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Citation	Oh, H., S. B. Coburn, C. E. Matthews, R. T. Falk, E. S. LeBlanc, J. Wactawski-Wende, J. Sampson, et al. 2017. "Anthropometric measures and serum estrogen metabolism in postmenopausal women: the Women's Health Initiative Observational Study." <i>Breast Cancer Research : BCR</i> 19 (1): 28. doi:10.1186/s13058-017-0810-0. <a href="http://dx.doi.org/10.1186/s13058-017-0810-0">http://dx.doi.org/10.1186/s13058-017-0810-0</a> .
Published Version	<a href="https://doi.org/10.1186/s13058-017-0810-0">doi:10.1186/s13058-017-0810-0</a>
Citable link	<a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:32072247">http://nrs.harvard.edu/urn-3:HUL.InstRepos:32072247</a>
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RESEARCH ARTICLE

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# Anthropometric measures and serum estrogen metabolism in postmenopausal women: the Women's Health Initiative Observational Study

Hannah Oh<sup>1\*</sup> , Sally B. Coburn<sup>1</sup>, Charles E. Matthews<sup>1</sup>, Roni T. Falk<sup>1</sup>, Erin S. LeBlanc<sup>2</sup>, Jean Wactawski-Wende<sup>3</sup>, Joshua Sampson<sup>1</sup>, Ruth M. Pfeiffer<sup>1</sup>, Louise A. Brinton<sup>1</sup>, Nicolas Wentzensen<sup>1</sup>, Garnet L. Anderson<sup>4</sup>, JoAnn E. Manson<sup>5</sup>, Chu Chen<sup>4</sup>, Oleg Zaslavsky<sup>6</sup>, Xia Xu<sup>7</sup> and Britton Trabert<sup>1</sup>

## Abstract

**Background:** Several anthropometric measures have been associated with hormone-related cancers. However, it is unknown whether estrogen metabolism plays an important role in these relationships. We examined whether measured current body mass index (BMI), waist-to-hip ratio (WHR), height, and self-reported BMI at age 18 years were associated with serum estrogens/estrogen metabolites using baseline, cross-sectional data from 1835 postmenopausal women enrolled in the Women's Health Initiative Observational Study.

**Methods:** Fifteen estrogens/estrogen metabolites were quantified using liquid chromatography-tandem mass spectrometry. Geometric means (GMs) of estrogens/estrogen metabolites (in picomoles per liter) were estimated using inverse probability weighted linear regression, adjusting for potential confounders and stratified on menopausal hormone therapy (MHT) use.

**Results:** Among never or former MHT users, current BMI ( $\geq 30$  vs.  $< 25$  kg/m<sup>2</sup>) was positively associated with parent estrogens (multivariable adjusted GM 432 vs. 239 pmol/L for estrone, 74 vs. 46 pmol/L for estradiol;  $p$ -trend  $< 0.001$  for both) and all of the 2-, 4-, and 16-pathway estrogen metabolites evaluated (all  $p$ -trend  $\leq 0.02$ ). After additional adjustment for estradiol, unconjugated methylated 2-catechols were inversely associated (e.g., 2-methoxyestrone multivariable GM 9.3 vs. 12.0 pmol/L;  $p$ -trend  $< 0.001$ ). Among current MHT users, current BMI was not associated with parent estrogens but was inversely associated with methylated catechols (e.g., 2-methoxyestrone multivariable GM 216 vs. 280 pmol/L;  $p$ -trend = 0.008). Similar patterns of association were found with WHR; however, the associations were not independent of BMI. Height and BMI at age 18 years were not associated with postmenopausal estrogens/estrogen metabolite levels.

**Conclusions:** Our data suggest that postmenopausal BMI is associated with increased circulating levels of parent estrogens and reduced methylation of catechol estrogen metabolites, the estrogen metabolism patterns that have previously been associated with higher breast cancer risk.

**Keywords:** BMI, WHR, Height, Estrogen, Estrogen metabolites, Sex hormones, Postmenopausal

\* Correspondence: hannah.oh@nih.gov

<sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, 6E332, Bethesda, MD 20892, USA

Full list of author information is available at the end of the article



## Background

Several anthropometric measures have been associated with increased risk of various hormone-related cancers (endometrial, ovarian, and postmenopausal breast cancers) [1–7]. Authors of meta-analyses have estimated a 54% increased risk of endometrial cancer [1] and a 12% increased risk of postmenopausal breast cancer [6] associated with every 5 kg/m<sup>2</sup> increase in body mass index (BMI). Abdominal adiposity estimated by waist-to-hip ratio (WHR) has also shown strong positive associations with endometrial and postmenopausal breast cancer risk [1, 4], although it is unclear whether the associations are independent of BMI. Further, every 10-cm increase in height has been associated with a 15% higher risk of endometrial [1] and ovarian cancers [3]. One of the hypothesized mechanisms for the BMI associations is that overweight and obese postmenopausal women have elevated levels of circulating estrogens [8], because adipocytes produce estrogens via aromatase activity [9]. Height may indicate early-life nutritional status and exposure to high levels of endogenous proliferative hormones such as estrogens in preadolescence [10].

Estrogens play a key role in endometrial, ovarian, and breast carcinogenesis [11, 12]. Parent estrogens (estradiol and estrone) stimulate cell proliferation largely via estrogen receptor-mediated mechanisms [13]. Parent estrogens are metabolized via the 2-, 4-, and 16-hydroxylation pathways, leading to an array of metabolites in each pathway. Experimental studies have shown that these metabolites can also stimulate cell proliferation via estrogen receptor-mediated mechanisms, and catechols of 2- and 4-pathways, if not methylated, can induce DNA damage directly by forming quinone DNA adducts or indirectly via redox cycling [14–16]. In recent epidemiologic studies, higher levels of parent estrogens and most estrogen metabolites have consistently been associated with higher risk of endometrial and postmenopausal breast cancers [17–20].

Parent estrogens and estrogen metabolites may play an important role in the associations between anthropometric measures and cancer risk. However, beyond parent estrogens [8, 21–23], little is known about the associations between anthropometric measures and circulating estrogen metabolites in postmenopausal women. Further, although current menopausal hormone therapy (MHT) users have higher circulating estrogen/estrogen metabolite levels [24, 25], it is unknown whether anthropometrics are associated with further differences in circulating levels. Using cross-sectional data from the Women's Health Initiative (WHI) Observational Study (OS), we examined whether current BMI, WHR, height, and BMI at age 18 years were associated with circulating levels of 15 estrogens/estrogen metabolites in postmenopausal women by MHT use.

## Methods

### Study population

This study includes participants in a case-control study of ovarian and endometrial cancers nested within the WHI-OS [20, 26]. The WHI-OS is a prospective cohort study in which researchers recruited 93,676 postmenopausal women aged 50–79 years at 40 clinical centers in the United States between 1993 and 1998 [27, 28]. At the baseline clinic visit, anthropometric measures (height, weight, and waist and hip circumferences) were measured and blood samples were collected. Additionally, baseline self-administered questionnaires were used to collect information on participants' demographics, medical history, and health behaviors.

Details of the nested case-control study are described elsewhere [20, 26]. In brief, cases were women with ovarian or endometrial cancer diagnosed between study enrollment and 2012. Control subjects were selected from among those women who remained cancer-free at the date of case diagnosis and were frequency-matched to cases on the basis of age at baseline (5-year categories), year of blood draw (1993–1996, 1997–1998), race/ethnicity (white, black, Hispanic, other/unknown), hysterectomy at baseline or during follow-up prior to the index date (for ovarian control subjects only), and MHT use (never,  $\leq 1$  year since last MHT use,  $> 1$  year since last MHT use, current). Participants had no history of cancer (except nonmelanoma skin cancer), bilateral oophorectomy, or hysterectomy (for endometrial control subjects only), and had  $\geq 1.1$  ml of serum available.

A total of 1835 women (507 cases and 450 control subjects among never/former MHT users, 457 cases and 421 control subjects among current MHT users), representing 56,109 women when weighted back to the entire cohort, were included in the study. Because all serum samples were collected at baseline prior to cancer diagnosis (mean 6.7 years from baseline to cancer diagnosis), we included both cases and control subjects in this cross-sectional analysis.

### Exposure assessment

Baseline measured height and weight as well as waist and hip circumferences were used to calculate current BMI (in kilograms per meter squared) and WHR, respectively. BMI at age 18 years was estimated using height and weight at age 18 years recalled via baseline questionnaire. Current BMI was categorized into three groups using the World Health Organization classification cutpoints for overweight and obesity:  $< 25.0$  kg/m<sup>2</sup> (normal), 25.0–29.9 kg/m<sup>2</sup> (overweight), and  $\geq 30$  kg/m<sup>2</sup> (obese) [29]. In secondary analyses, we subdivided the obesity category by class (class 1: 30.0–34.9 kg/m<sup>2</sup>, class 2: 35.0–39.9 kg/m<sup>2</sup>, class 3:  $\geq 40$  kg/m<sup>2</sup>) [29] to further

examine the potential dose-response relationship between current BMI and estrogens/estrogen metabolites. WHR, adult height, and BMI at age 18 years were categorized into tertile groups based on the distributions in the entire study population as follows: WHR <0.76, 0.76–0.82,  $\geq$ 0.83; height <160 cm, 160–164.9 cm,  $\geq$ 165 cm; and BMI at age 18 years <20 kg/m<sup>2</sup>, 20–21.9 kg/m<sup>2</sup>,  $\geq$ 22 kg/m<sup>2</sup>.

