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Body Size in Relation to Urinary Estrogens and Estrogen Metabolites (EM) among Premenopausal Women during the Luteal Phase

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Abstract

Estrogen metabolism profiles may play an important role in the relationship between body size and breast carcinogenesis. Previously, we observed inverse associations between current body mass index (BMI) and plasma levels of parent estrogens (estrone and estradiol) among premenopausal women during both follicular and luteal phases. Using data from the Nurses' Health Study II (NHS II), we assessed whether height, current BMI, and BMI at age 18 were associated with the urinary concentrations of 15 estrogens and estrogen metabolites (jointly referred to as EM) measured during the luteal phase among 603 premenopausal women. We observed inverse associations with total EM for height (Ptrend=0.01) and current BMI (Ptrend=0.01), but not BMI at age 18 (Ptrend=0.26). Six EMs were 18–27% lower in women with a height 68+ inches versus 62 inches, primarily in the methylated catechol pathway (Ptrend=0.04). Eight EMs were 18–50% lower in women with a BMI of 30+ versus <20, primarily in the 2-catechol and methylated catechol pathways (Ptrend<0.001 for both). Our results suggest that height and current BMI are associated with estrogen metabolism profiles in premenopausal women. Further studies with timed urine and blood collections are required to confirm and extend our findings.

Keywords

Body mass index; height; estradiol; estrogen metabolites; premenopausal women

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Introduction

Experimental studies have suggested that endogenous estrogens and estrogen metabolites may play an important role in breast carcinogenesis [43]. Mechanisms may include estrogen receptor-mediated signaling that increases breast cell proliferation as well as the genotoxic effects of some estrogen metabolites [42, 21, 26, 15]. Evidence from cell culture and animal experiments suggests that catechol estrogen metabolites derived from reactive quinones are genotoxic [42, 6]. While circulating estradiol and estrone consistently have been associated with increased risk of postmenopausal breast cancer in epidemiologic studies [12, 28, 44, 22], the relationship in premenopausal women is less clear [19, 31, 36, 25, 27, 9, 23, 24]. In the Nurses' Health Study II (NHS II) among premenopausal women, we observed that higher plasma follicular free estradiol levels were associated with an increased risk of breast cancer (highest versus lowest quartile relative risk (RR) = 2.4, P_{trend} = 0.01); however, luteal plasma estrogens were not associated clearly with risk [9]. Conversely, urinary excretion of conjugated estrone and estradiol during the mid-luteal phase was associated with a lower risk of breast cancer in the same population [10]. It is important to identify correlations of urinary estrogen metabolism, we reported that certain reproductive factors, including menstrual irregularity, were associated with estrogen metabolism patterns [16].

Body size characteristics are important in determining breast cancer risk. Among premenopausal women, BMI has been inversely associated with breast cancer risk; compared with a BMI of less than 21 kg/m², women with a BMI > 31 kg/m² had an relative risk of 0.54 in a pooled analysis of seven prospective cohort studies [40]. Height has been positively associated with breast cancer risk; the RR per 10 cm increase in height was 1.17, 95% CI: 1.15–1.19 in a recent meta-analysis of prospective studies [17]. Further, in the NHS II, we previously observed that current BMI was inversely and significantly associated with plasma levels of estrone, estradiol, and estrone sulfate in premenopausal women [39].

Estrogen metabolism profiles may play an important role in the relationship between body size and breast carcinogenesis. Thus, we evaluated the associations between adult body size measures and 15 estrogens and estrogen metabolites (jointly referred to as EM) in luteal urine among 603 women from NHS II.

Methods

Study Population

The NHS II is a prospective cohort study established in 1989, when 116,430 female registered nurses, ages 25 to 42, completed a questionnaire. Biennially, the cohort has been followed to update exposure variables and ascertain newly diagnosed diseases. The population is 94% Caucasian. Between 1996 and 1999, 29,611 cohort members, aged 32 to 54 y, provided urine samples. Of the women who were still having menstrual cycles (i.e., premenopausal), had not used oral contraceptives in the last six months, and were not pregnant or breastfeeding within the past six months, 18,521 provided their urine samples timed to the mid-luteal phase of the menstrual cycle (7 to 9 d before the anticipated start of their next menstrual cycle).

Urine samples were shipped to our laboratory, via overnight mail with a frozen water bottle, where the urine was aliquoted into labeled cryotubes and stored in liquid nitrogen freezers (−130 °C) without any preservative. Approximately 93% of luteal samples were received within 26 h of collection. Further details of sample collection, processing, and storage methods have been described elsewhere [33]. There were 603 participants with urinary EM measurements in this study: 493 were controls from a nested case-control study of breast cancer [38, 10], and 110 were from a biomarker reproducibility study [11]. The study was

approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Covariate Data

At the time of urine collection, participants completed a questionnaire recording their current weight, details about the urine collection date and time, smoking status, physical activity, and whether the urine was a first morning sample. In addition, 97% of the participants returned a postcard noting the first day of their next menstrual cycle, which allowed for a more accurate determination of the luteal day of the collection (date of next menstrual cycle minus date of urine collection) compared to forward-counting [1]. Reproductive history, oral contraceptive use, history of benign breast disease, family history of breast cancer, age at menarche, menstrual cycle regularity, usual menstrual cycle length, weight at age 18, and attained height were reported on the 1989 questionnaire; oral contraceptive use and reproductive factors were updated on subsequent biennial NHS II questionnaires. Current BMI at blood collection and BMI at age 18 were calculated as weight in kilograms divided by attained height in meters squared.

