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The Relation of Urinary Estrogen Metabolites with Mammographic Densities in Premenopausal Women

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Abstract

Background—Mammographic density is a strong predictor of breast cancer risk. The total amount and the metabolism of endogenous estrogens, e.g., the ratio of 2-hydroxyestrone (2-OHE_1) and 16α-OHE_1 may influence breast cancer risk. This study examined the association of urinary estrogen metabolites with breast density in premenopausal women.

Methods—Urine samples were collected at baseline and after 2 years, analyzed for 11 estrogen metabolites plus progesterone and testosterone by liquid chromatography mass spectrometry, and adjusted for creatinine levels. Mixed-effects regression was applied to examine the association of estrogens with breast density.

Results—Total estrogen metabolites (181±113 vs. 247±165 pmol/mg creatinine, p=0.01) and the 2/16α-OH ratio (8.4±10.4 vs. 13.0±17.1, p=0.02) were lower in the 74 Asian than in the 114 non-Asian women. In adjusted models, positive associations of total estrogen metabolites (p=0.002) and the 2/16α-OHE_1 ratio (p=0.08) with percent density were detected in Asians only. In all women, mammographic density was positively associated with the 2-OH pathway (p=0.01), inversely related to the 16α-OH pathway (p=0.01), and not associated with the 4-OH pathway, testosterone, and progesterone. Results for the size of the dense area weakly reflected the findings for percent density, while associations with the non-dense area were in the opposite direction.

Conclusions—The findings that the 2-OH pathway is associated with higher and the 16α-OH pathway with lower breast density contradicts the hypothesized risk profile of these metabolites, but, if a relation between estrogen metabolites and breast cancer risk exists, it may be mediated through pathways other than mammographic density.

Keywords

Urinary estrogen metabolites; breast cancer risk; mammographic density; premenopausal women

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

Introduction

Mammographic density is one of the strongest predictors of breast cancer risk [1]. Although a positive relation with steroid hormones is supported by findings of higher breast density in pre-than postmenopausal women, increased densities after hormone therapy and reduced densities after tamoxifen treatment, the relation with endogenous estrogen levels is less clear [2–5]. It was proposed that women who metabolize endogenous estrogens predominantly via 16α rather than via 2-hydroxylation and, as a result, have a low ratio of 2-hydroxyestrone (2-OHE_1) to 16α-OHE_1, are at higher risk of breast cancer because 16α-OHE_1 is considered more carcinogenic than 2-OHE_1 [6, 7]. Only three previous studies have explored the association between urinary estrogen metabolites and mammographic densities. Among 70 postmenopausal women [8], participants with Wolfe parenchymal patterns P2/DY (high risk) had, on average, 58% higher levels of 2-OHE_1 (p=0.002), 15% higher levels of 16α-OHE_1 (p=0.37), and a 35% higher 2/16α-OHE_1 ratio (p=0.005) than those with a low risk N1 pattern. In a cross-sectional study, 16α-OHE_1 and 2-OHE_1 were measured by competitive immunoassays in 305 women with a mean age of 47.2 years [9]. Although individual hormones were not associated with breast density, the 2/16α-OHE_1 ratio was 25% higher in the highest as compared to the lowest density category (p=0.01). In Asian-American women, a lower 2/16α-OHE_1 ratio appeared to be a marker of westernization [10]. The current analysis examined the association of urinary estrogen metabolites measured by liquid chromatography mass spectrometry (LCMS) [11–13] with mammographic measures in premenopausal women who participated in a 2-year soy intervention, in which the soy diet did not affect breast density [14].

MATERIALS AND METHODS

Study design

As described elsewhere [14, 15], the participants were recruited by sending out 10,022 invitations to women who had received a normal screening mammogram. Of these, 975 (9.73%) interested women replied and 352 women aged 35–46 years were eligible. Women were excluded from this study due to use of oral contraceptives or other sex hormones, diagnosis of cancer, hysterectomy, no intact ovary, or no regular menstrual periods. After a run-in period, 220 women were randomized to a soy diet or to the control group and 189 subjects completed 2 years of intervention [15]. The Institutional Review Boards of the University of Hawaii and the participating clinics approved the study protocol; participants signed informed consent and gave written permission to use their frozen samples for future analyses.

The 2-year trial examined the effect of consuming 2 servings of soy foods per day (approximately 50 mg aglycone equivalents of isoflavones) on hormonal outcomes [16]. Women in the control group were instructed to maintain their regular diet and limit their soy intake. Body weight was recorded at baseline and at each study visit. Since the dietary intervention did not change serum sex hormone levels and mammographic densities [14, 15], both groups were combined for the current analysis.