#### Laboratory assays

Aliquoted and batched serum samples were transferred to the Laboratory of Proteomics and Analytical Technologies, Cancer Research Technology Program, Leidos Biomedical Research, Inc. (Frederick, MD, USA), for testing. The assay quantifies the combined concentrations of conjugated and unconjugated forms of 2 parent estrogens (estrone, estradiol) and 13 estrogen metabolites (2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, 4-hydroxyestrone, 4-methoxyestrone, 4-methoxyestradiol, 16 $\alpha$ -hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, 17-epiestriol), as well as the unconjugated concentration of 5 analytes (estrone, estradiol, estriol, 2-methoxyestrone, 2-methoxyestradiol), in serum using a stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay [25]. For the five estrogens/estrogen metabolites with unconjugated measurements, their conjugated concentration was calculated by subtracting the unconjugated concentration from the measured (combined) concentration. For the other ten estrogen metabolites, unconjugated concentration was not separately measured, owing to their low abundance in unconjugated forms. (When we do not specify unconjugated or conjugated form, we refer to the combined measured concentration throughout the paper.) Assay reliability was monitored using masked technical replicates across batches. Coefficients of variation were <6% for all analytes; intraclass correlation coefficients (ICCs) ranged from 0.93 to 0.996 (median 0.98) [20, 26]. Correlations among the estrogens/estrogen metabolites ranged from 0.34 to 0.99 [20].

#### Statistical analyses

Because estrogen/estrogen metabolite levels vary by MHT use [24, 25], all analyses were stratified on MHT use ( $n = 957$  never/former users,  $n = 878$  current users). Inverse probability sampling weights were used to adjust the data to represent the population in the entire cohort using methods for secondary phenotype analysis of case-control data described by Li and Gail [30]. Sampling weights were calculated by taking the inverse of sampling fractions: 1 for all cases, and varying weights for control subjects, depending on their strata as defined by matching factors. After log transformation of data to improve normality, geometric means

(GMs) in picomoles per liter of individual serum estrogen/estrogen metabolite concentration by exposure categories were estimated using inverse probability weighted linear regression, adjusting for potential confounders: age at blood draw, blood draw year, race, smoking status (never, former, current), and time since menopause (<10 years, 10–19 years,  $\geq$ 20 years, missing). Additional adjustments for soy and alcohol intake did not change the results and thus were not included in the final models. For current BMI and WHR, we additionally adjusted for physical activity (0, 0.1–9.9,  $\geq$ 10.0 metabolic equivalents of task-h/week). We performed a test for trend by including the exposure in the model as a continuous variable. The percentage change in GMs between the highest and the lowest categories was estimated by taking the ratio of the GM difference between the two categories over the GM of the lowest category, multiplied by 100. We statistically tested for the difference using a Wald test.

Several secondary analyses were performed. First, for WHR and BMI at age 18 years (Spearman's  $r \leq 0.48$  with current BMI), we estimated the associations after additional adjustment for current BMI to examine their independent associations. Second, for current BMI among never/former MHT users, we additionally adjusted for unconjugated estradiol to examine whether the associations with individual estrogen metabolites were driven by their correlations with unconjugated estradiol, the estrogen/estrogen metabolite most strongly correlated with current BMI (Spearman's  $r = 0.46$ ). Next, using pathway groups, we investigated whether current BMI was associated with altered patterns of estrogen metabolism. We compared the mean proportions of parent estrogens out of summed estrogens/estrogen metabolites across BMI categories, with adjustment for the summed concentration of estrogens/estrogen metabolites. Further, because 2-, 4-, and 16-pathway metabolites ("child metabolites") are metabolized from a limited pool of shared precursors (parent estrogens), an increase in the level of one downstream pathway indicates a reduction in levels of other competing pathways. To address this, we modeled proportions of each child metabolite pathway group (2-catechols [2-hydroxyestrone, 2-hydroxyestradiol]; methylated 2-catechols [2-methoxyestrone and 2-methoxyestradiol]; 4-catechols [4-hydroxyestrone]; methylated 4-catechols [4-methoxyestrone, 4-methoxyestradiol]; 16-pathway metabolites [16 $\alpha$ -hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, 17-epiestriol]) out of summed child metabolites, with adjustment for the summed concentration of child metabolites. This approach estimates the association with replacement of one pathway group for other pathway groups while holding summed child metabolites constant. We tested for any difference across BMI

categories using a global *F* test; if there was a significant difference ( $p < 0.05$ ), we performed pairwise *t* tests for three different combinations of BMI comparisons ( $25\text{--}29.9\text{ kg/m}^2$  vs.  $<25\text{ kg/m}^2$ ;  $\geq 30\text{ kg/m}^2$  vs.  $<25\text{ kg/m}^2$ ;  $\geq 30\text{ kg/m}^2$  vs.  $25\text{--}29.9\text{ kg/m}^2$ ).

Finally, because subclinical or underlying diseases may influence the associations, we performed sensitivity analyses after excluding endometrial and ovarian cancer cases ( $n = 507$  never/former MHT users,  $n = 457$  current MHT users); excluding women with a history of diabetes at baseline ( $n = 57$  never/former MHT users,  $n = 28$  current MHT users); excluding women with low BMI ( $<18.5\text{ kg/m}^2$ ;  $n = 14$  never/former MHT users,  $n = 14$  current MHT users); and, among never/former MHT users, excluding women using any potential hormone-related medications (e.g., antiestrogens, glucocorticoid steroids;  $n = 303$ ). To test the robustness of our results, we also repeated analyses after excluding outliers ( $\leq 10$  values for never/former MHT users,  $\leq 10$  values for current MHT users) identified using the extreme Studentized deviate many-outlier procedure [31] and after further stratifying by never vs. former MHT users ( $n = 645$  never MHT users,  $n = 312$  former MHT users).

All statistical tests were two-sided with a 5% type I error rate. *Q* values reflecting the false discovery rates (FDRs) were calculated to account for multiple comparisons (25 tests per exposure). Analyses were conducted with SAS version 9 software (SAS Institute, Cary, NC, USA).

## Results

### Study population characteristics

The mean age was 64.6 years for never/former MHT users and 61.3 years for current MHT users (Table 1). Most women were white (89% among never/former users, 94% among current users). Compared with never/former MHT users, current users were more likely to have been postmenopausal for  $<10$  years (44% vs. 32%). Mean current BMI, BMI at age 18 years, height, and WHR were similar between the two groups. As expected, serum concentrations of all evaluated estrogens/estrogen metabolites were higher in current MHT users than in never/former users (Tables 2 and 3).

### Current body mass index

Among never/former MHT users, higher current BMI ( $\geq 30$  vs.  $<25\text{ kg/m}^2$ ) was significantly associated with higher levels of parent estrogens (GM 432 vs. 239 pmol/L for estrone, 73.9 vs. 45.9 pmol/L for estradiol;  $p$ -trend  $< 0.001$ ) and all of the 2-, 4-, and 16-pathway metabolites evaluated (all  $p$ -trend  $\leq 0.02$ ) (Table 2). However, after adjustment for unconjugated estradiol, these patterns did not persist, and inverse associations were observed for unconjugated methylated 2-catechols (9.29 vs. 12.0 pmol/L,

$p$ -trend  $< 0.001$  for unconjugated 2-methoxyestrone; 1.88 vs. 2.37 pmol/L,  $p$ -trend = 0.002 for unconjugated 2-methoxyestradiol) (Table 2). Similar dose-response trends were found across classes 1–3 obesity (Additional file 1: Table S1).

Among current MHT users, current BMI ( $\geq 30$  vs.  $<25\text{ kg/m}^2$ ) was not associated with parent estrogens (3201 vs. 3201 pmol/L for estrone; 414 vs. 444 pmol/L for estradiol;  $p$ -trend  $\geq 0.13$ ), but it was inversely associated with methylated catechols (216 vs. 280 pmol/L,  $p$ -trend = 0.008 for 2-methoxyestrone; 73.7 vs. 92.6 pmol/L,  $p$ -trend = 0.02 for 2-methoxyestradiol; 23.1 vs. 28.9 pmol/L,  $p$ -trend = 0.02 for 4-methoxyestrone) (Table 3).

In secondary analyses among never/former MHT users, the proportion of parent estrogens was significantly higher in obese women (BMI  $\geq 30$  vs.  $<25\text{ kg/m}^2$ , 50.8% vs. 46.7%, respectively;  $p = 0.006$ ) (Fig. 1). Further, obese women had lower proportions of methylated 2-catechols (15.4% vs. 17.7%;  $p = 0.001$ ) and methylated 4-catechols (1.5% vs. 1.8%;  $p = 0.003$ ) and higher proportions of 16-pathway metabolites (60.3% vs. 57.8%;  $p = 0.0002$ ) (Fig. 2). Similar results were found in overweight women; however, there was no statistically significant difference in proportions between overweight and obese women. Results were similar among current MHT users, although the association with proportion of parent estrogens was statistically nonsignificant (BMI  $\geq 30$  vs.  $<25\text{ kg/m}^2$ , 56.4% vs. 54.3%, respectively; overall *F* test  $p = 0.40$ ) (Additional file 2: Figure S1, Additional file 3: Figure S2).