We have conducted validation studies of self-reported current weight, weight at age 18, and waist and hip circumferences in our population [37, 30]. Mean self-reported weights were 1.5 kg less than the technician measurements and were, therefore, consistent with the difference between a random casual weight in clothing and a nude weight. The correlation between measured and self-reported weight was 0.96 and did not differ by BMI levels [37]. The correlation between recalled weight at age 18 and that documented in college or nursing school physical exam records was 0.84 [30].

Laboratory Assays

Details of the assay have been published previously [41, 13]. In brief, to measure urinary EM, 500 μ L of frozen urine was sent to the Laboratory of Proteomics and Analytical Chemistry, SAIC-Frederick, Inc., Frederick, MD. Each urine sample was thawed and mixed, and 400 μ L was immediately aliquoted into a clean screw-cap glass tube and 20 μ L of an internal standard solution containing 1.6 ng of each of five deuterated EM (17 β -estradiol-d₄, estriol-d₃, 2-hydroxy-17 β -estradiol-d₅, 2-methoxy-17 β -estradiol-d₅, 16-epiestriol-d₃) was added. An additional 0.5 mL of freshly prepared hydrolysis buffer containing 0.15 M acetate buffer, pH 4.1, 2 mg of ascorbic acid and 5 μ L of β -glucuronidase/sulfatase (*Helix pomatia*, Sigma-Aldrich, St. Louis, MO). After incubating the sample for 20 hours at 37 °C, 8 mL of dichloromethane was added. The sample was slowly rotated at 8 rpm (RKVSD™, ATR, Inc., Laurel, MD) for 30 min. After extraction, the organic solvent portion was transferred into a clean glass tube and evaporated to dryness at 60 °C under nitrogen gas (Reacti-Vap III™, Pierce, Rockford, IL). One hundred microliters of 0.1 M NaHCO₃ buffer (pH at 9.0) and 100 μ L of dansyl chloride solution (1 mg/mL in CH₃COCH₃) were added to the dried sample, followed by vortexing and heating at 60 °C (Reacti-Therm III™ Heating Module, Pierce, Rockford, IL) for 5 min to form dansyl derivatives of the EM and isotopically labeled EM. The LC-MS/MS measurements were acquired using a TSQ Quantum-AM triple quadrupole mass spectrometer coupled with a Surveyor high performance liquid chromatography system (Thermo Scientific, San Jose, CA). The entire LC-MS/MS system was controlled using Xcalibur software (Thermo Scientific). Quantification of urinary EM was carried out using Xcalibur Quan Browser software (Thermo Scientific). Calibration curves for the 15 EM were constructed by plotting EM/deuterium-labeled EM peak area ratios versus amounts of each EM. A linear function was used to interpolate the amount of each EM in the urine samples. Masked replicate quality control samples were placed in each batch to assess laboratory variability. The overall coefficients of variation (CVs) were <7% except for 4-methoxyestrone (17%) and 4-methoxyestradiol (15%), which are present at the

lowest concentrations amongst the EM measured. The lower level of quantitation for each EM was about 150 fmol/mL urine.

Creatinine was measured in three batches at the Endocrine Core Laboratory at Emory University (Atlanta, GA), Dr. Nader Rifai's laboratory at Boston Children's Hospital (Boston, MA), and Dr. Vincent Ricchiuti's laboratory at Brigham and Women's Hospital (Boston, MA). Overall CVs were 9.2% in all labs.

Statistical Analyses

We standardized urinary EM concentrations (pmol/mL) by urinary creatinine concentrations to account for urine dilution. There was no statistically significant fluctuation of creatinine concentrations across the primary exposure categories in our data (all $P_{trend} > 0.33$). We log-transformed the resultant values (pmol EM/mg creatinine) in all analyses. We identified a small number of statistical outliers using the generalized extreme studentized deviate many-outlier detection approach [32] and excluded individual EM results with extreme values from the analyses. In addition, several EM values were missing because of technical difficulties or low sample volume; thus, the final sample size for each analysis varied by EM from 563 to 596. EM were evaluated individually, as well as grouped by metabolic pathway and as pathway ratios. (See Table 1 for the specific groups and ratios).

