Brinton LA, Felix AS, McMeekin DS, Creasman WT, Sherman ME, Mutch D, et al. Etiologic heterogeneity in endometrial cancer: evidence from a Gynecologic Oncology Group trial. *Gynecologic oncology*. 2013; 129:277–84. [PubMed: 23485770]
18. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, et al. Type I and II endometrial cancers: have they different risk factors? *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2013; 31:2607–18. [PubMed: 23733771]
19. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Controlled clinical trials*. 1998; 19:61–109. [PubMed: 9492970]

20. Langer RD, White E, Lewis CE, Kotchen JM, Hendrix SL, Trevisan M. The Women's Health Initiative Observational Study: baseline characteristics of participants and reliability of baseline measures. *Annals of epidemiology*. 2003; 13:S107–21. [PubMed: 14575943]
21. Trabert B, Brinton LA, Anderson GL, Pfeiffer RM, Falk RT, Strickler HD, et al. Circulating estrogens and postmenopausal ovarian cancer risk in the Women's Health Initiative Observational Study. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2016
22. Xu X, Roman JM, Issaq HJ, Keefer LK, Veenstra TD, Ziegler RG. Quantitative measurement of endogenous estrogens and estrogen metabolites in human serum by liquid chromatography-tandem mass spectrometry. *Analytical chemistry*. 2007; 79:7813–21. [PubMed: 17848096]
23. Loud JT. Circulating estrogens and estrogens within the breast among postmenopausal BRCA1/2 mutation carriers. *Breast cancer research and treatment*. 2014; 148:691–2. [PubMed: 25403808]
24. Newbold RR, Liehr JG. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. *Cancer research*. 2000; 60:235–7. [PubMed: 10667565]
25. Reddy VV, Hanjani P, Rajan R. Synthesis of catechol estrogens by human uterus and leiomyoma. *Steroids*. 1981; 37:195–203. [PubMed: 6261427]
26. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis*. 1998; 19:1–27. [PubMed: 9472688]
27. Takahashi M, Shimomoto T, Miyajima K, Yoshida M, Katashima S, Uematsu F, et al. Effects of estrogens and metabolites on endometrial carcinogenesis in young adult mice initiated with N-ethyl-N'-nitro-N-nitrosoguanidine. *Cancer letters*. 2004; 211:1–9. [PubMed: 15194211]
28. Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *The Lancet Oncology*. 2012; 13:476–86. [PubMed: 22401913]
29. Trabert B, Wentzensen N, Yang HP, Sherman ME, Hollenbeck AR, Park Y, et al. Is estrogen plus progestin menopausal hormone therapy safe with respect to endometrial cancer risk? *International journal of cancer Journal international du cancer*. 2013; 132:417–26. [PubMed: 22553145]
30. Siiteri PK. Adipose tissue as a source of hormones. *The American journal of clinical nutrition*. 1987; 45:277–82. [PubMed: 3541569]
31. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *Journal of the National Cancer Institute*. 2003; 95:1218–26. [PubMed: 12928347]
32. Rinaldi S, Key TJ, Peeters PH, Lahmann PH, Lukanova A, Dossus L, et al. Anthropometric measures, endogenous sex steroids and breast cancer risk in postmenopausal women: a study within the EPIC cohort. *International journal of cancer Journal international du cancer*. 2006; 118:2832–9. [PubMed: 16385576]
33. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecologic oncology*. 1983; 15:10–7. [PubMed: 6822361]
34. Felix AS, Weissfeld JL, Stone RA, Bowser R, Chivukula M, Edwards RP, et al. Factors associated with Type I and Type II endometrial cancer. *Cancer causes & control: CCC*. 2010; 21:1851–6. [PubMed: 20628804]
35. McCullough ML, Patel AV, Patel R, Rodriguez C, Feigelson HS, Bandera EV, et al. Body mass and endometrial cancer risk by hormone replacement therapy and cancer subtype. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2008; 17:73–9.
36. Yang HP, Wentzensen N, Trabert B, Gierach GL, Felix AS, Gunter MJ, et al. Endometrial cancer risk factors by 2 main histologic subtypes: the NIH-AARP Diet and Health Study. *American journal of epidemiology*. 2013; 177:142–51. [PubMed: 23171881]
37. Sherman ME, Sturgeon S, Brinton LA, Potischman N, Kurman RJ, Berman ML, et al. Risk factors and hormone levels in patients with serous and endometrioid uterine carcinomas. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc*. 1997; 10:963–8.

38. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc.* 2000; 13:295–308.
39. Zannoni GF, Vellone VG, Arena V, Prisco MG, Scambia G, Carbone A, et al. Does high-grade endometrioid carcinoma (grade 3 FIGO) belong to type I or type II endometrial cancer? A clinical-pathological and immunohistochemical study. *Virchows Archiv: an international journal of pathology.* 2010; 457:27–34. [PubMed: 20552366]
40. Stanczyk FZ, Mathews BW, Sherman ME. Relationships of sex steroid hormone levels in benign and cancerous breast tissue and blood: A critical appraisal of current science. *Steroids.* 2015; 99:91–102. [PubMed: 25554581]

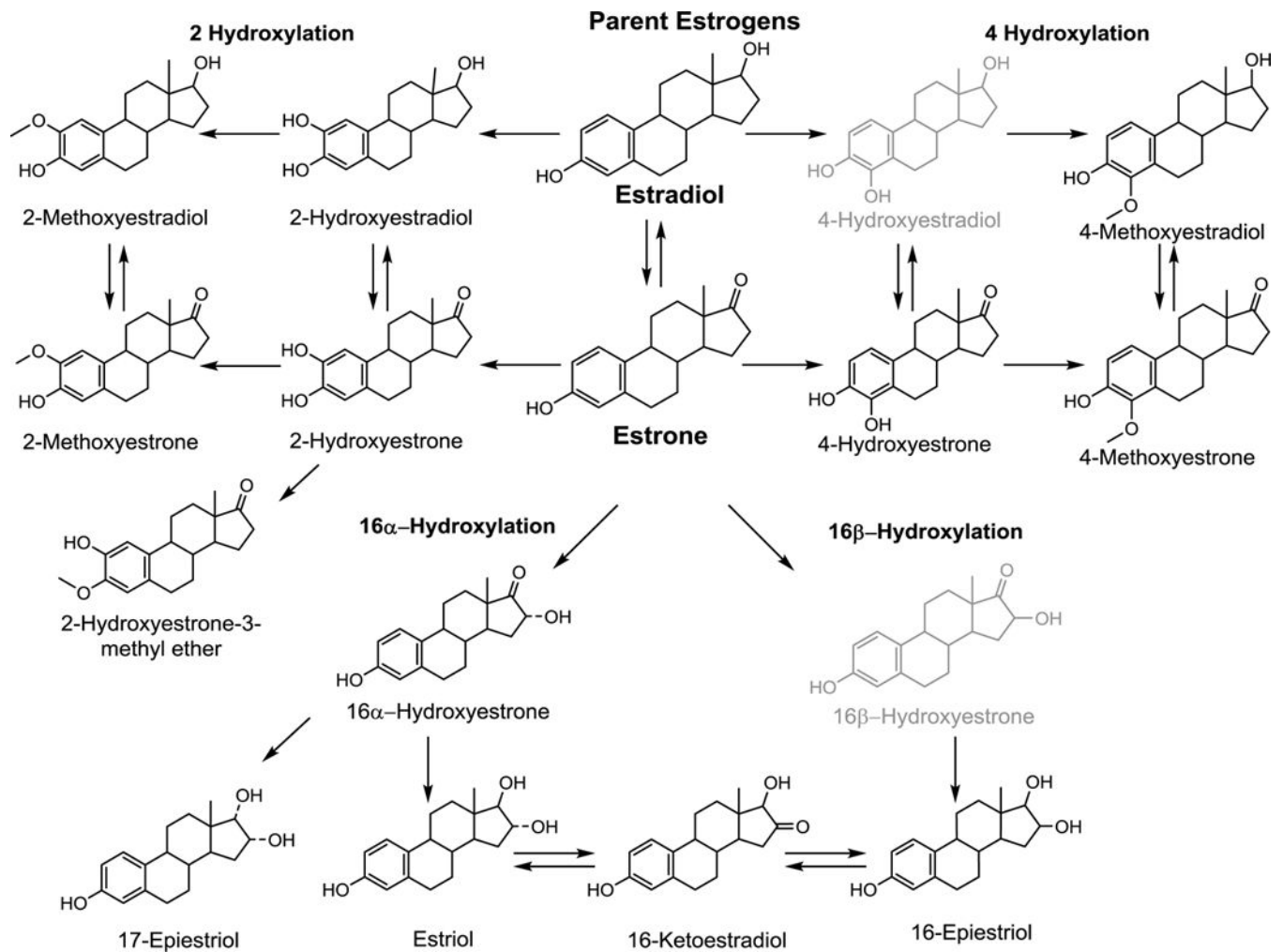


Figure 1. Formation of 2-, 4-, and 16-hydroxylation pathway estrogen metabolites from parent estrogens. The current serum estrogen metabolite assay measures 15 of the 17 metabolites pictured. 4-Hydroxyestradiol and 16 β -Hydroxyestrone (in light gray) are not measured with the current assay due to very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

Table 1

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

	Endometrial cancer cases		Controls	
	n=313		n=354	
	Mean	SD	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				

	Endometrial cancer cases		Controls	
	n=313		n=354	
	Mean	SD	Mean	SD
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

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Table 2

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

Estrogens and estrogen metabolites (pmol/L)	Cases		Controls	
	median	(IDR)	median	(IDR)
<i>Parent Estrogens</i>				
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)
<i>2-Hydroxylation Pathway</i>				
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)
<i>4-Hydroxylation Pathway</i>				
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)
<i>16alpha-Hydroxylation Pathway</i>				
16alpha-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)

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

Table 3

	OR ¹	(95% CI)	p-trend ²	FDR	OR ³	(95% CI)	p-trend ²
<i>Parent Estrogens</i>							
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			
<i>2-Hydroxylation Pathway</i>							
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
<i>4-Hydroxylation Pathway</i>							
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
<i>16alpha-Hydroxylation Pathway</i>							
16alpha-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

	OR ¹	(95% CI)	p-trend ²	FDR	OR ³	(95% CI)	p-trend ²
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

¹OR (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.

²p-values for trend across quintile (median value of category)

³Same 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 ¹	(95% CI)	p-trend	p-intx	Age	OR ¹	(95% CI)	p-trend	p-intx	Years of OC Use	OR ¹	(95% CI)	p-trend	p-intx
<i>Parent estrogens</i>															
	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 yrs	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 yrs	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 yrs	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 yrs	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 yrs	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 yrs	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 yrs	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 yrs	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 yrs	**		0.002	
	30	6.60	(1.02–42.75)	0.05		70	12.61	(3.17–50.13)	0.001		5 yrs	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 yrs	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 yrs	7.41	(0.54–100.96)	0.19	

¹OR (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.

