Published in final edited form as:

Biomark Med. 2015 May ; 9(5): 417–424. doi:10.2217/bmm.15.10.

Association of estrogen measurements in serum and urine of premenopausal women

Gertraud Maskarinec^{*,1}, Fanchon Beckford¹, Yukiko Morimoto¹, Adrian A Franke¹, and Frank Z Stanczyk²

¹University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, HI 96813, USA

²University of Southern California, Los Angeles, CA 90095, USA

Abstract

Aims—We evaluated conjugated and unconjugated urinary estrogen metabolites as surrogate biomarkers for serum levels of unconjugated E₁ and E₂ in premenopausal women.

Materials & methods—Repeated blood and urine samples were analyzed for estrogens and their metabolites using radioimmunoassays and liquid chromatography/mass spectrometry.

Results—The strongest correlation ($r = 0.39$) was observed between serum E₁ and urinary E₁ and E₂. The correlations of urinary E₂ ($r = 0.35$), E₁ ($r = 0.29$), all E₂ metabolites ($r = 0.30$), all E₁ metabolites ($r = 0.23$) and total estrogens ($r = 0.26$) with serum E₂ were only moderate although statistically significant. All correlations were substantially stronger for Whites than Asians.

Conclusion—Urinary E₂ emerged as the best predictor for serum E₁ and E₂, but the large intra-subject variability in urinary estrogen levels limits its use as a biomarker.

Keywords

biomarker; breast cancer risk; correlation; estrogens; ethnicity; steroid hormones

Background

The positive association between elevated levels of endogenous estrogens and increased breast cancer risk is well recognized in post but not premenopausal women [1]. Assessments in serum have been commonly performed, but it is easier to collect urine samples for large

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*Author for correspondence: Tel.: +1 808 586 3078 Fax: +1 808 586 2982 gertraud@cc.hawaii.edu.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interest disclosure

This work was supported by grants R01CA80843 (PI: G Maskarinec) and P30CA71789 (PI: M Carbone) from the National Cancer Institute. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

population-based studies, and relatively higher estrogen concentrations in urine than in serum make measurement in urine easier [2]. However, there is limited research in determining the best urinary biomarker(s) of serum endogenous estrogens. Since the parent estrogens estrone (E_1) and estradiol (E_2) are metabolized along two quantitatively important pathways (2- or 16-hydroxylation) [3], differences in metabolism and routes of excretion (urine/feces) among women as well as timing within the menstrual cycle lead to large variations in urinary excretion patterns [4]. Using radioimmunoassays (RIAs) first and enzyme immunoassays (EIAs) later, numerous investigations in small groups of women demonstrated the clinical utility of urinary markers to assess reproductive function and predict ovulation [5–12], but did not address the question of estrogen exposure assessment in larger epidemiologic studies. Using RIA and EIA technology, early studies focused on a few key urinary estrogen metabolites. For example, urinary E_1 conjugates (E_1C), in other words, the sum of E_1 sulfate and E_1 glucuronide (E_1 -3-G) was found to be highly correlated with serum E_2 levels [9,12–13]. In other studies, urinary E_2 -17-glucuronide (E_2 -17-G) [5,7], E_1 -3-G [6,10–11], and estriol-16-glucuronide were the optimal representatives of serum E_2 [8]. In recent years, high-performance liquid chromatography-mass spectrometry (LC/MS) assays were developed to measure more than a dozen estrogen metabolites in urine simultaneously [1,14–15] and offer the opportunity to examine a wider range of conjugated and unconjugated urinary estrogen metabolites. The objective of the current study was to determine the urinary estrogen metabolite profile that best predicts levels of serum unconjugated E_1 and E_2 measured in repeated samples collected from premenopausal women using Orbitrap LC/MS.