Primary analyses used generalized linear models to calculate adjusted geometric means of individual EM, metabolic pathway groups, and pathway ratios by category of attained height, BMI at urine collection, BMI at age 18, and secondarily of waist-to-hip ratio (WHR) in 1993. Height was categorized into quintiles. BMI was categorized into 6 categories based on biological cutpoints. BMI at age 18 was categorized into 4 categories, since there were very few girls have BMI greater than 25 at age 18. Sample sizes for each category were shown in Table 1–3. Test for trend analyses were conducted by modeling continuous exposure variables and calculating the Wald statistic. Multivariate models were adjusted for age at urine collection (continuous, in years), time of day of urine collection (1–8am, 9am–noon, 1pm–midnight), month of collection (categorical), history of benign breast disease (yes, no), parity (0, 1+ children), duration of past oral contraceptive use (continuous), smoking (never, past, and current), physical activity (<3, 3–8, 9–17, 18–26, 27–41, and 42+ MET hours/week), menstrual cycle irregularity (extremely regular, very regular, regular, and usually/always irregular), and ovulatory status of menstrual cycle during which urine was collected (an ovulatory cycle was defined as one with plasma progesterone collected on the same day as the urine sample ≥ 400 ng/dL). We also considered other potential confounders including family history of breast cancer, usual menstrual cycle length, age at menarche, and age at first birth; however, these did not change the estimates substantially and therefore were not included in the final models. Secondary analyses were restricted to those with ovulatory cycles (mid-luteal plasma progesterone ≥ 400 ng/dL), women who reported having regular menstrual cycles, women who provided sample during luteal days 4–10, parous women, and women who did not become menopausal until 2001 or later (thus were less likely to be perimenopausal at urine collection). We also stratified by the median age (43 y) and, for the height analyses, the median BMI (23.7 kg/m²). All P values were two-sided and considered to be statistically significant if ≤ 0.05 . Given that there are 15 metabolites being evaluated, we secondarily considered adjustment for multiple comparison using the Bonferroni method ($p < 0.05/15 = 0.003$) [3]. Analyses were conducted with SAS version 9 (SAS Institute, Cary, NC).

Results

Among the 603 women available for analysis, the average age was 42.7 y (range 33.3–50.9 y; Table 1). 89% of women had an ovulatory cycle at urine collection, 15% had a family

history of breast cancer, 44% had a history of benign breast disease, 81% had given birth to at least one child, 86% were 4–10 days before the next cycle, and 80% gave a first morning urine. The average height was 65.1 inches (standard deviation=2.7). On average, BMI at age 18 and current BMI were 21.1 kg/m² (standard deviation=2.9) and 25.0 kg/m² (standard deviation=5.5), respectively.

Height was inversely associated with total urinary EM ($P_{\text{trend}}=0.01$) and with 6 of 15 urinary EM, including estrone ($P_{\text{trend}}=0.02$), estradiol ($P_{\text{trend}}<0.001$), 2-hydroxyestradiol ($P_{\text{trend}}=0.02$), 2-methoxyestrone ($P_{\text{trend}}=0.01$), 4-methoxyestrone ($P_{\text{trend}}=0.02$), and 16-epiestriol ($P_{\text{trend}}=0.01$). (Table 1) Compared to women with a height ≤ 62 inches, levels in those with height > 68 inches were 18% lower for estrone, 27% lower for estradiol, 27% lower for 2-hydroxyestradiol, 26% lower for 2-methoxyestrone, 27% lower for 4-methoxyestrone, and 18% lower for 16-epiestriol. Height was associated with the 2-hydroxylation pathway ($P_{\text{trend}}=0.05$) and the methylated catechol pathway ($P_{\text{trend}}=0.04$), but not with the other pathways or any metabolic pathway ratios. The associations between height and EM appeared to be driven by the high EM values in the lowest category (≤ 62 in). Therefore, in a *post-hoc* analysis we evaluated the possibility of a non-linear association by dichotomizing height at above or below 62 inches and presenting the results of the Wald test from multivariate linear regression models. The interpretation of the results based on this approach was similar to the linear trend tests, except that the associations for the 2-hydroxyestrone-3-methyl ether, 16 α -hydroxyestrone, estriol, and 16-ketoestradiol were statistically significant when evaluating associations dichotomized at 62 inches versus the trend test.