### Waist-to-hip ratio

Among never/former MHT users, WHR ( $\geq 0.83$  vs.  $<0.76$ ) was positively associated with parent estrogens (348 vs. 286 pmol/L for estrone, 62.5 vs. 50.2 pmol/L for estradiol;  $p$ -trend  $\leq 0.01$ ) and nearly all of the 16-pathway metabolites; however, in models mutually adjusting for current BMI and WHR, only current BMI remained significantly associated (Table 4). Among current MHT users, WHR was not associated with parent estrogens but was inversely associated with methylated catechols, namely 2-methoxyestrone (219 vs. 272 pmol/L,  $p$ -trend = 0.03), 2-hydroxyestrone-3-methyl ether (35.4 vs. 44.1 pmol/L,  $p$ -trend = 0.03), and 4-methoxyestrone (22.8 vs. 28.6 pmol/L,  $p$ -trend = 0.04); the associations did not remain significant after adjustment for current BMI (Additional file 4: Table S2).

### Height

Height ( $\geq 165$  vs.  $<160$  cm) was not associated with circulating estrogens/estrogen metabolites in postmenopausal women, except for inverse associations with conjugated 2-methoxyestrone (27.0 vs. 33.2 pmol/L,  $p$ -trend = 0.01) and

**Table 1** Characteristics of study population in the Women's Health Initiative Observational Study

Characteristic	Never or former menopausal hormone therapy users (n = 957)			Current menopausal hormone therapy users (n = 878)		
	n	%	Weighted % <sup>a</sup>	n	%	Weighted % <sup>a</sup>
Age at baseline blood draw						
<55 years	82	8.6	8.7	101	11.5	16.8
55–59 years	177	18.5	17.3	194	22.1	25.8
60–64 years	230	24.0	21.0	218	24.8	23.9
65–69 years	201	21.0	23.1	178	20.3	18.3
70–74 years	170	17.8	19.3	135	15.4	11.2
75–79 years	97	10.1	10.6	52	5.9	4.0
Race						
White	841	87.9	89.4	825	94.0	93.5
Year at blood draw						
1993–1996	586	61.2	61.4	544	62.0	61.9
1997–1998	371	38.8	38.6	334	38.0	38.1
Smoking status						
Never	488	51.0	50.2	427	48.6	45.9
Former	402	42.0	41.3	416	47.4	48.8
Current	67	7.0	8.5	35	4.0	5.3
Time since menopause						
<10 years	299	32.9	31.5	349	39.7	44.0
10–19 years	359	39.5	38.7	330	37.6	34.8
≥ 20 years	250	27.5	29.9	199	22.7	21.1
Moderate- to vigorous-intensity physical activity						
0 MET-h/week	205	21.4	19.9	145	16.5	18.4
0.1–9.9 MET-h/week	304	31.8	30.9	279	31.8	34.2
≥10.0 MET-h/week	448	46.8	49.3	454	51.7	47.4
Characteristic	n	Weighted n <sup>a</sup>	Weighted mean (SD)	n	Weighted n <sup>a</sup>	Weighted mean (SD)
Current BMI, kg/m <sup>2</sup>	953	30,814	27.0 (0.27)	877	25,152	26.6 (0.28)
BMI at age 18 years, kg/m <sup>2</sup>	933	30,149	20.6 (0.15)	859	24,661	20.5 (0.12)
Waist-to-hip ratio	952	30,720	0.81 (0.004)	877	25,152	0.79 (0.004)
Height, cm	956	30,817	162.2 (0.30)	877	25,152	163.1 (0.34)

BMI Body mass index, MET Metabolic equivalent of task

<sup>a</sup>Weighted percentages and weighted n reflect weighted counts and refer to the study cohort

4-methoxyestrone (3.90 vs. 4.59 pmol/L, *p*-trend = 0.03) among never/former MHT users (Table 5).

#### Body mass index at age 18 years

Among never/former MHT users, BMI at age 18 years (≥22 vs. <20 kg/m<sup>2</sup>) was positively associated with estradiol (64.1 vs. 49.8 pmol/L; *p*-trend = 0.04) but was not associated with other estrogens/estrogen metabolites (Additional file 5: Table S3). The association with estradiol did not remain after adjustment for current BMI (data not shown), suggesting that the association may be accounted for by current BMI. Among current MHT users, BMI at age 18 years was not associated with estrogens/estrogen metabolites (Additional file 5: Table S3).

There was no statistically significant interaction between BMI at age 18 years and current BMI (*p*-interaction >0.05) (data not shown).

Most associations remained at 5% FDR (Tables 2, 3, 4 and 5, Additional file 1: Table S1, Additional file 4: Table S2, Additional file 5: Table S3). Results were similar after excluding endometrial or ovarian cancer cases at the time of last follow-up (Additional file 6: Table S4), after excluding women who reported a history of diabetes at baseline (data not shown), after excluding women with low BMI (<18.5 kg/m<sup>2</sup>) (data not shown), after excluding women using any potential hormone-related medications (data not shown), and after further stratifying by never vs. former MHT users (data not

**Table 2** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by current body mass index in postmenopausal women not using menopausal hormone therapy in the Women's Health Initiative Observational Study

	Model 1 <sup>a</sup>				Model 1 + unconjugated estradiol <sup>b</sup>				
	Geometric mean (95% CI)		p-trend <sup>c</sup>	%Δ <sup>d</sup>	Geometric mean (95% CI)		p-trend <sup>c</sup>	%Δ <sup>d</sup>	p-diff <sup>e</sup>
	<25 kg/m <sup>2</sup>	≥30 kg/m <sup>2</sup>			<25 kg/m <sup>2</sup>	≥30 kg/m <sup>2</sup>			
Median, kg/m <sup>2</sup>	22.5	33.6			22.5	33.6			
n	345	314			345	314			
Weighted n <sup>f</sup>	13,571	7893			13,571	7893			
Estrone	239 (201–284)	432 (355–525)	<0.001 <sup>g</sup>	80.8	313 (266–368)	344 (296–399)	0.91	4.2	0.64
Conjugated	179 (147–218)	348 (281–431)	<0.001 <sup>g</sup>	94.4	237 (197–284)	271 (229–320)	0.59	9.7	0.35
Unconjugated	51.3 (45.0–58.6)	75.6 (65.2–87.8)	<0.001 <sup>g</sup>	47.4	64.4 (57.5–72.1)	63.4 (58.0–69.3)	0.13	-7.1	0.24
Estradiol	45.9 (37.6–56.1)	73.9 (60.0–91.0)	<0.001 <sup>g</sup>	61.0	60.7 (52.5–70.2)	60.3 (52.7–68.8)	0.33	-8.9	0.19
Conjugated	31.0 (25.2–38.2)	45.9 (36.6–57.6)	<0.001 <sup>g</sup>	48.1	38.5 (31.7–46.9)	40.7 (34.1–48.7)	0.99	-4.9	0.61
Unconjugated	10.2 (8.13–12.7)	23.5 (18.8–29.4)	<0.001 <sup>g</sup>	130	NA	NA	NA	NA	NA
2-Hydroxyestrone	55.6 (47.8–64.8)	82.9 (70.5–97.4)	<0.001 <sup>g</sup>	49.1	66.1 (56.7–77.0)	72.6 (63.3–83.3)	0.83	4.8	0.59
2-Hydroxyestradiol	14.2 (12.3–16.5)	20.4 (17.3–24.0)	<0.001 <sup>g</sup>	43.7	16.8 (14.4–19.5)	18.0 (15.6–20.6)	0.69	2.4	0.76
2-Methoxyestrone	37.8 (33.2–43.0)	47.7 (41.6–54.6)	0.001 <sup>g</sup>	26.2	44.6 (39.5–50.3)	44.2 (39.4–49.5)	0.05	-9.9	0.15
Conjugated	27.5 (23.9–31.6)	34.5 (29.7–40.2)	0.002 <sup>g</sup>	25.5	31.8 (27.9–36.2)	32.5 (28.6–37.0)	0.25	-6.6	0.42
Unconjugated	9.70 (8.39–11.2)	11.6 (10.0–13.5)	0.02 <sup>h</sup>	19.6	12.0 (10.5–13.8)	10.6 (9.29–12.2)	<0.001 <sup>g</sup>	-22.6	0.001 <sup>h</sup>
2-Methoxyestradiol	11.4 (9.63–13.5)	16.4 (13.7–19.7)	<0.001 <sup>g</sup>	43.9	13.7 (11.8–16.0)	13.7 (12.0–15.6)	0.50	-0.7	0.91
Conjugated	9.02 (7.49–10.9)	13.4 (11.0–16.4)	<0.001 <sup>g</sup>	48.6	10.8 (9.01–12.8)	10.9 (9.17–12.8)	0.83	3.7	0.70
Unconjugated	1.91 (1.63–2.23)	2.36 (2.02–2.76)	<0.001 <sup>g</sup>	23.6	2.37 (2.12–2.65)	2.24 (1.99–2.53)	0.002 <sup>h</sup>	-20.7	<0.001 <sup>g</sup>
2-Hydroxyestrone-3-methyl ether	6.74 (5.93–7.67)	8.72 (7.59–10.0)	0.004 <sup>g</sup>	29.4	7.68 (6.71–8.79)	8.01 (7.02–9.15)	0.24	-0.8	0.92
4-Hydroxyestrone	6.77 (5.80–7.89)	10.2 (8.57–12.1)	<0.001 <sup>g</sup>	50.7	7.97 (6.82–9.32)	8.80 (7.65–10.1)	0.96	7.5	0.44
4-Methoxyestrone	3.93 (3.45–4.48)	4.86 (4.18–5.65)	0.02 <sup>h</sup>	23.7	4.47 (3.89–5.13)	4.40 (3.91–4.96)	0.19	-4.9	0.58
4-Methoxyestradiol	1.66 (1.41–1.95)	2.22 (1.86–2.65)	<0.001 <sup>g</sup>	33.7	1.95 (1.66–2.29)	1.89 (1.65–2.16)	0.30	-4.1	0.64
16α-Hydroxyestrone	27.5 (23.4–32.4)	41.6 (35.0–49.4)	<0.001 <sup>g</sup>	51.3	32.8 (27.8–38.6)	36.3 (31.4–41.9)	0.92	5.8	0.54
Estril	114 (97.0–133)	178 (151–211)	<0.001 <sup>g</sup>	56.1	137 (117–160)	157 (137–181)	0.94	7.3	0.45
Conjugated	87.1 (73.0–104)	143 (118–172)	<0.001 <sup>g</sup>	64.2	105 (88.2–125)	126 (108–147)	0.60	12.4	0.28
Unconjugated	23.2 (20.1–26.6)	33.0 (28.3–38.4)	<0.001 <sup>g</sup>	42.2	27.9 (24.5–31.7)	27.8 (24.5–31.6)	0.29	-2.5	0.74