Materials & methods

Study design & procedures

As described in detail elsewhere, two nutritional interventions, the Breast, Estrogen, and Nutrition (BEAN1) and BEAN2 studies collected repeated serum and urine samples for estrogen analysis [16,17]. In brief, BEAN1 was a 2-year randomized trial with 220 women of whom 173 provided samples for this analysis [16]. BEAN2 was a crossover study of 6-month high and low soy diet separated by a 1-month washout period; 76 of the 96 participants contributed data to our current study [17]. Participant requirements for both studies included a normal mammogram, no breast implants, no oral contraceptives, not pregnant, no previous cancer diagnosis, intact uterus and ovaries, regular menstrual periods and low soy intake. Production of at least 10 μ l nipple aspirate fluid (NAF) was an additional inclusion criterion for BEAN2 participants [17].

At baseline all participants completed a food frequency questionnaire that included a dietary history, demographic characteristics, anthropometric measures and reproductive health. Both studies utilized the same dietary intervention protocol. The high soy diet consisted of two servings of soy foods providing approximately 50 mg of isoflavones per day. During the low soy diet, participants continued their regular diet and were counseled to minimize soy intake. The protocols of both studies were approved by the Institutional Review Boards of the University of Hawaii (HI, USA) and the participating clinics. All women signed an informed consent form before entry into the trial and gave written permission to use frozen samples

for future analyses. A data safety monitoring committee reviewed the progress of the studies, reasons for dropouts and any reported symptoms annually.

Urine & serum sample collection & analysis

If possible, blood and urine samples were collected 5 days after ovulation, determined by ovulation kits at baseline and in month 24 in BEAN1 [18], while in BEAN2 self-reported menstruation information was used to confirm ovulation by the onset of the next period at baseline and in months 6 and 13. Due to scheduling problems, 18% of specimens were obtained outside the luteal phase. All serum specimens were collected in the morning, and aliquots of 0.5 ml were prepared for estrogen measurements, which were stored at -80°C . Serum levels of unconjugated E_1 and E_2 were measured by radioimmunoassay (RIA) after extraction with hexane: ethyl acetate (3:2) and Celite column partition chromatography to separate E_1 and E_2 [16,19]. In both studies, repeated overnight urine samples (months 0, 6, 13, 24) covering approximately 8–10 h of the night before the blood draws were collected in containers, with added ascorbic and boric acid to control bacterial growth [18]. For the 173 BEAN1 participants, two samples (baseline and month 24) were evaluated after 7–10 years, while three samples (baseline, month 6 and 13) from 76 BEAN2 participants were analyzed after 0–3 years of storage (Table 1). The samples were divided into three sets and analyzed during 2010. Consistency across rounds was checked by including external urines. The nine predominant steroidal urinary estrogen metabolites [20], E_1 , E_2 , 2-hydroxyestrone (2-OHE₁), 2-hydroxyestradiol (2-OHE₂), 2-methylestrone (2-MeOE₁), 4-hydroxyestrone (4-OHE₁), E_3 , 16-ketoestradiol (16-ketoE₂) and 16 α -hydroxyestrone (16 α -OHE₁), were measured by orbitrap LC/MS (model Exactive, Thermo Fisher Scientific, Waltham, MA) following enzymatic hydrolysis of the conjugated estrogens, using five isotopically labeled internal standards as described previously [15]. As a result, this measurement determined total urinary E_1 and E_2 concentrations, that is, sulfated plus glucuronidated estrogens plus negligible amounts of unconjugated estrogens in urine. We did not assess six less common metabolites (2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, 4-methoxyestrone, 4-methoxyestradiol, 17-epiestriol and 16-epiestriol) that constituted only 6.5% of all metabolites in a report from a cohort of premenopausal women [20]. The respective percentages and 5th–95th percentiles of these metabolites were 0.40 (0.17–0.74), 0.80 (0.25–1.55), 0.09 (0.03–0.21), 0.02 (0.00–0.07), 1.51 (0.24–5.28) and 3.65% (1.60–6.33). In the current analysis, we used 564 paired sets of serum and urine samples. To adjust for urine volume, all measurements were expressed as nmol/mg creatinine. Urinary creatinine concentrations were measured using a Roche-Cobas MiraPlus clinical chemistry autoanalyzer (Roche Diagnostics, Switzerland) [21].