Current BMI also was inversely associated with total urinary EM ($P_{\text{trend}}=0.01$) and with 8 out of 15 urinary EM, including 2-hydroxyestrone ($P_{\text{trend}}<0.001$), 2-hydroxyestradiol ($P_{\text{trend}}<0.001$), 2-methoxyestrone ($P_{\text{trend}}<0.001$), 2-methoxyestradiol ($P_{\text{trend}}<0.001$), 2-hydroxyestrone-3-methyl ether ($P_{\text{trend}}<0.001$), 4-methoxyestrone ($P_{\text{trend}}<0.001$), 16 α -hydroxyestrone ($P_{\text{trend}}=0.01$), and 16-ketoestradiol ($P_{\text{trend}}=0.04$). (Table 2) Compared to women with a BMI < 20 kg/m² levels in those with BMI ≥ 30 were 44% lower for 2-hydroxyestrone, 33% lower for 2-hydroxyestradiol, 38% lower for 2-methoxyestrone, 35% lower for 2-methoxyestradiol, 35% lower for 2-hydroxyestrone-3-methyl ether, 50% lower for 4-methoxyestrone, 32% lower for 16 α -hydroxyestrone, and 18% lower for 16-ketoestradiol. BMI was associated with the 2-hydroxylation pathway ($P_{\text{trend}}<0.001$), the catechol pathway ($P_{\text{trend}}<0.001$), 2-catechol pathway ($P_{\text{trend}}<0.001$), and the methylated catechol pathways ($P_{\text{trend}}<0.001$), but not with the 4- or 16-hydroxylation pathways. In addition, higher current BMI was associated with a higher 4-pathway/2-pathway ratio ($P_{\text{trend}}=0.001$), a lower 2-pathway/16-pathway ratio ($P_{\text{trend}}<0.001$), a lower 2,4-pathway/16-pathway ratio ($P_{\text{trend}}<0.001$), and a higher parent estrogen/EM ratio ($P_{\text{trend}}<0.001$), among others.

BMI at age 18 was not associated with total urinary EM concentrations ($P_{\text{trend}}=0.26$). However, BMI at age 18 was inversely associated with 2-hydroxyestrone ($P_{\text{trend}}=0.01$), 2-methoxyestrone ($P_{\text{trend}}=0.02$), and 4-methoxyestrone ($P_{\text{trend}}=0.03$), but not other EM. (Table 3) BMI at age 18 was not associated with the 2-, 4- or 16-hydroxylation pathways. It also was positively associated with ratios of 4-catechol/2-catechols ($P_{\text{trend}}<0.001$) and 4-pathway/2-pathway ($P_{\text{trend}}=0.04$).

Overall, results for height, current BMI, and BMI at age 18 did not change when excluding women with anovulatory cycles (10% of the population) or irregular menstrual cycles (5%), when restricting to parous women (81%), women with mid-luteal urine samples collected 4–10 days prior to the next menstrual cycle (85%), or women who did not become menopausal within 4 years after sample collection (88%) (data not shown). Further the results did not

vary by age, and the height results did not vary by current BMI (data not shown). When both height and current BMI were included in the same multivariate models, each was a statistically significant predictor for certain urinary EM (data not shown). When both current BMI and BMI at age 18 were included in the same multivariate models, current BMI remained statistically significant for most urinary EM (data not shown). We assessed different ways of adjusting for urine dilution in the statistical models: standardizing individual EM and grouped EM (pmol/mL urine) by creatinine (mg/mL urine) as the outcome variables (primary analysis) and using EM and grouped EM (pmol/mL urine) as the outcome variables with creatinine (mg/mL urine) as a covariate. These methods produced similar results (data not shown).

Using the Bonferroni correction p-value of 0.003, height remained significantly associated with estradiol, and BMI remained statistically significantly associated with 6 EM including 2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, and 4-methoxyestrone.

Discussion

To our knowledge, this study is the first to examine relationships between anthropometric measures and urinary estrogen metabolites. Attained height and current BMI were strongly inversely associated with total urinary EM as well as multiple specific EM and related pathways among premenopausal women. The patterns of associations with individual EM were different for height and current BMI. This is summarized in Figure 1, which combines knowledge about endogenous estrogen metabolism with our study results. Height was inversely associated with parent EM, catechol EM, and the 2-hydroxylation pathway. Current BMI was inversely associated with nearly all EM along the 2- and 4-pathways, though there was a U-shaped relationship with 4-hydroxyestrone. BMI at age 18 was not associated clearly with urinary EM with the possible exceptions of 2-catechols.

Our results suggest possibly different underlying mechanisms of associations for height and BMI with EM. For example, BMI was strongly inversely associated with 2-hydroxylation pathway, but not associated with the 16-hydroxylation pathway, which is thought to have strong estrogenic and genotoxic activity [35]. Metabolism of the parent estrogens, estrone and estradiol, has several mutually exclusive pathways that produce different metabolic products. Oxidation of estrone and estradiol can occur at the C-2, C-4, or C-16 positions on the steroid ring to create catechol estrogens [45]. With further metabolism, the catechol EMs can be methylated at one of the adjacent hydroxyl groups, generating methylated catechol EMs. EMs in the 16-hydroxylation pathway are metabolized into 17-epiestriol, estriol, 16-ketoestradiol, and 16-epiestriol [45]. Experimental studies suggest that metabolites along these pathways may have differential levels of estrogenic and genotoxic activities [45]. It has been hypothesized that estrogens metabolized through the 2-hydroxylation pathway may have a more favorable breast cancer risk profile over the 16-hydroxylation pathway [5, 2, 4, 10]. In our study, current BMI, but not height, was inversely associated with the ratio of 2-pathway/16-pathway.