**Table 2** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by current body mass index in postmenopausal women not using menopausal hormone therapy in the Women's Health Initiative Observational Study (Continued)

16-Ketoestradiol	29.1 (24.7–34.3)	38.2 (32.4–45.1)	44.3 (37.2–52.9)	<0.001 <sup>19</sup>	52.2	<0.001 <sup>19</sup>	34.7 (29.4–40.9)	38.7 (33.3–45.0)	37.0 (31.3–43.7)	0.95	6.6	0.50
16-Epiestriol	12.9 (11.1–14.9)	16.4 (14.0–19.2)	19.3 (16.5–22.6)	<0.001 <sup>19</sup>	49.6	<0.001 <sup>19</sup>	15.1 (13.1–17.4)	16.6 (14.4–19.1)	16.4 (14.1–19.0)	0.78	8.6	0.31
17-Epiestriol	10.8 (9.37–12.3)	13.5 (11.6–15.6)	16.4 (14.0–19.3)	<0.001 <sup>19</sup>	51.9	<0.001 <sup>19</sup>	12.6 (11.0–14.4)	13.6 (12.0–15.5)	13.9 (12.0–16.2)	0.72	10.3	0.21

NA Not applicable

<sup>19</sup>Model 1: adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, 75–79 years), blood draw year (1993–1996, 1997–1998), race (white, nonwhite), smoking status (never, former, current), time since menopause (<10 years, 10–19 years, ≥20 years, missing), moderate- to vigorous-intensity physical activity (0, 0.1–9.9, ≥10 metabolic equivalents of task-h/week)

<sup>20</sup>Model 1 + unconjugated estradiol (log-transformed, continuous)

<sup>21</sup>p-trend was estimated using the Wald test for continuous body mass index (BMI; in kilograms per meter squared)

<sup>22</sup>%Δ indicates the percentage change in estrogen/estrogen metabolite levels, comparing women with current BMI ≥30 vs. <25 kg/m<sup>2</sup>, and was estimated by taking the ratio of the geometric mean difference in estrogen/estrogen metabolite levels between women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> to the geometric mean of women with current BMI <25 kg/m<sup>2</sup>, multiplied by 100

<sup>23</sup>p-diff was estimated using the Wald test and indicates a p-value for comparing estrogen/estrogen metabolite levels of women with current BMI ≥30 vs. <25 kg/m<sup>2</sup>

<sup>24</sup>Weighted n reflects weighted counts and refers to the study cohort

<sup>25</sup>false discovery rate (FDR) q value <0.01

<sup>26</sup>FDR q values <0.05 and ≥0.01

Note: Italicized p-values indicate nominal statistical significance at 0.05



**Table 3** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by current body mass index in postmenopausal women currently using menopausal hormone therapy in the Women's Health Initiative Observational Study

	Geometric means (95% CI) <sup>a</sup>			<i>p</i> -trend <sup>b</sup>	%Δ <sup>c</sup>	<i>p</i> -diff <sup>d</sup>
	<25 kg/m <sup>2</sup>	25–29.9 kg/m <sup>2</sup>	≥30 kg/m <sup>2</sup>			
Median (kg/m <sup>2</sup> )	22.4	27.2	33.1			
<i>n</i>	445	257	175			
Weighted <i>n</i> <sup>e</sup>	11,498	7844	5810			
Estrone	3201 (2433–4212)	2668 (1960–3632)	3201 (2291–4472)	0.46	0.0	1.00
Conjugated	2930 (2208–3887)	2473 (1801–3396)	2896 (2046–4098)	0.48	–1.2	0.94
Unconjugated	227 (186–277)	191 (150–244)	205 (158–264)	0.18	–9.7	0.38
Estradiol	444 (339–582)	390 (292–522)	414 (300–572)	0.13	–6.8	0.62
Conjugated	368 (273–497)	327 (237–450)	341 (238–486)	0.13	–7.3	0.63
Unconjugated	42.5 (33.8–53.5)	42.9 (33.1–55.7)	50.8 (38.5–66.9)	0.15	19.5	0.17
2-Hydroxyestrone	462 (378–565)	382 (300–486)	422 (331–539)	0.21	–8.7	0.42
2-Hydroxyestradiol	109 (90.6–131)	89.1 (70.9–112)	98.3 (77.7–124)	0.16	–9.8	0.35
2-Methoxyestrone	280 (236–333)	219 (180–266)	216 (174–268)	0.008	–22.9	0.02
Conjugated	174 (142–215)	135 (108–169)	139 (109–177)	0.04	–20.1	0.05
Unconjugated	78.8 (62.4–99.5)	62.5 (45.6–85.8)	56.3 (41.3–76.8)	<0.001 <sup>f</sup>	–28.6	0.02
2-Methoxyestradiol	92.6 (72.7–118)	70.1 (54.1–90.8)	73.7 (57.4–94.6)	0.02	–20.4	0.03
Conjugated	80.9 (62.0–105)	58.3 (43.8–77.5)	60.8 (46.3–79.7)	0.001 <sup>g</sup>	–24.8	0.01
Unconjugated	8.90 (7.56–10.5)	8.43 (6.86–10.4)	8.08 (6.49–10.1)	0.19	–9.2	0.38
2-Hydroxyestrone-3-methyl ether	44.2 (36.7–53.2)	34.8 (28.3–42.6)	36.8 (29.3–46.3)	0.04	–16.7	0.09
4-Hydroxyestrone	61.6 (50.2–75.5)	51.3 (40.2–65.4)	57.0 (44.4–73.1)	0.26	–7.5	0.50
4-Methoxyestrone	28.9 (24.2–34.6)	23.1 (19.0–28.0)	23.1 (18.7–28.6)	0.02	–20.1	0.04
4-Methoxyestradiol	12.3 (9.51–16.0)	9.61 (7.26–12.7)	9.98 (7.52–13.2)	0.04	–18.9	0.07
16α-Hydroxyestrone	247 (200–304)	200 (155–257)	227 (177–292)	0.28	–8.1	0.48
Estriol	1136 (897–1438)	966 (740–1261)	1075 (812–1424)	0.36	–5.4	0.67
Conjugated	988 (772–1264)	844 (638–1117)	914 (680–1227)	0.35	–7.5	0.57
Unconjugated	129 (106–158)	113 (89.3–142)	116 (90.3–148)	0.12	–10.1	0.29
16-Ketoestradiol	286 (231–354)	229 (178–295)	262 (203–339)	0.30	–8.4	0.47
16-Epiestriol	86.7 (71.1–106)	70.2 (56.5–87.3)	79.5 (62.7–101)	0.21	–8.3	0.44
17-Epiestriol	58.2 (47.6–71.3)	46.5 (37.2–58.1)	51.9 (40.6–66.4)	0.28	–10.8	0.31

<sup>a</sup>Adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, 75–79 years), blood draw year (1993–1996, 1997–1998), race (white, nonwhite), smoking status (never, former, current), time since menopause (<10 years, 10–19 years, ≥20 years, missing), moderate- to vigorous-intensity physical activity (0, 0.1–9.9, ≥10 metabolic equivalents of task-h/week)

<sup>b</sup>*p*-trend was estimated using the Wald test for continuous body mass index (BMI; kilograms per meter squared)

<sup>c</sup>%Δ indicates the percentage change in estrogen/estrogen metabolite levels, comparing women with current BMI ≥30 vs. <25 kg/m<sup>2</sup>, and was estimated by taking the ratio of the geometric mean difference in estrogen/estrogen metabolite levels between women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> to the geometric mean of women with current BMI <25 kg/m<sup>2</sup>, multiplied by 100