** Could not be estimated; too few cases in reference category

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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)		
	OR ²	(95% CI)	p-trend ³	OR ²	(95% CI)	p-trend ³	OR ²	(95% CI)	p-trend ³	OR ²	(95% CI)	p-trend ³
Parent Estrogens												
Estrone	3.41	(1.79–6.50)	<0.001	3.39	(0.90–12.73)	0.14	3.38	(1.65–6.96)	<0.001	3.16	(1.09–9.13)	0.01
Unconjugated Estrone	2.91	(1.58–5.37)	<0.001	1.50	(0.46–4.87)	0.69	2.65	(1.34–5.21)	<0.001	2.51	(0.89–7.08)	0.11
Conjugated Estrone	3.04	(1.62–5.70)	<0.001	4.22	(1.02–17.54)	0.15	3.00	(1.49–6.06)	<0.001	2.67	(0.98–7.29)	0.03
Estradiol	1.70	(0.89–3.25)	0.13	0.76	(0.22–2.62)	0.67	1.48	(0.72–3.04)	0.16	1.89	(0.54–6.62)	0.98
Unconjugated Estradiol	6.97	(3.20–15.20)	<0.001	1.80	(0.43–7.49)	0.83	8.92	(3.38–23.54)	<0.001	3.27	(1.03–10.36)	0.09
Conjugated Estradiol	1.16	(0.62–2.19)	0.64	0.48	(0.13–1.77)	0.28	1.05	(0.52–2.13)	0.50	1.26	(0.39–4.08)	0.57
2-Hydroxylation Pathway												
2-Hydroxyestrone	2.99	(1.55–5.78)	<0.001	3.38	(0.58–19.67)	0.78	3.56	(1.65–7.69)	0.001	2.30	(0.81–6.48)	0.06
2-Hydroxyestradiol	2.43	(1.28–4.63)	0.008	2.56	(0.56–11.72)	0.71	3.16	(1.47–6.82)	0.006	1.63	(0.62–4.29)	0.16
2-Methoxyestrone	2.51	(1.36–4.65)	<0.001	0.90	(0.28–2.90)	0.81	2.27	(1.16–4.45)	0.001	3.94	(1.30–11.92)	0.01
Unconjugated 2-Methoxyestrone	3.47	(1.82–6.62)	<0.001	0.95	(0.30–3.05)	0.74	3.40	(1.63–7.11)	0.003	3.45	(1.16–10.23)	0.03
Conjugated 2-Methoxyestrone	1.71	(0.95–3.08)	0.007	1.19	(0.37–3.84)	0.53	1.62	(0.85–3.09)	0.01	1.99	(0.77–5.13)	0.07
2-Methoxyestradiol	4.19	(2.18–8.07)	<0.001	1.48	(0.43–5.08)	0.66	3.50	(1.68–7.28)	<0.001	5.14	(1.76–14.99)	0.00
Unconjugated 2-Methoxyestradiol	3.85	(1.99–7.45)	<0.001	1.68	(0.52–5.43)	0.55	3.43	(1.63–7.25)	<0.001	4.07	(1.33–12.45)	0.01
Conjugated 2-Methoxyestradiol	3.79	(1.97–7.29)	<0.001	1.97	(0.52–7.41)	0.51	3.35	(1.61–6.94)	<0.001	4.03	(1.38–11.78)	0.01
2-Hydroxyestrone-3-methyl ether	3.58	(1.80–7.11)	0.004	1.08	(0.25–4.60)	0.81	3.93	(1.80–8.58)	0.003	2.33	(0.73–7.43)	0.43
4-Hydroxylation Pathway												
4-Hydroxyestrone	3.19	(1.67–6.11)	<0.001	2.73	(0.59–12.63)	0.82	4.17	(1.93–9.03)	<0.001	1.91	(0.72–5.08)	0.07
4-Methoxyestrone	3.54	(1.91–6.59)	<0.001	0.84	(0.23–3.06)	0.96	3.20	(1.61–6.37)	<0.001	4.13	(1.6–10.66)	0.00
4-Methoxyestradiol	4.00	(2.06–7.79)	<0.001	2.23	(0.59–8.45)	0.44	3.70	(1.75–7.83)	<0.001	3.60	(1.31–9.90)	0.00
16alpha-Hydroxylation Pathway												
16alpha-Hydroxyestrone	2.84	(1.49–5.41)	0.001	2.23	(0.47–10.61)	0.91	3.10	(1.48–6.50)	0.002	2.50	(0.90–6.96)	0.05
Estril	4.14	(2.14–8.00)	<0.001	3.38	(0.75–15.20)	0.34	4.85	(2.20–10.66)	<0.001	3.53	(1.32–9.46)	0.00

	Type I (n=271)			Type II (n=42)			Low grade (n=189)			High Grade (n=65)				
	OR ²	(95% CI)	p-trend ³	OR ²	(95% CI)	p-trend ³	P _{het}	OR ²	(95% CI)	p-trend ³	OR ²	(95% CI)	p-trend ³	P _{het}
Parent Estrogens														
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

¹Type 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.

²OR (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 contraceptive use, age at menarche, gravidity, age at menopause, and previous tubal ligation.

³p-values for trend across quintile (median value of category)