Our results suggest that the positive association between height and premenopausal breast cancer risk [46] may be explained, at least in part, by estrogen metabolism profile, especially for the shortest women who had the most distinct hormone profile. Our data suggest that height may be inversely related to urinary concentrations of many EMs, particularly the methylated catechols, which may be beneficial for breast carcinogenesis [10]. These EMs generally were inversely related to risk of premenopausal cancer in the same population [10]. Further studies should explore the potential mechanism linking height to premenopausal estrogen metabolism.

With respect to BMI, our results are consistent with previous studies of circulating estrogens. Some studies reported that obese premenopausal women had lower plasma estradiol concentrations than that of normal weight women [47, 8, 7, 18, 29]. We observed that BMI was inversely associated with plasma levels of estradiol both during follicular and luteal phase among premenopausal women [39]. Other studies also reported that obesity was associated with significant decreases in 2-hydroxylation of estradiol in premenopausal women [14, 34]. This is consistent with our current results that BMI was inversely associated with total urinary estrogens, total estrone and estradiol levels, and a series of downstream metabolites of estradiol in urine, including a reduction in 2-hydroxylation. This seems to suggest that the regulatory mechanism linking BMI and estrogens is similar for circulating estrogen metabolism as well as for excretion in urine.

In the NHS II, we reported that both EM and BMI were inversely associated with breast cancer risk in premenopausal women [10, 20]. This appears to conflict with our results that BMI was associated with lower EM. Thus, it is possible that estrogen metabolism is not a mechanism through which BMI influences breast cancer risk in premenopausal women. Alternate etiologic pathways for the BMI-breast cancer association in premenopausal women should be explored.

Limitations

The primary limitation of our study is its cross-sectional nature without ability to examine causal relationships. However, our study is large, with 603 women. In addition, participants in our study were all healthy women with carefully timed urine samples, which is helpful to reduce misclassification by time in the menstrual cycle of the collection. Another limitation may be multiple comparisons. We evaluated associations with a number of *a priori* derived measures, including grouping of individual EM and ratios of these groupings. However, we did so based on what is known about estrogen metabolism; the metabolic pathway groups and pathways ratios that we derived are based on recognized metabolic pathways, shared biochemistry, and/or etiologic hypotheses. Even under Bonferroni correction for multiple comparisons, many of the associations remained statistically significant; however since the metabolic levels are correlated, Bonferroni correction is a conservative estimate of statistical significance. Third, the associations between height and several EM were driven by differences in the lowest category of height. We cannot rule out the possibility of residual confounding or a threshold effect. However, we adjusted for multiple potential confounders, including menstrual irregularity [16]. In general, the associations observed in the individual EMs were recapitulated in the summary pathway measures. A final limitation is that little is known about the relationships of urinary EM to circulating parent estrogens or EM.

Conclusions

Our results suggest that taller height and higher current BMI were associated with lower levels of urinary EM in the mid-luteal phase among premenopausal women. As this is the first analysis to examine body size with these 15 urinary EM, further studies with appropriately timed samples are required to confirm our findings.

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Figure 1.
Associations of Height and BMI with Endogenous Estrogen Metabolism

Table 1

Characteristics of study population: NHSII (n=603)

Characteristics	Mean (SD)
Age, y	42.8 (3.8)
Height, in	65.1 (2.7)
BMI at urine collection, kg/m ²	25.0 (5.5)
BMI at age 18, kg/m ²	21.1 (2.9)
Physical activity, MET-hr/wk	21.6 (20.5)
	Percentage
First morning urine	79.6
Family history of breast cancer	15.4
History of benign breast disease	44.0
Parous	81.1
Ovulatory cycle [†]	89.1
Current smoker	7.0
Irregular menstrual cycle at age 18–22	4.5
Past oral contraceptive user	83.9

[†] An ovulatory cycle was defined as one with plasma progesterone collected on the same day as the urine sample 400 ng/dL

Table 2

Adjusted* geometric means of urinary estrogen and estrogen metabolite (EM) measures by categories of height in premenopausal women