Statistical analysis

The statistical analysis was performed using the SAS software package version 9.3 (SAS Institute, Inc., NC, USA). Total urinary estrogens, both conjugated and unconjugated, were calculated as the sum of E_1 , E_2 , 2-OHE₁, 2-OHE₂, 2-MeOE₁, 4-OHE₁, estriol (E_3), 16-ketoE₂ and 16 α -OHE₁, all urinary E_1 metabolites as the sum of E_1 , 2-OHE₁, 2-MeOE₁, 4-OHE₁ and 16 α -OHE₁, and all urinary E_2 metabolites as the sum of E_2 , 2-OHE₂ and 16-ketoE₂. Using the 249 baseline samples, we computed Spearman correlation coefficients between serum estrogens (E_1 and E_2) and total urinary estrogens for the total population as

well as stratified by ethnicity. To account for the repeated sample design, mixed models that included all study samples were applied to evaluate the association between paired serum estrogens and urinary estrogen metabolites and adjusted for age, ethnicity, body mass index, parity, age at first live birth, timing within the luteal phase and participant ID as a random variable. Due to nonnormal distributions, all estrogen values were log-transformed. The Akaike information criterion (AIC) obtained from the mixed models was used to compare the different models. The AIC is a measure of the relative quality of different statistical models and describes their goodness of fit; a lower AIC indicates a better model fit. We also calculated intraclass correlation coefficients (ICC) to evaluate the intra- versus intersubject variability in serum and urinary estrogen levels.

Results

The mean age of the 249 premenopausal women was 41.9 ± 4.5 years, and the mean BMI was 26.0 ± 5.6 kg/m² (Table 1). The majority of women were of White (41%) or Asian descent (36%). A total of 184 (74%) women in both groups had given birth; only 65 (26%) were nulliparous. The overall mean values for serum E₁, E₂, and total urinary estrogens were 365 ± 227 pmol/l, 541 ± 374 pmol/l and 201 ± 156 pmol/mg creatinine, respectively, with considerable within-person variation over time. The ICCs of serum E₁ and E₂ and total urinary estrogens were 0.32, 0.09 and 0.40, respectively. Whereas the serum values did not differ across ethnic groups, total urinary estrogens were significantly higher ($p < 0.0001$) in Whites (240 ± 183 pmol/mg creatinine) and others (229 ± 178 pmol/mg creatinine) than in Asians (155 ± 111 pmol/mg creatinine) and native Hawaiians (174 ± 107 pmol/mg creatinine).

Correlations between 249 paired baseline serum and urine samples were moderate with the strongest significant correlation of $r = 0.39$ between serum E₁ and urinary E₁ or urinary E₂ in all women (Table 2). The correlations of serum E₂ with other urinary metabolites were lower: $r = 0.35$ with urinary E₂, $r = 0.29$ with urinary E₁, $r = 0.30$ with all urinary E₂ metabolites, $r = 0.23$ with all urinary E₁ metabolites and $r = 0.26$ with total urinary estrogens. Similar correlations were seen for serum E₁ with all urinary E₁ metabolites ($r = 0.28$), all E₂ metabolites ($r = 0.29$) and total urinary estrogens ($r = 0.28$). When stratified by ethnicity, the correlations were substantially stronger (20–30%) for Whites or other ethnicities than for Asians. For example, the respective correlations for serum E₁ and E₂ with urinary E₂ were 0.44 and 0.40 in Whites but only 0.28 and 0.31 in Asians.

When all pairs of serum and urine samples were evaluated with adjustment for covariates, the best model fits corresponded to the highest correlations. For serum E₁ and E₂, the lowest AICs of 578 and 845 were obtained for urinary E₂ followed by 588 and 929 for urinary E₁, respectively (Table 3). The AICs for all urinary E₂ metabolites and total urinary estrogens were also low. In adjusted serum E₁ models, Asian ethnicity emerged as a significant predictor; compared with Whites, Asians had higher serum E₁ levels after taking into account all covariates. In the serum E₂ models, luteal phase was significantly associated with higher E₂ levels.