<sup>d</sup>*p*-diff was estimated using the Wald test and indicates a *p* value for comparing estrogen/estrogen metabolite levels of women with current BMI ≥30 vs. <25 kg/m<sup>2</sup>

<sup>e</sup>Weighted *n* reflects weighted counts and refers to the study cohort

<sup>f</sup>False discovery rate (FDR) *q* value <0.01

<sup>g</sup>FDR *q* values <0.05 and ≥0.01

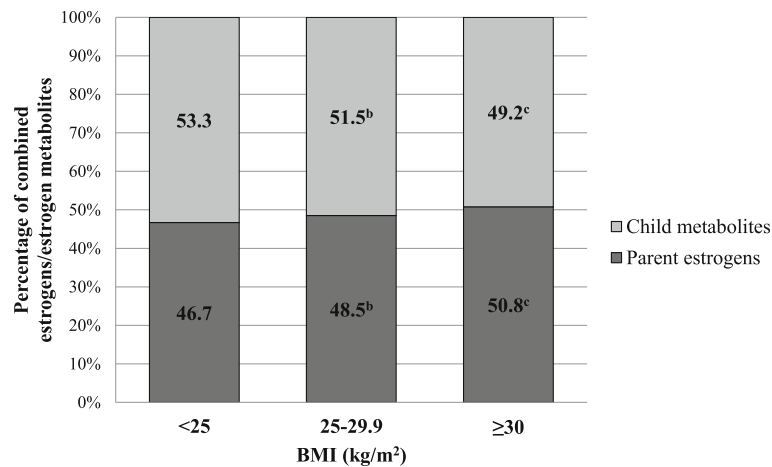
Note: Italicized *p*-values indicate nominal statistical significance at 0.05

shown). Results also did not change after excluding outliers (data not shown).

## Discussion

To the best of our knowledge, this study is the first to examine the relationships of anthropometric measures with detailed serum estrogen/estrogen metabolite

measures in postmenopausal women by MHT use. In this cross-sectional analysis, current measured BMI was positively associated with parent estrogens and all estrogen metabolites evaluated among never/former MHT users. Adjusted for unconjugated estradiol, current BMI was inversely associated with methylated 2-catechols but not with the other estrogen metabolites measured,



**Fig. 1** Percentages of parent estrogens (estradiol and estrone) and child estrogen metabolites (2-, 4-, and 16-hydroxylation pathway metabolites) out of summed estrogens/estrogen metabolites by current body mass index (BMI) among never or former menopausal hormone therapy users<sup>a</sup>. Adjusted for age at blood draw (<55, 55-59, 60-64, 65-69, 70-74, 75-79 years), blood draw year (1993-1996, 1997-1998), race (white, non-white), smoking status (never, former, current), time since menopause (<10, 10-19, ≥20 years, missing), moderate- to vigorous-intensity physical activity (0, 0.1-9.9, ≥10 MET-hr/wk), summed concentration of estrogens/estrogen metabolites (continuous, pmol/L).

Note: Summed estrogens/estrogen metabolites include the summed concentration of parent estrogens (estrone, estradiol) and child metabolites (2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, 4-hydroxyestrone, 4-methoxyestrone, 4-methoxyestradiol, 16 $\alpha$ -hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, 17-epiestriol).

<sup>a</sup>Overall F-test  $p=0.02$ .

<sup>b</sup> $P$ -value for comparing percent parent estrogens between women with current BMI 25-29.9 vs. <25 kg/m<sup>2</sup> was 0.11.

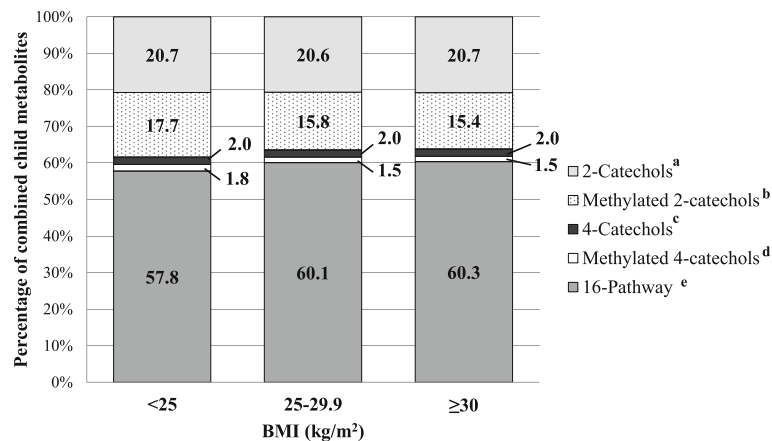
<sup>c</sup> $P$ -value for comparing percent parent estrogens between women with current BMI ≥30 vs. 25-29.9 kg/m<sup>2</sup> was 0.10.  $P$ -value for comparing percent parent estrogens between women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> was 0.006.

BMI body mass index

suggesting that associations with individual estrogen metabolites among never/former MHT users were driven largely by their correlations with unconjugated estradiol. Associations with measured WHR were not independent of current BMI, suggesting that the associations were driven by overall adiposity rather than by fat distribution per se. BMI at age 18 years and height were not associated with postmenopausal estrogen/estrogen metabolite levels.

Our findings of positive associations between current BMI and parent estrogens are consistent with those from previous studies [8, 21–23, 32–34]. A pooled analysis of eight studies estimated 83% higher levels of circulating total estradiol and 60% higher levels of total estrone in postmenopausal obese women (BMI ≥30 kg/m<sup>2</sup>) compared with lean women (BMI <22.5 kg/m<sup>2</sup>) [8]. These findings are also in line with biological evidence that supports the major source of estrogens in postmenopausal women being via aromatization of androgens in adipose tissue [35, 36]. Further, although few studies have examined the associations in current MHT users, our findings are consistent with data from the Nurses' Health Study regarding unconjugated estradiol BMI (≥30 vs. <25 kg/m<sup>2</sup>) associations in both never/former and current MHT users and the finding of stronger positive associations in never/former users (62% vs. 22% change in

estradiol levels) [23]. In the present study, unconjugated estradiol was consistently positively associated with current BMI (≥30 vs. <25 kg/m<sup>2</sup>) among both never/former (130% change) and current MHT users (20% change), although the association was not statistically significant in current MHT users. The differential associations between BMI and parent estrogens by MHT use mirror the similar differential associations of BMI with cancer risk [37, 38], further supporting the notion that endogenous estrogens may mediate the BMI-cancer association, although alternative mechanisms may also exist [24, 39, 40]. The positive associations of BMI with postmenopausal breast cancer [37, 38] and endometrial cancer [41, 42] risk have been found consistently in women not using MHT; however, the associations have been weakly positive or near null among current MHT users [37, 38, 41, 42], possibly owing to a threshold effect of circulating estrogens in current MHT users. Current MHT users have higher circulating levels of estrogens; thus, increased estrogen production by adipose tissue may not contribute to further increase in cancer risk. Moreover, given the high estrogen levels in current MHT users, the association with BMI on the relative scale may be masked, whereas an absolute difference in estrogen concentration may be apparent, as in never/former users.



**Fig. 2** Percentages of each pathway estrogen metabolites (2-catechols, methylated 2-catechols, 4-catechols, methylated 4-catechols, 16-pathway metabolites) out of summed child estrogen metabolites by current body mass index (BMI) among never/former menopausal hormone therapy users. Adjusted for age at blood draw (<55, 55-59, 60-64, 65-69, 70-74, 75-79 years), blood draw year (1993-1996, 1997-1998), race (white, non-white), smoking status (never, former, current), time since menopause (<10, 10-19, ≥20 years, missing), moderate- to vigorous-intensity physical activity (0, 0.1-9.9, ≥10 MET-hr/wk), summed concentration of child estrogen metabolites (continuous, pmol/L). Note: Summed child estrogen metabolites include the following estrogen metabolites: 2-catechols (2-hydroxyestrone, 2-hydroxyestradiol), methylated 2-catechols (conjugated and unconjugated 2-methoxyestrone, conjugated and unconjugated 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether), 4-catechols (4-hydroxyestrone), methylated 4-catechols (4-methoxyestrone, 4-methoxyestradiol), 16-pathway metabolites (16 $\alpha$ -hydroxyestrone, conjugated and unconjugated estriol, 16-ketoestradiol, 16-epiestriol, 17-epiestriol). <sup>a</sup>For 2-catechols, overall F-test  $p=0.89$ . <sup>b</sup>For methylated 2-catechols, overall F-test  $p=0.001$ . P-values for comparing women with current BMI 25-29.9 vs. <25 kg/m<sup>2</sup> was 0.002, comparing women with current BMI ≥30 vs. 25-29.9 kg/m<sup>2</sup> was 0.57, and comparing women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> was 0.001. <sup>c</sup>For 4-catechols, overall F-test  $p=0.59$ . <sup>d</sup>For methylated 4-catechols, overall F-test  $p=0.0002$ . P-values for comparing women with current BMI 25-29.9 vs. <25 kg/m<sup>2</sup> was <0.0001, comparing women with current BMI ≥30 vs. 25-29.9 kg/m<sup>2</sup> was 0.82, and comparing women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> was 0.003. <sup>e</sup>For 16-pathway metabolites, overall F-test  $p<0.0001$ . P-values for comparing women with current BMI 25-29.9 vs. <25 kg/m<sup>2</sup> was 0.0003, comparing women with current BMI ≥30 vs. 25-29.9 kg/m<sup>2</sup> was 0.78, and comparing women with current BMI ≥30 vs. <25 kg/m<sup>2</sup> was 0.0002. BMI body mass index