EM measure	N	Categories of height (inches)						Change per inch (%) ^Φ	P trend [‡]	P [€]
		62	63 to >64	65	>66 to 67	68+	100~114			
	N	92~102	136~148	83~89	135~143	100~114				
Individual EM and metabolic pathway groups (pmol/mg creatinine)										
Total EM	590	147	126	129	127	123		-2.1	0.01	0.01
Parent estrogens	585	44	36	36	39	36		-2.2	0.01	0.003
Estrone	593	28	23	23	25	23		-2.1	0.02	0.01
Estradiol	587	15	12	12	13	11		-3.1	<0.001	0.001
Catechol EM	589	40	32	31	33	30		-3.1	0.03	0.02
2-Catechol EM	591	33	27	27	28	27		-2.4	0.06	0.03
2-Hydroxyestrone	592	26	22	23	22	22		-2.2	0.09	0.07
2-Hydroxyestradiol	592	5.2	4.0	3.8	4.4	3.8		-2.9	0.02	0.004
4-Catechol EM										
4-Hydroxyestrone	589	4.7	3.8	3.7	4.0	3.9		-1.6	0.32	0.11
Methylated catechol EM	567	11	9.6	9.2	9.5	9.3		-2.2	0.04	0.02
Methylated 2-catechol EM	577	11	9.3	9.0	9.4	8.9		-2.2	0.03	0.02
2-Methoxyestrone	592	9.1	7.0	7.2	7.7	6.7		-2.9	0.01	0.01
2-Methoxyestradiol	580	0.73	0.64	0.57	0.69	0.6		-1.7	0.12	0.08
2-Hydroxyestrone-3-methyl ether	592	1.2	0.97	0.94	1.1	0.99		-1.6	0.16	0.02
Methylated 4-catechol EM	585	0.16	0.14	0.14	0.14	0.13		-2.4	0.10	0.13
4-Methoxyestrone	596	0.11	0.09	0.10	0.10	0.08		-4.0	0.02	0.06
4-Methoxyestradiol	585	0.03	0.03	0.03	0.03	0.03		-0.6	0.74	0.85
2-Hydroxylation pathway EM[§]	576	46	40	40	39	39		-2.2	0.05	0.05
4-Hydroxylation pathway EM[¶]	578	5.3	4.3	4.3	4.4	4.2		-2.2	0.13	0.06
16-Hydroxylation pathway EM	580	50	40	45	41	42		-1.8	0.06	0.01
16 α -Hydroxyestrone	594	6.9	5.1	6.2	5.8	6.0		-1.0	0.43	0.04
Estril	588	23	19	22	19	20		-1.3	0.26	0.04
17-Epiestril	589	1.1	0.96	1.1	0.98	0.99		-1.2	0.43	0.41

EM measure	N	Categories of height (inches)					Change per inch (%) [Ⓞ]	P trend [Ⓢ]	P [Ⓣ]
		62	63 to >64	65	>66 to 67	68+			
16-Ketoestradiol	594	10	7.8	8.7	8.6	8.7	-0.9	0.33	0.01
16-Epiestriol	591	6.1	5.0	5.3	4.9	5.0	-2.3	0.01	0.002
Metabolic pathway ratios									
4-Catechol/2-Catechols	585	0.14	0.15	0.14	0.15	0.16		0.31	0.52
2-Catechols/16-Pathway	577	0.64	0.69	0.59	0.65	0.60		0.43	0.99
Catechols/16-Pathway	571	0.73	0.80	0.64	0.74	0.69		0.42	0.96
4-Pathway/2-Pathway	561	0.11	0.10	0.11	0.11	0.11		0.32	0.73
2-Pathway/16-Pathway	564	0.95	1.1	0.93	0.97	0.92		0.53	0.80
4-Pathway/16-Pathway	562	0.11	0.10	0.10	0.11	0.09		0.70	0.69
2,4-Pathway/16-Pathway	549	1.0	1.1	0.97	1.0	0.96		0.37	0.94
2-Pathway/4,16-Pathway	549	0.83	0.86	0.78	0.80	0.78		0.48	0.77
2-Catechols/Methylated 2-catechols	576	3.2	3.2	3.3	3.0	3.3		0.92	0.83
4-Catechol/Methylated 4-catechols	578	30	27	26	28	29		0.97	0.66
Catechols/Methylated Catechols	561	3.5	3.6	3.6	3.4	3.6		0.71	0.96
Parent estrogens/estrogen metabolites	539	0.43	0.42	0.39	0.43	0.40		0.24	0.44
2-Pathway/Parent estrogens	567	1.1	1.1	1.1	1.1	1.1		0.73	0.61
4-Pathway/Parent estrogens	567	0.12	0.13	0.12	0.12	0.13		0.70	0.78
16-Pathway/Parent estrogens	570	1.1	1.1	1.3	1.1	1.3		0.22	0.75

* Adjusted for age at urine collection, first morning urine, month of urine collection, history of benign breast diseases, parity, oral contraceptive use, smoked within last month, ovulatory status, physical activity, and menstrual irregularity

[Ⓞ] Percentage change for one unit increase of height when treating it as a continuous variable

[Ⓢ] Linear trend across height using the Wald test determined the p-trend

[Ⓣ] P values from Wald test on dichotomous height (62, >62 inches)

[Ⓣ] Includes 2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether

[Ⓣ] Includes 4-hydroxyestrone, 4-methoxyestrone, and 4-methoxyestradiol

Table 3

Adjusted* geometric means of urinary estrogen and estrogen metabolite (EM) measures by current BMI in premenopausal women