Mammographic density analysis

As described in detail before [14], mammograms at baseline and after 2 years were collected for each woman. After scanning films from both breasts with a Kodak LS85 Film Digitizer, one of the authors performed computer-assisted density assessment while being blinded to group status and time sequence [17]. Percent density was calculated as the ratio of dense area to total area of the breast. We averaged the values for the right and the left breast. A

sample of 219 mammograms read in duplicate indicated high reproducibility with an intraclass correlation coefficient of 0.95 for percent density (95% CI: 0.93–0.96).

Urinary Analysis

This study made use of existing urine samples that were collected during the trial period [15]. Participants donated midluteal urine samples at baseline and at 3, 6, 12 and 24 months after randomization timed to occur 4–6 days after ovulation as determined by an ovulation kit [15]. For the current analysis only the baseline and the final samples were used. As described in the original study [15], serum progesterone was measured to confirm ovulation. Urinary estrogen results were available for 186 baseline urines and 185 follow-up samples collected from 188 women; 5 women contributed only one sample due to missing values.

The 11 most predominant steroidal estrogens in premenopausal women, namely E_1, E_2, 2-OHE_1, 2-OHE_2, 2-$MeOE_1$, 2-OHE_1-3-methyl ether, 4-OHE_1, 4-OHE_2, 16α-OHE_1, 16keto-E_2, and E_3 [18], as well as progesterone, testosterone, and equol were measured by orbitrap LCMS (model Exactive, ThermoFisher Scientific, Waltham, MA) as described in detail previously [13]. In brief, 0.3 mL urine was enzymatically hydrolyzed with β-glucuronidase and sulfatase after addition of ascorbic acid and deuterated internal standards. This was followed by extraction with methyl tertiary butyl ether and redissolving the dried ether phase in 75 μL dansyl chloride (3 mg/mL in acetone) followed by mixing with 75 μL aq. sodium bicarbonate (100 mM, pH 9) and 15 μL 1% aq. ascorbic acid and incubation for 15 min at 64°C. This mixture was analyzed by orbitrap LCMS in the positive mode after electrospray ionization using exact masses as described in detail previously [13].

Analysis of an external urine pool from premenopausal women repeated on 9 different days revealed the following coefficients of variation (CV): E_3 (13%), 16-ketoE$_2$ (14%), 16α-OHE_1 (21%), E_2 (4%), 2-$MeOE_1$ (9%), 2-OHE_1-3-methyl ether (10%), E_1 (5%), 4-OHE_2 (20%), 2-OHE_2 (15%), 4-OHE_1 (6%), 2-OHE_1 (11%), progesterone (20%), and testosterone (9%). Urinary concentrations were expressed as pmol/mg creatinine to adjust for urine volume. Creatinine levels were analyzed with a Roche-Cobas MiraPlus chemistry analyzer using a kit from Randox Laboratories (Crumlin, UK) that is based on a kinetic modification of the Jaffé reaction.

Urinary isoflavonoids as a biomarker for soy intake had been measured previously by HPLC [16]. Since equol producers are thought to experience more protective effects of isoflavones than non-producers [19], equol producer status was determined based on two criteria: urinary daidzein excretion ≥2 nmol/mg and urinary equol to daidzein ratio ≥0.018 [20, 21]. The 23 participants who meet both criteria at least once during the study were considered equol producers; 7 were of Asian ethnicity and 16 were Caucasians.

Statistical Analysis

The SAS statistical software package version 9.2 (SAS Institute Inc., Cary, NC) was used for the statistical analysis. Subjects were classified into two ethnic categories: Asians and non-Asians; the number of women with other ethnic backgrounds was too small for separate analyses. For consistency with the literature [18], we calculated the sum of all 11 urinary metabolites and computed relative percentages for the individual analytes. Metabolites were grouped by pathway (2, 4, and 16α), the 2/16α-OHE_1 ratio was computed by dividing 2-OHE_1 by 16α-OHE_1, and quartiles were created for all urinary measures. Variables with non-normal distributions were log-transformed (non-dense breast area and all urinary variables except E_1, 2-OHE_1, and the 2-OH pathway) or square root-transformed (dense breast area). Percent breast density had an acceptable distribution.