When we restricted the analysis to the 216 (87%) of samples collected during the luteal phase, the correlations between serum unconjugated E₁ and urinary E₂ ($r = 0.33$) and all urinary E₂ metabolites ($r = 0.24$) as well as those between serum unconjugated E₂ and urinary E₁ ($r = 0.25$), urinary E₂ ($r = 0.30$) and all urinary E₂ metabolites ($r = 0.25$) were generally lower than for the complete set of 249 (Table 2).

Discussion

The moderate associations detected in this analysis suggest that urinary estrogen metabolites are not very robust predictors of circulating estrogen levels. The strongest correlation and lowest AIC were seen for serum unconjugated E₁ ($r = 0.39$, AIC = 578) and E₂ ($r = 0.35$, AIC = 845) with urinary E₂, which corresponds to the sum of sulfated and glucuronidated metabolites, that is, E₂-3-sulfate, E₂-17-sulfate, E₂-3-G and E₂-17-G. Although urinary E₂ emerged as the best predictor for both parent estrogens in this analysis using RIA and EIA methodology, urinary E₁ and total urinary estrogen metabolites showed very similar associations, likely due to the interconversion of E₂ and E₁ and their metabolites before and after hydroxylation. Unfortunately, adjustment for demographic and reproductive factors, which may not always be available, did not improve the prediction of serum levels. Given the significant differences by ethnic group for urinary but not serum estrogen levels, measuring estrogens in urine may contribute to our understanding of disparities in estrogen metabolism and hormone-related cancers, possibly due to differential metabolism of estrogens [22].

The correlations between serum and urine values in the current study are weaker than the results reported by investigations assessing the clinical utility of urinary markers [5–12]. There are several reasons for this discrepancy. In reports that assessed reproductive function and predicted ovulation [5–12], specimens were collected daily during 1–2 menstrual cycles and E₂ levels varied 5–6-fold due to the low levels in the early follicular phase and the high levels prior to ovulation [13]. Correlation of serum E₂ with different urinary metabolites varied between 0.60 [6], 0.63 [11] and 0.73 [10] for E₁-3-G; 0.75 [5] for E₂-17-G; and 0.93 [13] for E₁-C. In contrast, the current report examined E₂ concentrations during a more restricted time period within the cycle, that is, during the luteal phase approximately 5 days after ovulation. Reducing the range within the cycle results in weaker correlations due to the smaller range of estrogen levels and lower interindividual variation as compared with concentrations in reproductive studies that collected samples throughout the menstrual cycle.

The time lag between serum and urine collection is another possible reason for our findings [9,13]. For example, the strongest correlation of 0.93 was seen when serum samples were evaluated in relation to urine values collected 1 day later, whereas the correlation was only 0.47 when urine samples from the previous day were considered [13]. A comparison of paired hormone values in the late follicular phase indicated that estrogen metabolites reach the urine 12–24 h after estrogen appears in blood [9]. Our study design with overnight urines collected in the morning of the same day as blood was drawn corresponds to collecting urine before the blood draw. In order to achieve adequate comparisons, blood values should be collected at the start and the end time of urine collection in order to obtain an integrated assessment of serum levels for the time of urine collection [23]. In further differences, comparisons in previous studies were based on serum E₂ and one or two urinary conjugates,

usually a glucuronide [5–7,10–11], whereas we also evaluated the relation with E₁ plus seven of the main oxidized urinary metabolites of E₂ or E₁ [1] using a modern LC/MS approach, but given the interconversion between E₁ plus E₂ this may be a minor issue.

The present study had several strengths, in particular the well-established, sensitive and accurate assays to measure multiple urinary estrogens and their metabolites. Additional metabolites not measured appear to contribute only a small percentage to total urinary excretion [20]. Compared to previous studies that followed 7–33 women for 1–2 menstrual cycles [5–13], the sample size of 249 women followed for 13 or 24 months represented a more population-based design relevant to etiologic investigations. The study design allowed us to collect multiple samples per woman over a relatively long period of time, which may have corrected for intraindividual variability to some degree, but as the low ICCs indicate, the within-person variability remains large. The low ICCs of estrogen measures also confirm the weakness of one time serum measures commonly used in epidemiologic studies [24,25]. As circulating estrogen levels reflect a single time point, urinary levels have the advantage to integrate excretion over longer time periods, in our case at least 6–8 h but not the several weeks or years necessary to capture intraindividual variation in estrogen exposure due to differences in metabolism and routes of excretion independent of assay method [26,27]. Therefore, it is not clear if the concentration of E₁C or other conjugated analytes in urine can accurately reflect the long-term exposure to the unconjugated parent compound in the circulation.