EM measure	N	Current BMI (kg/m ²)								Change per kg/m ² (%), ϕ	P trend [‡]
		<20	20 to <22.5	22.5 to <25	25 to <27.5	27.5 to <30	30+				
	N	60~64	151~161	126~140	73~77	45~53	80~97				
Individual EM and metabolic pathway groups (pmol/mg creatinine)											
Total EM	586	146	144	132	128	113	120		-1.0	0.01	
Parent estrogens	581	40	40	39	41	35	37		-0.5	0.29	
Estrone	589	27	26	25	25	23	23		-0.8	0.11	
Estradiol	583	11	12	12	12	11	12		0.1	0.80	
Catechol EM	585	43	39	36	31	30	28		-2.4	<0.001	
2-Catechol EM	587	38	36	31	31	22	23		-3.2	<0.001	
2-Hydroxyestrone	588	32	30	26	26	18	18		-3.4	<0.001	
2-Hydroxyestradiol	588	48	5.5	4.5	4.6	3.1	3.2		-3.1	<0.001	
4-Catechol EM											
4-Hydroxyestrone	585	4.9	4.0	3.9	3.2	3.8	4.2		0	0.96	
Methylated catechol EM	563	13	11	10	9.5	8.3	8.4		-2.4	<0.001	
Methylated 2-catechol EM	573	12	11	10	9.3	8.1	8.2		-2.4	<0.001	
2-Methoxyestrone	588	9.8	9.2	8.1	7.3	5.8	6.1		-3.2	<0.001	
2-Methoxyestradiol	576	0.86	0.73	0.71	0.61	0.56	0.56		-2.2	<0.001	
2-Hydroxyestrone-3-methyl ether	588	1.4	1.2	1.1	1.1	0.95	0.91		-2.3	<0.001	
Methylated 4-catechol EM	581	0.16	0.17	0.16	0.13	0.15	0.10		-2.8	<0.001	
4-Methoxyestrone	592	0.12	0.13	0.11	0.10	0.12	0.06		-3.9	<0.001	
4-Methoxyestradiol	581	0.04	0.04	0.04	0.03	0.03	0.03		-1.8	0.06	
2-Hydroxylation pathway EM[§]	572	52	49	45	42	35	34		-2.6	<0.001	
4-Hydroxylation pathway EM[¶]	574	5.6	4.7	4.6	3.7	3.9	4.4		-0.6	0.40	
16-Hydroxylation pathway EM	576	44	46	42	42	40	43		-0.3	0.58	
16 α -Hydroxyestrone	590	7.7	7.3	6.3	6.7	5.3	5.2		-1.7	0.01	
Estriol	584	19	20	19	19	18	22		0.6	0.29	

EM measure	N	Current BMI (kg/m ²)						Change per kg/m ² (%) ϕ	P trend [‡]
		<20	20 to <22.5	22.5 to <25	25 to <27.5	27.5 to <30	30+		
17-Epiestriol	585	0.98	1.0	0.99	0.79	1.1	0.99	0.1	0.96
16-Ketoestradiol	590	9.4	9.9	9.1	9.3	8.7	7.7	-1.0	0.04
16-Epiestriol	587	4.7	5.4	5.0	5.3	5.1	5.2	0.5	0.34
Metabolic pathway ratios									
4-Catechol/2-Catechols	581	0.13	0.12	0.13	0.11	0.17	0.20		<0.001
2-Catechols/16-Pathway	573	0.81	0.74	0.71	0.67	0.55	0.52		<0.001
Catechols/16-Pathway	567	0.86	0.79	0.79	0.70	0.68	0.63		0.01
4-Pathway/2-Pathway	557	0.10	0.09	0.10	0.09	0.10	0.13		<0.001
2-Pathway/16-Pathway	560	1.2	1.1	1.1	0.97	0.88	0.80		<0.001
4-Pathway/16-Pathway	558	0.12	0.10	0.10	0.09	0.10	0.10		0.61
2,4-Pathway/16-Pathway	545	1.2	1.1	1.1	0.98	0.99	0.90		0.01
2-Pathway/4,16-Pathway	545	0.99	0.93	0.92	0.85	0.85	0.67		<0.001
2-Catechols/Methylated 2-catechols	572	3.2	3.3	3.3	3.5	3.2	3.1		0.59
4-Catechol/Methylated 4-catechols	574	30	23	24	23	23	39		0.02
Catechols/Methylated Catechols	557	3.4	3.6	3.5	3.6	3.5	3.6		0.48
Parent estrogens/estrogen metabolites	535	0.35	0.38	0.42	0.44	0.44	0.44		<0.001
2-Pathway/Parent estrogens	563	1.5	1.3	1.3	1.1	1.0	0.94		<0.001
4-Pathway/Parent estrogens	563	0.15	0.13	0.12	0.10	0.12	0.12		0.52
16-Pathway/Parent estrogens	566	1.2	1.2	1.1	1.2	1.2	1.2		0.87

* Adjusted for age at urine collection, first morning urine, month of urine collection, history of benign breast diseases, parity, oral contraceptive use, smoked within last month, ovulatory status, physical activity, and menstrual irregularity

ϕ Percentage change for one unit increase of BMI when treating it as a continuous variable