We evaluated means and frequencies at baseline between the two randomization groups and by ethnicity using χ^2 and *t* tests. To examine the association between urinary and mammographic measures (percent density, dense area, non-dense area), we applied mixed-effects regression models with breast measures as dependent variable, computed adjusted least-square means for quartiles of urinary measures, and performed trend tests using continuous variables. Due to known associations with breast density and to control for differences by group, we included group assignment (intervention vs. control), time (baseline vs. end of study), age (continuous), body mass index (BMI) (continuous), Asian ethnicity, number of children (0, 1, 2, 3+), age at first-live birth (</≥30 years), alcohol intake (</≥1 drink/month), age at menarche (≤/>13 years), and family history of breast cancer (yes/no) as covariates. To examine effect modification, we included interaction terms and performed stratified analyses by ethnicity, weight and equol status using the same quartiles as for the total population.

RESULTS

Of the 188 women included in the study, 114 (60%) were Caucasian and Native Hawaiian and 74 (40%) were of Asian ancestry (Table 1). Their mean ages were similar, but Asian women had lower BMIs, smaller total breast areas, and smaller dense areas than non-Asians. On the other hand, mean percent density was higher for Asians than non-Asians (49.0 vs. 43.4; p=0.12). Total estrogen metabolites (181±113 vs. 247±165 pmol/mg creatinine, p=0.01) and the 2/16α-OH ratio (8.4±10.4 vs. 13.0±17.1, p=0.02) were lower in Asians than non-Asians. The relative percentages of urinary metabolites also differed by ethnicity. Whereas Asians had higher proportions of E_1, E_2, E_3, and 16α-OH metabolites, non-Asians excreted relatively more 2-OH metabolites. A comparison between the control and the soy intervention group showed that urinary estrogen metabolites did not differ significantly (data not shown). For example, the sum of all metabolites was 223±136 pmol/mg creatinine for the control group and 220±164 pmol/mg creatinine for the intervention group (p=0.57). The respective values for the 2/16α-OH ratio were 11.1±16.3 and 11.2±13.8 (p=0.58). BMI was not significantly related to total estrogen metabolites or the 2/16 α-OH ratio, but it was the most influential confounder; alcohol intake, parity, and age at menarche were also significant in several models.

After adjustment for covariates (Table 2), higher percent densities were observed in the highest as compared to the lowest quartile of total estrogen metabolite excretion in Asian women only (55.4% vs. 44.2%; p_{trend}=0.002). The interaction of ethnicity with total estrogen metabolites was highly significant ($p_{interaction}$=0.003). Further examinations found no interaction effects for randomization group, weight status, and equol producer status.

In all women, positive associations were observed for the 2-OH pathway (p=0.01), in particular 2-$MeOE_1$ (p=0.07) and 2-OHE_1 (p=0.06). Significant inverse associations were seen for the 16α-OH pathway (p=0.01), especially for E_3 (p=0.002) with mean percent densities of 46.3 and 40.5% in the lowest and highest quartile. The 4-OH pathway was not associated with percent density. For the 2/16α-OHE_1 ratio, a trend toward higher percent density with higher quartiles was borderline significant in Asians (p=0.08) but no association was detected in the other women. Progesterone and testosterone were also not associated with percent densities. Models with molar concentrations of estrogen metabolites showed weaker associations in the same direction as those for relative percentages. For example, the p-values for the 2-OH pathway were 0.02, 0.68, and 0.004 among all, non-Asian, and Asian women, respectively, and 0.81, 0.20, and 0.07 for the 16α-OH pathway (data not shown).

When we repeated the analyses with the absolute dense area as dependent variable (Table 3), a positive association with total estrogen metabolites (p=0.01) was seen in Asian women only and an inverse relation for the 16α-OH pathway (p=0.03) and E_3 (p=0.0007) in non-Asians only. In all women, the size of the non-dense area was inversely associated with the 2-OH pathway (p=0.03) and positively associated with the 16α-OH pathway (p=0.008), whereas inverse associations of total estrogen metabolites (p=0.01), the 4-OH pathway (p=0.05), and the 2/16α-OHE_1 ratio (p=0.003) were restricted to Asian women.

DISCUSSION

This analysis of 11 urinary estrogen metabolites detected lower levels total estrogen metabolites in Asian than non-Asian women. Percent densities were positively associated with total estrogen metabolites only in Asian women who also had a lower BMI, smaller breasts and smaller dense areas than non-Asians. Contrary to the hypothesized carcinogenic effects of the 16α-OH metabolites [6, 7], the 16α-OH pathway including E_3 was associated with lower and the 2-OH pathway with higher percent densities in all women. A borderline significant association of the 2/16α-OHE_1 ratio and percent density in Asian women also conflicts with its hypothesized protective effect but agrees with previous findings [8, 9]. The associations between metabolites and the dense area were in the same direction as percent density, but considerably weaker and often not significant, while the relation with the non-dense areas was in the opposite direction. This observation agrees with a recent publication describing a protective effect of the nondense area, in contrast to the higher risk associated with the dense area, on breast cancer risk [22].