To date, few studies have examined current BMI in relation to estrogen metabolism beyond parent estrogens. Earlier studies measured only two estrogen metabolites that have been thought to be the most and the least carcinogenic: 16 $\alpha$ -hydroxyestrone and 2-hydroxyestrone, respectively [43–46]. These earlier studies have shown an inverse association between adiposity and the ratio of urinary 2-hydroxyestrone to 16 $\alpha$ -hydroxyestrone in both pre- and postmenopausal women [44, 45, 47]. In a more recent analysis in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial cohort, self-reported BMI was positively correlated with all 15 serum estrogens/estrogen metabolites among postmenopausal women who had never used MHT [34]; however, whether the associations with estrogen metabolites remained after accounting for correlations with parent estrogens was not assessed. Although we observed similar positive associations with estrogens/estrogen metabolites using measured BMI among never/former MHT users in the present study, the associations did not remain after adjustment for unconjugated estradiol. Similarly to our findings of inverse associations between BMI and methylated 2-catechols after adjusting for unconjugated estradiol in postmenopausal women, researchers in a study of premenopausal women found that BMI was

inversely associated with most methylated catechols measured in urine [48]. In our secondary analyses, we also observed that obese women appeared to be less likely to metabolize parent estrogens into child metabolites in general but more likely to favor metabolism of parent estrogens into 16-pathway estrogen metabolites over 2- or 4-methylated catechols. Our findings provide novel, additional detailed information about patterns of estrogen metabolism. They are in line with results from a previous study that evaluated 12 estrogens/estrogen metabolites in prepubertal girls and observed higher levels of 16 $\alpha$ -hydroxyestrone and lower levels of 2-methoxyestradiol in obese girls (BMI >95th percentile vs. 10th–85th percentile) [49]. Although the exact mechanisms for this relationship are not clear, reduced catechol-O-methyltransferase (COMT) activity associated with obesity [50] and/or suppressed COMT activity by estradiol [51, 52] may explain the decreased methylation of catechols with increased BMI observed in the present study. Methylation prevents catechols from metabolizing into quinones, which can form quinone adducts and induce oxidative DNA damage [53]. Further, reduced levels of methylated 2-catechols in obese women (BMI ≥30 vs. <25 kg/m<sup>2</sup>) are also in line with studies that have consistently shown lower ratios of 2-pathway metabolites to parent estrogens to be

**Table 4** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by waist-to-hip ratio in postmenopausal women not using menopausal hormone therapy in the Women's Health Initiative Observational Study

	Model 1 <sup>a</sup>				Model 1 + current BMI <sup>b</sup>				
	Geometric means (95% CI)		p-trend <sup>c</sup>	%Δ <sup>d</sup>	Geometric means (95% CI)		p-trend <sup>c</sup>	%Δ <sup>d</sup>	
	<0.76	0.76–0.82			≥0.83	<0.76			0.76–0.82
Median	0.74	0.79	0.87		0.74	0.79	0.87		
n	229	340	383		229	340	383		
Weighted n <sup>f</sup>	7616	10,905	12,199		7616	10,905	12,199		
Estrone	286 (231–354)	290 (243–347)	348 (292–415)	0.003 <sup>g</sup>	345 (276–431)	320 (268–382)	327 (276–388)	0.80	–5.2
Conjugated	216 (170–274)	229 (188–279)	271 (223–329)	0.002 <sup>g</sup>	265 (207–341)	255 (209–312)	253 (209–306)	0.77	–4.5
Unconjugated	62.4 (53.6–72.5)	53.0 (46.4–60.6)	66.7 (58.7–75.8)	0.05	70.8 (61.1–82.0)	56.7 (49.9–64.4)	63.9 (56.6–72.2)	0.53	–9.7
Estradiol	50.2 (39.5–63.8)	53.1 (43.9–64.2)	62.5 (51.3–76.1)	0.01 <sup>g</sup>	59.0 (47.0–74.1)	57.8 (48.1–69.5)	59.2 (48.7–72.0)	0.98	0.3
Conjugated	31.8 (24.7–40.9)	36.5 (30.0–44.6)	40.6 (32.8–50.3)	0.04	36.4 (28.5–46.5)	39.2 (32.3–47.7)	38.8 (31.4–48.1)	0.88	6.6
Unconjugated	13.9 (10.7–18.2)	11.6 (9.20–14.5)	17.5 (14.3–21.4)	<0.001 <sup>h</sup>	18.5 (14.5–23.4)	13.4 (10.9–16.5)	15.9 (13.2–19.3)	0.89	–14.1
2-Hydroxyestrone	62.6 (52.1–75.1)	64.5 (55.7–74.7)	72.1 (62.2–83.7)	0.01 <sup>g</sup>	69.9 (57.9–84.4)	68.4 (58.9–79.3)	69.5 (59.8–80.8)	0.64	–0.6
2-Hydroxyestradiol	16.0 (13.4–19.1)	16.3 (14.1–18.9)	17.8 (15.3–20.6)	0.05	17.8 (14.8–21.3)	17.3 (14.9–20.0)	17.2 (14.8–20.0)	0.96	–3.4
2-Methoxyestrone	45.4 (39.0–52.7)	40.2 (35.5–45.7)	43.1 (38.1–48.7)	0.69	49.3 (42.1–57.7)	42.0 (37.0–47.8)	41.9 (37.1–47.5)	0.18	–15.0
Conjugated	32.0 (27.1–37.9)	30.3 (26.4–34.7)	31.1 (27.1–35.6)	0.64	34.9 (29.3–41.5)	31.7 (27.6–36.3)	30.2 (26.4–34.7)	0.26	–13.5
Unconjugated	12.4 (10.5–14.7)	9.21 (7.96–10.7)	10.9 (9.61–12.3)	1.00	13.3 (11.2–15.8)	9.56 (8.24–11.1)	10.6 (9.38–12.0)	0.22	–20.3
2-Methoxyestradiol	13.3 (11.1–16.0)	12.7 (10.8–14.9)	14.0 (11.8–16.5)	0.04	15.0 (12.5–18.0)	13.5 (11.5–15.9)	13.4 (11.4–15.9)	0.98	–10.7
Conjugated	10.4 (8.49–12.6)	10.2 (8.47–12.2)	11.2 (9.32–13.6)	0.02 <sup>g</sup>	11.7 (9.54–14.3)	10.8 (9.02–13.0)	10.8 (8.94–13.1)	0.70	–7.7
Unconjugated	2.41 (2.01–2.89)	1.96 (1.69–2.27)	2.17 (1.88–2.50)	0.96	2.69 (2.26–3.20)	2.07 (1.80–2.40)	2.10 (1.81–2.42)	0.06	–21.9
2-Hydroxyestrone-3-methyl ether	7.80 (6.62–9.19)	7.29 (6.40–8.31)	7.89 (6.96–8.95)	0.31	8.35 (7.01–9.94)	7.56 (6.60–8.65)	7.72 (6.79–8.77)	0.76	–7.5
4-Hydroxyestrone	7.66 (6.35–9.23)	7.79 (6.71–9.03)	8.82 (7.59–10.2)	0.01 <sup>g</sup>	8.57 (7.05–10.4)	8.26 (7.10–9.60)	8.50 (7.30–9.89)	0.48	–0.8
4-Methoxyestrone	4.60 (3.96–5.35)	4.01 (3.55–4.54)	4.46 (3.92–5.08)	0.56	4.90 (4.19–5.73)	4.15 (3.66–4.71)	4.37 (3.84–4.98)	0.51	–10.8
4-Methoxyestradiol	1.95 (1.62–2.34)	1.74 (1.49–2.02)	1.95 (1.67–2.29)	0.22	2.14 (1.79–2.57)	1.82 (1.57–2.13)	1.89 (1.61–2.22)	0.74	–11.7
16α-Hydroxyestrone	31.2 (25.7–37.8)	32.0 (27.4–37.3)	36.0 (30.8–42.1)	0.01 <sup>g</sup>	35.0 (28.7–42.8)	34.0 (29.0–39.7)	34.7 (29.6–40.7)	0.60	–0.9
Estril	130 (107–159)	133 (114–155)	156 (134–181)	0.005 <sup>g</sup>	148 (120–181)	142 (121–166)	149 (129–173)	0.46	0.7
Conjugated	97.3 (78.1–121)	106 (89.8–126)	123 (105–146)	0.002 <sup>g</sup>	111 (89.0–140)	114 (96.2–136)	118 (99.8–139)	0.34	6.3
Unconjugated	29.3 (24.8–34.7)	23.3 (20.3–26.8)	29.1 (25.5–33.1)	0.13	32.7 (27.5–39.0)	24.7 (21.5–28.4)	28.0 (24.6–31.9)	0.67	–14.4

**Table 4** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by waist-to-hip ratio in postmenopausal women not using menopausal hormone therapy in the Women’s Health Initiative Observational Study (Continued)