Logistical issues were responsible for several weaknesses in our study. The strict eligibility criteria for the two BEAN studies were a serious limitation and may have led to selection bias limiting the generalizability of the observations to other women. As only premenopausal women were included, the associations may differ in postmenopausal women and men who have lower levels of circulating estrogens. It would have been better to draw blood on one day and then start the urine collection for the next day to take care of the lag issue [9,13]. Ideally, samples would have been collected at the same time during the menstrual cycle for each woman because estrogen metabolites are significantly affected by menstrual cycle phase [4], but with schedules and availability that was not always possible; approximately 18% of samples were collected outside the luteal phase. Although it is well known that 24-h urines better account for circadian variations than overnight samples [5], overnight urine samples rather than 24-h samples had to be collected to reduce the burden on our participants.

Conclusion & future perspective

The current study contributes to our understanding of how urinary estrogen assessment may be useful for epidemiologic studies that evaluate the association of estrogen status with disease risk. However, due to the large intrasubject variability in estrogen levels, urinary concentrations of estrogens and their metabolites appear to provide only limited information about exposure to endogenous unconjugated estrogens; no single analyte performed much better than the others, most likely due to the continuous interconversion between estrogens. Since the goal in epidemiologic studies is to rank healthy women by estrogen exposure and not to determine fertility or diagnose reproductive disorders [2], relative levels are

acceptable to associate exposure with risk of disease, whereas for clinical questions, absolute estrogen values are more important. Further validation, which ideally includes postmenopausal women and men, of urinary metabolites representing serum estrogen levels may promote the use of urinary samples to assess estrogen and make it easier to conduct large epidemiologic investigations into the effects of hormonal status. Ideally, estrogen measurement will be standardized, which is now being done with serum E₂ assays, so that absolute values could be used in epidemiologic studies. Although LC/MS assays are more expensive than EIA measurements, they have the advantage that multiple metabolites of interest can be measured simultaneously. Since the ultimate goal is to assess estrogen concentrations in breast tissue, it would also be desirable to validate potential biomarkers for tissue concentrations [28]. Genetic profiles, which were not available for these women, might provide further insight into mechanistic aspects of estrogen metabolism in future studies.

Acknowledgments

The authors thank Laurie Custer (University of Hawaii Cancer Center) for the skillful performance of LC/MS analyses. We are very grateful to the dedicated study participants.

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Executive summary

Background

- In large epidemiologic studies investigating sex hormone levels, the collection of urine samples is often more feasible than drawing blood samples.

Results

- The strongest correlations ($r = 0.39$) were observed for serum levels of E_1 with urinary E_1 and E_2 .
- Moderate correlations ($r = 0.23$ – 0.35) were observed between serum E_2 and different combinations of urinary estrogen metabolites.
- Urinary E_2 appears to be the best predictor for serum levels of E_1 and E_2 .
- Across ethnic groups, the correlations were generally stronger among Whites than Asians.

Conclusions

- Large intrasubject variability in urinary estrogen levels over time remains problematic for any investigation of endogenous estrogens in premenopausal women.
- Further validation of urinary metabolites as surrogate markers for serum estrogen levels in women and men of all ages may promote use in epidemiologic investigations.

Table 1

Characteristics of the study participants.