The levels of total urinary estrogen metabolites measured in our study population and the relative proportions were in a similar range as previous reports from premenopausal women [18, 23]. Our finding that breast density is higher in Asian than Caucasian women agrees with previous studies [24, 25]. Repeated evidence has also demonstrated lower urinary and circulating estrogen levels in Japanese women [26–28]. The positive association between total estrogen metabolites and percent densities in Asian women is in conflict with a recent report from the Nurses' Health Study [23] describing a lower breast cancer risk with parent estrogens and the 2- and 4-OH pathway but not with the 16α-OH pathway and the 2/16α-OH metabolite ratio. As the authors stated, it remains unclear how conjugate urine metabolites reflect breast tissue activity. A higher rate of excretion of parent estrogens before conversion into more estrogenic metabolites may lower breast cancer risk. As shown recently, polymorphisms in genes coding for glucuronidation and sulfation enzymes may be responsible for the clearance of endogenous estrogens at different rates and modify breast cancer risk through an effect on circulating estrogen levels [29] or breast density [30].

There is no obvious explanation for our finding that several of the significant associations with percent density and non-dense area were limited to women with Asian ancestry. Since all models were adjusted for BMI, the lower body weight of Asian women does not explain the discrepant findings. One possibility is that the lower breast cancer risk in Asian women is a result of the low absolute levels of estrogens rather than the relative proportion of metabolites [7, 27, 28]. On the other hand, recent incidence data suggest that women of Asian ancestry in Hawaii, such as in this study, have similar circulating estrogen levels and a breast cancer risk as Caucasians [31, 32]. Alternatively, genetic polymorphisms in phase II enzymes may play a role [29, 30]. Despite the fairly large body of literature, the relation of the 2/16α-OHE_1 ratio with breast cancer remains unclear. A systematic review of 6 prospective and 3 retrospective studies [7] suggested a weak protective association in premenopausal women. Although many smaller studies reported supporting results [33], flaws in study design, e.g., matching of controls, question their validity.

Limitations of the current study include the issue of false positives due to multiple testing, the fact that urine was not collected on the same day as the mammograms, the limited sample size, the small number of Native Hawaiian women who had to be combined with Caucasians into one group, possible selection bias due to the strict eligibility criteria for the intervention study, and the relatively high CVs for some of the estrogen metabolites with low concentrations that decrease the likelihood of detecting a modest difference. We ruled out an effect of the soy diet by testing for an interaction effect and by including group membership into the model. As shown in the original analysis, the high soy diet did not affect mammographic densities [14]. On the other hand, this study had several strengths. Urine collection was timed according to menstrual cycle, and most samples were collected during the midluteal phase as confirmed by progesterone testing [15]. Two measurements for mammographic density and urinary estrogen over 2 years were available for each participant; this approach reduces concerns about intra-individual variability over time. As shown in the Nurses' Health Study, reproducibility of urinary excretion appears to be relatively high [18]. The use of LCMS as compared to ELISA assays provides more accurate measurements of 2- and 16α-OH metabolites and assess many different urinary estrogen metabolites [13]. Therefore, it would be desirable to conduct more studies that utilize the LCMS assessment methods instead of ELISA assays as in most published reports [7].

In conclusion, our findings that the 2-OH pathway is associated with higher and the 16α-OH pathway with lower breast density contradict the hypothesized risk profile of these metabolites [6, 7], but it is possible that estrogen metabolite patterns, if associated with breast cancer risk, and mammographic densities operate through different pathways. To elucidate the complex relations, a prospective study design that assesses breast density, urinary estrogen metabolites, and breast cancer incidence is needed.