16-Ketoestradiol	33.5 (27.5–40.8)	33.6 (28.7–39.3)	38.5 (32.9–45.1)	0.01 <sup>9</sup>	14.9	0.16	37.6 (30.6–46.2)	35.7 (30.4–42.0)	37.1 (31.5–43.6)	0.53	–1.3	0.88
16-Epiestriol	14.4 (12.1–17.1)	14.8 (12.8–17.1)	16.7 (14.4–19.4)	0.02 <sup>9</sup>	16.0	0.07	16.1 (13.4–19.3)	15.7 (13.5–18.2)	16.1 (13.9–18.7)	0.93	0.0	0.99
17-Epiestriol	12.1 (10.2–14.4)	12.1 (10.5–13.9)	14.1 (12.2–16.3)	0.01 <sup>9</sup>	16.5	0.07	13.6 (11.4–16.2)	12.9 (11.1–14.9)	13.6 (11.8–15.7)	0.84	0.0	0.98

<sup>9</sup>Model 1: adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, 75–79 years), blood draw year (1993–1996, 1997–1998), race (white, nonwhite), smoking status (never, former, current), time since menopause (<10 years, 10–19 years, ≥20 years, missing), moderate- to vigorous-intensity physical activity (0, 0.1–9.9, ≥10 metabolic equivalents of task-h/week)

<sup>8</sup>p-trend was estimated using the Wald test for continuous waist-to-hip ratio

<sup>7</sup>%Δ indicates the percentage change in estrogen/estrogen metabolite levels, comparing women with waist-to-hip ratio ≥0.83 vs. <0.76, and was estimated by taking the ratio of the geometric mean difference in estrogen/estrogen metabolite levels between women with waist-to-hip ratio ≥0.83 vs. <0.76 to the geometric mean of women with waist-to-hip ratio <0.76, multiplied by 100

<sup>6</sup>p-diff was estimated using the Wald test and indicates a p value for comparing estrogen/estrogen metabolite levels of women with waist-to-hip ratio ≥0.83 vs. <0.76

<sup>5</sup>Weighted n reflects weighted counts and refers to the study cohort

<sup>4</sup>False discovery rate (FDR) q values <0.05 and ≥0.01

<sup>3</sup>FDR q value <0.01

Note: Italicized p-values indicate nominal statistical significance at 0.05

**Table 5** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by height in postmenopausal women in the Women's Health Initiative Observational Study

Study	Never and former menopausal hormone therapy users					Current menopausal hormone therapy users						
	Geometric means (95% CI) <sup>a</sup>		p-trend <sup>b</sup>	%Δ <sup>c</sup>	p-diff <sup>d</sup>	Geometric means (95% CI) <sup>a</sup>		p-trend <sup>b</sup>	%Δ <sup>c</sup>	p-diff <sup>d</sup>		
	<160 cm	160–164.9 cm				≥165 cm	<160 cm				160–164.9 cm	≥165 cm
Median, cm	155.9	162.4	167.9		156.5	162.5	168.7					
n	333	310	313		289	269	319					
Weighted n <sup>e</sup>	10,171	10,625	10,021		7,764	7,588	9,800					
Estrone	288 (240–345)	338 (285–402)	300 (241–373)	0.27	4.2	0.67	3256 (2420–4383)	2926 (2079–4117)	3019 (2215–4117)	0.77	–7.3	0.57
Conjugated	220 (180–270)	267 (221–324)	233 (183–297)	0.25	5.9	0.61	2963 (2185–4018)	2735 (1920–3895)	2728 (1985–3750)	0.69	–7.9	0.55
Unconjugated	586 (51.1–67.2)	62.6 (55.0–71.4)	57.7 (49.4–67.5)	0.69	–1.5	0.85	229 (181–289)	196 (152–253)	213 (167–271)	0.94	–7.0	0.51
Estradiol	53.3 (42.9–66.1)	61.2 (50.7–73.9)	51.6 (41.4–64.4)	0.51	–3.2	0.75	443 (327–600)	399 (288–555)	425 (313–578)	0.91	–4.1	0.75
Conjugated	34.9 (27.6–44.1)	41.2 (33.9–50.1)	33.5 (26.6–42.1)	0.65	–4.0	0.69	370 (266–515)	335 (233–482)	346 (247–483)	0.64	–6.5	0.63
Unconjugated	13.8 (11.0–17.2)	14.9 (12.0–18.6)	13.8 (10.7–17.8)	0.45	0.0	0.99	45.5 (35.5–58.3)	42.2 (32.2–55.3)	43.0 (33.3–55.7)	0.41	–5.5	0.64
2-Hydroxyestrone	64.9 (55.1–76.5)	69.5 (60.1–80.3)	62.8 (53.1–74.2)	0.98	–3.2	0.68	448 (358–560)	407 (315–527)	439 (347–556)	0.90	–2.0	0.86
2-Hydroxyestradiol	16.2 (13.7–19.1)	17.4 (15.1–20.0)	16.0 (13.5–18.8)	0.88	–1.2	0.86	103 (82.9–128)	96.9 (76.3–123)	105 (83.8–132)	0.82	1.9	0.87
2-Methoxyestrone	44.6 (39.0–50.9)	41.9 (37.1–47.2)	37.6 (32.6–43.3)	0.23	–15.7	0.02	269 (220–329)	227 (183–280)	264 (212–327)	0.75	–1.9	0.84
Conjugated	33.2 (28.8–38.3)	30.3 (26.5–34.7)	27.0 (23.1–31.5)	0.14	–18.7	0.01	165 (133–207)	145 (114–183)	164 (129–208)	0.96	–0.6	0.91
Unconjugated	10.2 (8.83–11.8)	10.7 (9.36–12.2)	9.92 (8.48–11.6)	0.82	–2.7	0.74	75.7 (56.4–102)	64.1 (47.8–85.9)	73.3 (53.9–99.6)	0.66	–3.2	0.82
2-Methoxyestradiol	13.7 (11.4–16.4)	13.0 (11.2–15.2)	12.3 (10.3–14.8)	0.66	–10.2	0.24	84.0 (65.4–108)	78.5 (60.2–102)	89.2 (68.5–116)	0.69	6.2	0.58
Conjugated	11.1 (9.10–13.6)	10.4 (8.71–12.3)	9.72 (7.96–11.9)	0.61	–12.4	0.17	71.3 (54.3–93.5)	68.1 (51.0–91.0)	76.0 (57.0–101)	0.81	6.6	0.58
Unconjugated	2.08 (1.76–2.45)	2.11 (1.81–2.45)	2.06 (1.75–2.43)	0.91	–1.0	0.91	9.31 (7.68–11.3)	7.48 (6.12–9.15)	8.96 (7.35–10.9)	0.62	–3.8	0.72
2-Hydroxyestrone-3-methyl ether	7.50 (6.55–8.58)	7.68 (6.75–8.73)	7.44 (6.45–8.58)	0.71	–0.8	0.92	41.4 (33.7–50.9)	36.8 (29.6–45.8)	43.1 (34.6–53.7)	0.46	4.1	0.69
4-Hydroxyestrone	7.99 (6.75–9.46)	8.46 (7.31–9.80)	7.55 (6.39–8.92)	0.90	–5.5	0.50	59.2 (47.3–74.1)	54.9 (42.2–71.4)	59.6 (47.0–75.5)	0.96	0.7	0.95
4-Methoxyestrone	4.59 (3.96–5.33)	4.24 (3.78–4.76)	3.90 (3.41–4.46)	0.21	–15.0	0.03	27.7 (23.0–33.4)	23.0 (18.5–28.6)	28.9 (23.5–35.6)	0.51	4.3	0.66
4-Methoxyestradiol	1.92 (1.60–2.30)	1.84 (1.59–2.13)	1.74 (1.47–2.04)	0.55	–9.4	0.22	11.3 (8.75–14.7)	10.2 (7.54–13.7)	12.4 (9.39–16.3)	0.63	9.7	0.46
16α-Hydroxyestrone	32.3 (27.1–38.4)	34.8 (29.9–40.5)	31.1 (26.1–37.1)	0.98	–3.7	0.67	241 (191–304)	214 (163–281)	233 (182–298)	0.74	–3.3	0.77
Estril	135 (114–159)	148 (127–173)	135 (113–161)	0.51	0.0	1.00	1127 (880–1444)	1019 (751–1381)	1076 (828–1399)	0.51	–4.5	0.69
Conjugated	104 (86.7–125)	119 (100–140)	105 (86.6–128)	0.50	1.0	0.90	973 (752–1261)	887 (643–1223)	928 (705–1222)	0.44	–4.6	0.71
Unconjugated	27.4 (23.6–31.7)	26.5 (23.1–30.4)	25.6 (22.1–29.7)	0.81	–6.6	0.40	134 (108–166)	109 (83.7–141)	121 (96.7–152)	0.66	–9.7	0.30

**Table 5** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by height in postmenopausal women in the Women’s Health Initiative Observational Study (Continued)