Characteristic	BEAN1	BEAN2	Total		
Number of participants	173	76	249		
BMI	26.1 ± 5.8	25.8 ± 5.1	26.0 ± 5.6		
Age (years)	43.0 ± 2.9	39.3 ± 6.0	41.9 ± 4.5		
Ethnicity:					
– White	65 (38%)	38 (50%)	103 (41%)		
– Asian	70 (40%)	20 (26%)	90 (36%)		
– Native Hawaiian	20 (12%)	14 (19%)	34 (14%)		
– Other	18 (10%)	4 (5%)	22 (9%)		
Parity:					
– Yes	129 (75%)	55 (72%)	184 (74%)		
– No	44 (25%)	21 (28%)	65 (26%)		
Analyte	Baseline n = 249	Month 6 [†] n = 75	Month 13 [‡] n = 77	Month 24 [‡] n = 163	All months
Serum E ₁ , pmol/l	353 ± 188	408 ± 183	427 ± 366	332 ± 206	365 ± 227
Serum E ₂ , pmol/l	531 ± 295	579 ± 318	627 ± 656	498 ± 314	541 ± 374
Urinary estrone (E ₁) metabolites [§] , pmol/mg:	132 ± 103	142 ± 127	143 ± 117	137 ± 160	136 ± 126
– E ₁	42 ± 28	50 ± 43	50 ± 41	39 ± 30	43 ± 33
– 2-OHE ₁	54 ± 59	58 ± 66	59 ± 59	50 ± 50	54 ± 57
– 2-MeOE ₁	18 ± 19	12 ± 15	13 ± 17	31 ± 124	20 ± 68
– 4-OHE ₁	9 ± 11	7 ± 11	6 ± 7	9 ± 13	8 ± 11
– 16α-OHE ₁	9 ± 9	16 ± 19	15 ± 13	8 ± 8	11 ± 12
Urinary estradiol (E ₂) metabolites [¶] , pmol/mg:	29 ± 20	36 ± 34	35 ± 30	27 ± 17	30 ± 23
– E ₂	15 ± 10	17 ± 14	18 ± 17	14 ± 10	15 ± 12

Characteristic	BEAN1	BEAN2	Total
- 2-OHE ₂	7 ± 9	9 ± 12	5 ± 5
- 16-ketoE ₂	8 ± 6	10 ± 11	7 ± 6
Urine E ₃ , pmol/mg	45 ± 40	54 ± 51	42 ± 34
Total urinary estrogens [#] , pmol/mg	191 ± 125	232 ± 197	187 ± 164
			201 ± 156

[†] BEAN2 samples only.

[‡] BEAN1 samples only.

[§] E₁ metabolites = sum of E₁, 2-OHE₁, 2-MeOE₁, 4-OHE₁, and 16α-OHE₁.

[¶] E₂ metabolites = sum of E₂, 2-OHE₂, and 16-ketoE₂.

[#] Total urinary estrogens = sum of E₁, E₂, 2-OHE₁, 2-OHE₂, 2-MeOE₁, 4-OHE₁, E₃, 16-ketoE₂ and 16α-OHE₁.

Table 2

Correlations between serum and urine values of estrogens and their metabolites at baseline.[†]

Analyte in urine (pmol/mg)	Serum E ₁ (pmol/l)					Serum E ₂ (pmol/l)				
	All women	Whites	Asians	Others	All women	Whites	Asians	Others	All women	Whites
Estrone (E ₁) metabolites [¶] :	0.28 [‡]	0.35 [‡]	0.21 [§]	0.37 [§]	0.23 [‡]	0.32 [‡]	0.23 [§]	0.22	0.23 [‡]	0.27 [§]
– E ₁	0.39 [‡]	0.48 [‡]	0.26 [§]	0.54 [‡]	0.29 [‡]	0.39 [‡]	0.22 [§]	0.26 [§]	0.29 [‡]	0.31 [§]
– 2-OHE ₁	0.15 [§]	0.21 [§]	0.09	0.21	0.16 [§]	0.25 [§]	0.17	0.08	0.16 [§]	0.21 [§]
– 2-MeOE ₁	0.27 [‡]	0.27 [§]	0.17	0.46 [‡]	0.15 [§]	0.14	0.15	0.16	0.15 [§]	0.15
– 4-OHE ₁	0.24 [‡]	0.33 [‡]	0.12	0.37	0.24 [‡]	0.28 [§]	0.14	0.32	0.24 [‡]	0.28 [§]
– 16α-OHE ₁	0.10	0.15	0.06	0.10	0.21 [‡]	0.24 [§]	0.17	0.20	0.21 [‡]	0.24 [§]
Estradiol (E ₂) metabolites [#] :	0.29 [‡]	0.35 [‡]	0.17	0.37 [§]	0.30 [‡]	0.39 [‡]	0.27 [§]	0.22	0.30 [‡]	0.37 [§]
– E ₂	0.39 [‡]	0.44 [‡]	0.28 [§]	0.45 [‡]	0.35 [‡]	0.40 [‡]	0.31 [§]	0.29 [§]	0.35 [‡]	0.40 [‡]
– 2-OHE ₂	0.12	0.24 [§]	0.04	0.12	0.10	0.26 [§]	0.09	–0.08	0.10	0.26 [§]
– 16-ketoE ₂	0.11	0.08	0.07	0.25	0.19 [§]	0.18	0.19	0.24	0.19 [§]	0.24
– Estriol (E ₃)	0.09	0.03	0.08	0.21	0.16 [§]	0.19	0.15	0.11	0.16 [§]	0.19
Total estrogens ^{††}	0.28 [‡]	0.35 [‡]	0.23 [§]	0.34 [§]	0.26 [‡]	0.36 [‡]	0.27 [§]	0.18	0.26 [‡]	0.36 [‡]