[‡] Linear trend across BMI using the Wald test determined the p-trend

[§] Includes 2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether

[¶] Includes 4-hydroxyestrone, 4-methoxyestrone, and 4-methoxyestradiol

Table 4

Adjusted* geometric means of urinary estrogen and estrogen metabolite (EM) measures by BMI at age 18 in premenopausal women

EM measure	N	BMI at age 18 (kg/m ²)				Change per kg/m ² (%) ϕ	P trend [‡]
		<20	20 to <22.5	22.5 to <25	25+		
N		205~227	217~232	78~89	39~47		
Individual EM and metabolic pathway groups (pmol/mg creatinine)							
Total EM	589	132	122	119	130	−0.9	0.26
Parent estrogens	585	40	36	36	37	−1.3	0.17
Estrone	592	26	22	23	23	−1.3	0.19
Estradiol	587	13	11	12	12	−1.0	0.26
Catechol EM	588	35	29	32	30	−2.1	0.14
2-Catechol EM	591	31	26	26	24	−3.1	0.01
2-Hydroxyestrone	592	26	21	21	19	−3.4	0.01
2-Hydroxyestradiol	592	4.6	3.9	3.9	3.6	−2.2	0.07
4-Catechol EM							
4-Hydroxyestrone	588	3.9	3.5	4.3	4.4	1.1	0.50
Methylated catechol EM	567	10	9.1	9.8	9.1	−1.5	0.15
Methylated 2-catechol EM	577	10	8.8	9.5	8.8	−1.5	0.15
2-Methoxyestrone	591	8.2	6.9	7.5	6.3	−2.7	0.02
2-Methoxyestradiol	580	0.68	0.61	0.67	0.56	−1.2	0.30
2-Hydroxyestrone-3-methyl ether	591	1.1	0.99	1	0.91	−1.4	0.22
Methylated 4-catechol EM	584	0.14	0.12	0.14	0.14	−2.0	0.18
4-Methoxyestrone	595	0.1	0.09	0.1	0.08	−3.6	0.03
4-Methoxyestradiol	584	0.03	0.03	0.03	0.04	0	0.97
2-Hydroxylation pathway EM [§]	576	43	37	38	39	−2.0	0.10
4-Hydroxylation pathway EM [¶]	577	4.4	3.9	4.6	4.8	0.4	0.76
16-Hydroxylation pathway EM	579	44	44	44	44	−0.3	0.78
16 α -Hydroxyestrone	593	6.2	5.9	5.7	5.7	−1.2	0.33
Estriol	587	20	21	20	20	0	0.97
17-Epiestriol	588	0.96	1.1	1.1	1.1	1.0	0.52

EM measure	N	BMI at age 18 (kg/m ²)				Change per kg/m ² (%) ^Φ	P trend [‡]
		<20	20 to <22.5	22.5 to <25	25+		
16-Ketostadiol	593	9.1	8.6	8.1	8.1	-1.3	0.14
16-Epiestriol	590	5.2	5.3	5	5.4	0.3	0.74
Metabolic pathway ratios							
4-Catechol/2-Catechols	585	0.13	0.15	0.18	0.19		<0.001
2-Catechols/16-Pathway	577	0.68	0.58	0.58	0.56		0.11
Catechols/16-Pathway	571	0.77	0.65	0.69	0.65		0.17
4-Pathway/2-Pathway	561	0.1	0.11	0.13	0.12		0.04
2-Pathway/16-Pathway	564	1.0	0.88	0.91	0.91		0.18
4-Pathway/16-Pathway	561	0.1	0.09	0.1	0.11		0.82
2,4-Pathway/16-Pathway	549	1.1	0.91	0.96	0.99		0.21
2-Pathway/4,16-Pathway	549	0.86	0.75	0.73	0.74		0.10
2-Catechols/Methylated 2-catechols	576	3.2	3.1	3.0	3.3		0.60
4-Catechol/Methylated 4-catechols	577	26	27	28	32		0.20
Catechols/Methylated Catechols	561	3.6	3.4	3.4	3.8		0.76
Parent estrogens/estrogen metabolites	539	0.42	0.41	0.41	0.4		0.59
2-Pathway/Parent estrogens	567	1.2	1.1	1.1	1.1		0.09
4-Pathway/Parent estrogens	567	0.12	0.12	0.13	0.14		0.36
16-Pathway/Parent estrogens	570	1.2	1.3	1.3	1.2		0.64

* Adjusted for age at urine collection, first morning urine, month of urine collection, history of benign breast diseases, parity, oral contraceptive use, smoked within last month, ovulatory status, physical activity, and menstrual irregularity

^Φ Percentage change for one unit increase of BMI at age 18 when treating it as a continuous variable

[‡] Linear trend across BMI at age 18 using the Wald test determined the p-trend

[§] Includes 2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether

[¶] Includes 4-hydroxyestrone, 4-methoxyestrone, and 4-methoxyestradiol