16-Ketoestradiol	34.4 (28.9–41.0)	36.5 (31.3–42.7)	33.8 (28.3–40.5)	0.70	–1.7	0.85	279 (221–352)	252 (191–332)	265 (207–341)	0.60	–5.0	0.67
16-Epiestriol	14.5 (12.4–16.9)	16.4 (14.2–18.8)	14.8 (12.6–17.5)	0.47	2.1	0.78	83.0 (66.8–103)	77.1 (60.4–98.5)	82.5 (65.5–104)	1.00	–0.6	0.96
17-Epiestriol	11.9 (10.2–13.9)	13.7 (11.9–15.6)	12.9 (10.9–15.2)	0.30	8.4	0.30	56.4 (45.3–70.4)	49.7 (38.7–63.6)	54.9 (43.3–69.6)	0.90	–2.7	0.81

<sup>a</sup>Adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, 75–79 years), blood draw year (1993–1996, 1997–1998), race (white, nonwhite), smoking status (never, former, current), time since menopause (<10 years, 10–19 years, ≥20 years, missing)

<sup>b</sup>p-trend was estimated using the Wald test for continuous height (cm)

<sup>c</sup>%Δ indicates the percentage change in estrogen/estrogen metabolite levels, comparing women with height ≥165 vs. <160 cm, and was estimated by taking the ratio of the geometric mean difference in estrogen/estrogen metabolite levels between women with height ≥165 vs. <160 cm to the geometric mean of women with height <160 cm, multiplied by 100

<sup>d</sup>p-diff was estimated using the Wald test and indicates a p value for comparing estrogen/estrogen metabolite levels of women with height ≥165 vs. <160 cm

<sup>e</sup>Weighted n reflects weighted counts and refers to the study cohort

Note: All false discovery rate q values >0.05

Italicized p-values indicate nominal statistical significance at 0.05

associated with higher postmenopausal breast cancer risk [17–19].

In the present study, we did not observe associations of WHR with estrogens/estrogen metabolites after adjustment for current BMI. Similarly, in studies where body fat distribution was measured by dual-energy X-ray absorptiometry [32] or measured WHR [33], central obesity was not associated with circulating unconjugated estradiol independent of BMI among postmenopausal women not using MHT, suggesting that body fat distribution does not provide additional information about circulating estradiol beyond what overall adiposity (as measured by BMI) provides.

In the present study, adult height and BMI at age 18 years were not associated with estrogens/estrogen metabolites after adjustment for current BMI. BMI at age 18 years and height may indicate early-life nutritional status, which may not influence estrogen metabolism in postmenopausal women. In a study of premenopausal women, BMI at age 18 years was not associated with urinary estrogen/estrogen metabolite levels; however, contrary to our findings, height was inversely associated with urinary parent estrogens and 2-pathway metabolites in the same study [48]. The difference in results may be due to the variation in study populations (e.g., age, menopausal status) or biospecimen used (circulating vs. excreted levels).

We acknowledge several limitations of this study. We measured circulating estrogens/estrogen metabolites in a single baseline serum sample. However, in a previous study using the same assay we used, researchers showed moderate to high 1-year ICCs in postmenopausal women for parent estrogens (0.72 for estrone, 0.65 for estradiol) and most estrogen metabolites of 2-, 4-, and 16-pathways (range 0.35–0.53), with the exception of 2-methoxyestrone (0.10) and 2-hydroxyestrone-3-methyl ether (0.14) [17], suggesting that measured serum estrogens/estrogen metabolites may also adequately represent postmenopausal levels over at least 1 year. BMI at age 18 years was based on self-reported height and weight. However, measurement error in this context is unlikely to be related to serum estrogen levels and, if present, would likely underestimate the associations. Although we cannot exclude the possibility of false-positives, most associations with current BMI remained at 5% FDR.

Despite these limitations, this study has important strengths. Whereas most epidemiologic studies have used self-reported measures, measurement error in the present study was reduced by using measured height, weight, and waist and hip circumferences. Use of a high-performance LC-MS/MS assay also allowed comprehensive evaluation of individual estrogens/estrogen metabolites with high reliability, sensitivity, and specificity. By stratifying the analysis by MHT use, we were

able to take into account variations in estrogen/estrogen metabolite levels by exogenous hormone use. Further, use of a large sample size and careful adjustment for potential confounders assessed at blood collection increased the validity of the results.

## Conclusions

In this comprehensive analysis of measured anthropometrics and serum estrogens/estrogen metabolites, we observed strong positive associations between current BMI and parent estrogens in postmenopausal women not using MHT. Adjusted for unconjugated estradiol, current BMI was also associated with lower levels of methylated catechols among both never/former and current MHT users. Similar estrogen metabolism patterns have previously been associated with higher breast cancer risk; thus, these findings further support the need to prospectively evaluate the roles of endogenous estrogens/estrogen metabolites in the BMI-breast cancer risk association.

## Additional files

**Additional file 1: Table S1.** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by current BMI (<25, 25–29.9, 30–34.9, ≥40 kg/m<sup>2</sup>) in postmenopausal women not using menopausal hormone therapy in the Women's Health Initiative Observational Study. (PDF 93 kb)

**Additional file 2: Figure S1.** Percentages of parent estrogens (estradiol and estrone) and child estrogen metabolites (2-, 4-, and 16-hydroxylation pathway metabolites) out of summed estrogens/estrogen metabolites by current BMI among current menopausal hormone therapy users. (PPTX 63 kb)

**Additional file 3: Figure S2.** Percentages of each pathway estrogens/estrogen metabolites (2-catechols, methylated 2-catechols, 4-catechols, methylated 4-catechols, 16-pathway metabolites) out of summed child estrogen metabolites by current BMI among current menopausal hormone therapy users. (PPTX 70 kb)

**Additional file 4: Table S2.** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by waist-to-hip ratio in postmenopausal women currently using menopausal hormone therapy in the Women's Health Initiative Observational Study. (PDF 95 kb)

**Additional file 5: Table S3.** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by BMI at age 18 years in postmenopausal women in the Women's Health Initiative Observational Study. (PDF 94 kb)

**Additional file 6: Table S4.** Geometric means (pmol/L) and 95% CIs of serum estrogens/estrogen metabolites by current BMI in postmenopausal women not using menopausal hormone therapy among control subjects only in the Women's Health Initiative Observational Study. (PDF 103 kb)

**Additional file 7:** Women's Health Initiative clinical centers. (DOCX 13 kb)

## Abbreviations

BMI: Body mass index; COMT: Catechol-O-methyltransferase; FDR: False discovery rate; GM: Geometric mean; ICC: Intraclass correlation coefficient; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; MET: Metabolic equivalent of task; MHT: Menopausal hormone therapy; OS: Observational Study; WHI: Women's Health Initiative; WHR: Waist-to-hip ratio



### Acknowledgements

The WHI program is supported by contracts from the National Heart, Lung, and Blood Institute, National Institutes of Health, Program Office (Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller). The authors thank the WHI Clinical Coordinating Center (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) and the WHI investigators (Garnet Anderson, Ross Prentice, Andrea LaCroix, Charles Kooperberg, JoAnn E. Manson, Barbara V. Howard, Marcia L. Stefanick, Rebecca Jackson, Cynthia A. Thomson, Jean Wactawski-Wende, Marian Limacher, Jennifer Robinson, Lewis Kuller, Sally Shumaker, Robert Brunner, and Karen L. Margolis) for their dedication, as well as the study participants for making the program possible. A full list of WHI investigators can be found at <https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf>.

### Funding

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services, through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. This study was also supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics of the National Cancer Institute.

### Authors' contributions

HO performed the statistical analyses and drafted the manuscript. BT developed and designed the study aims and made substantial contributions to statistical analysis, interpretation of data, and critical revision and editing of the manuscript. SBC made substantial contributions to statistical analysis and revision of the manuscript. CEM made substantial contributions to interpretation of data and revision of the manuscript. RTF made substantial contributions to interpretation of data and revision of the manuscript. ESL made substantial contributions to interpretation of data and revision of the manuscript. JWW made substantial contributions to interpretation of data and revision of the manuscript. JS made substantial contributions to statistical analysis and revision of the manuscript. RMP made substantial contributions to statistical analysis and revision of the manuscript. LAB developed the study aims and made substantial contributions to revision of the manuscript. NW developed the study aims and made substantial contributions to revision of the manuscript. GLA made substantial contributions to interpretation of data and revision of the manuscript. JEM made substantial contributions to interpretation of data and revision of the manuscript. CC made substantial contributions to interpretation of data and revision of the manuscript. OZ made substantial contributions to interpretation of data and revision of the manuscript. XX developed and performed the assay and contributed to revision of the manuscript. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

### Authors' information

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

The Office of Human Subjects Research at the National Institutes of Health approved this analysis. As required by the WHI protocol, informed consent was obtained from all study subjects. Informed consent documents and procedures were approved by the institutional review board (IRB) of the Fred Hutchinson Cancer Research Center and by the IRBs of each of the participating clinical centers (see Additional file 7).

### Author details

<sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, 6E332, Bethesda, MD 20892, USA. <sup>2</sup>Center for Health Research, Kaiser Permanente NW, Portland, OR, USA. <sup>3</sup>Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY, USA. <sup>4</sup>Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. <sup>5</sup>Department of

Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. <sup>6</sup>School of Nursing, University of Washington, Seattle, WA, USA. <sup>7</sup>Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA.

Received: 14 August 2016 Accepted: 28 January 2017

Published online: 11 March 2017

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