[†] Spearman correlation coefficients for all women at baseline.[‡] p < 0.001.[§] p < 0.05.[¶] E₁ metabolites = sum of E₁, 2-OHE₁, 2-MeOE₁, 4-OHE₁ and 16α-OHE₁.[#] E₂ metabolites = sum of E₂, 2-OHE₂ and 16-ketoE₂.^{††} Total estrogens = sum of E₁, E₂, 2-OHE₁, 2-OHE₂, 2-MeOE₁, 4-OHE₁, E₃, 16-ketoE₂ and 16α-OHE₁.

Table 3

Mixed models for serum and urine values of estrogens and their metabolites.[†]

Analyte in urine (pmol/mg)	Serum E ₁ (pmol/l)				Serum E ₂ (pmol/l)			
	AIC [‡]	Estimate	Standard error	p-value	AIC [‡]	Estimate	Standard error	p-value
Estron (E ₁) metabolites [§] :	650	0.31	0.02	<0.001	954	0.36	0.03	<0.001
– E ₁	588	0.40	0.02	<0.001	929	0.44	0.03	<0.001
– 2-OHE ₁	729	0.17	0.02	<0.001	1016	0.18	0.03	<0.001
– 4-OHE ₁	711	0.22	0.02	<0.001	993	0.26	0.03	<0.001
– 2-MeOE ₁	705	0.22	0.02	<0.001	1010	0.21	0.03	<0.001
– 16α-OHE ₁	743	0.20	0.03	<0.001	1005	0.25	0.03	<0.001
Estradiol (E ₂) metabolites [¶] :	629	0.38	0.03	<0.001	892	0.49	0.03	<0.001
– E ₂	578	0.43	0.03	<0.001	845	0.57	0.03	<0.001
– 2-OHE ₂	745	0.19	0.03	<0.001	1028	0.20	0.03	<0.001
– 16-ketoE ₂	729	0.26	0.03	<0.001	994	0.33	0.04	<0.001
Estriol (E ₃)	745	0.19	0.03	<0.001	998	0.26	0.03	<0.001
Total estrogens [#]	649	0.38	0.03	<0.001	924	0.48	0.03	<0.001

[†] Obtained through mixed models and adjusted for age, ethnicity, body mass index, parity, age at first live birth, luteal phase using log-transformed variables for estrogens and metabolites from all repeated samples.

[‡] Akaike Information Criterion.

[§] E₁ metabolites = sum of E₁, 2-OHE₁, 2-MeOE₁, 4-OHE₁ and 16α-OHE₁.

[¶] E₂ metabolites = sum of E₂, 2-OHE₂ and 16-ketoE₂.

[#] Total estrogens = sum of E₁, E₂, 2-OHE₁, 2-OHE₂, 2-MeOE₁, 4-OHE₁, E₃, 16-ketoE₂ and 16α-OHE₁.