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Table 1

Characteristics of the Study Population

	All women		Non-Asian		Asian		p-value[e]
	N	%	N	%	N	%	
Number of participants	188	100	114	60.6	74	39.4	--
Age at menarche >13 years	80	42.6	50	43.9	30	40.5	0.65
Parous	140	74.5	83	72.8	57	77.0	0.52
Age at first-live birth <30 years	144	76.6	94	82.5	50	67.6	0.02
Alcohol intake (≥1 drink/month)	106	56.4	70	61.4	36	48.6	0.09
	Mean±SD		Mean±SD		Mean±SD		
Age (years)	43.0±2.9		42.7±2.9		43.4±2.7		0.10
Body mass index (kg/m^2)	26.1±5.9		27.0±6.3		24.8±4.9		0.01
Mammographic density measures							
Total breast area (cm^2)	109.5±59.1		126.2±62.7		84.4±42.8		<0.001
Dense area (cm^2)	42.5±25.5		46.2±28.1		37.0±19.8		<0.001
Non-dense area (cm^2)	66.9±58.3		80.0±64.4		47.3±41.8		<0.001
Percent density (%)	45.6±23.9		43.4±23.5		49.0±24.3		0.12
Total estrogen metabolites (pmol/mg creatinine)	221±150		247±165		181±113		0.01
Estrone (E_1)[a]	20.0±5.8		18.7±5.3		21.9±6.1		<0.001
Estradiol (E_2)	7.5±3.4		6.7±3.3		8.7±3.2		<0.001
2-OH pathway[b]	37.5±15.8		41.3±15.6		31.6±14.4		<0.001
2-OHE_1	23.8±12.6		26.6±12.5		19.3±11.4		<0.001
2-OHE_2	2.4±2.1		2.7±1.9		2.1±2.3		0.01
2-MeOE_1	9.6±6.0		10.2±6.7		8.5±4.7		0.14
2-OHE_1-3-methyl ether	1.7±0.8		1.7±0.8		1.7±0.8		0.44
4-OH pathway[c]	5.3±3.1		5.1±2.4		5.7±4.1		0.55
4-OHE_1	4.9±3.0		4.7±2.2		5.3±4.0		0.65
4-OHE_2	0.4±0.3		0.4±0.3		0.1±0.2		0.27

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	All women		Non-Asian		Asian		p-value[e]
	N	%	N	%	N	%	
16-OH pathway[d]	29.7±15.3		28.1±15.7		32.1±14.4		0.02
16α-OHE_1	4.4±3.7		4.5±4.2		4.1±2.8		0.78
16-ketoE_2	3.9±2.0		3.8±2.0		4.1±2.0		0.29
Estriol (E_3)	21.4±11.8		19.9±11.7		23.8±11.6		0.006
2/16α-OHE_1 ratio	11.2±15.0		13.0±17.1		8.4±10.4		0.02
Testosterone (pmol/mg creatinine)	9.7±15.4		11.5±10.1		7.0±20.9		<0.001
Progesterone (pmol/mg creatinine)	1.7±1.2		1.6±1.1		1.8±1.2		0.12

[a] Individual metabolites are expressed as percent of total estrogen metabolites

[b] (2-OHE_1 + 2-OHE_2 + 2-MeOE_1 + 2-OHE_1-3-methyl ether)

[c] (4-OHE_2 + 4-OHE_1)

[d] (E_3 + 16α-OHE_1 + 16-ketoE_2)

[e] p-values obtained by chi-square test for categorical variables and by t-test for continuous variables

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Table 2

Mean Percent Density by Quartiles of Urinary Metabolites

Estrogen Metabolites	Quartile range[a]	All women			Non-Asian			Asian	
		%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]
Total estrogen metabolites (pmol/mg creatinine)	0.5–116.1	43.3	0.12	*19*	43.2	0.64	*22*	44.2	**0.002**
	116.2–180.4	42.7		*23*	42.4		*20*	42.6	
	180.8–265.6	44.7		*31*	42.3		*20*	49.8	
	265.9–1538	44.3		*40*	41.0		*11*	55.4	
Estrone (E_1) (%)	3.9 – 16.0	43.5	0.64	*33*	42.0	0.62	*10*	45.2	0.68
	16.1 – 18.9	45.7		*31*	43.1		*18*	50.5	
	19.0 – 23.9	43.0		*29*	42.4		*20*	44.2	
	24.0 – 46.2	42.8		*20*	40.4		*25*	45.7	
Estradiol (E_2) (%)	1.1 – 5.0	44.5	0.32	*37*	43.8	0.55	*6*	44.3	0.24
	5.0 – 6.7	43.6		*32*	41.2		*15*	47.8	
	6.7 – 9.0	43.8		*28*	41.1		*25*	47.7	
	9.0 – 24.2	43.1		*16*	41.4		*27*	45.5	
2-OH pathway[d] (%)	2.0 – 24.9	40.1	**0.01**	*14*	37.4	**0.05**	*26*	43.6	**0.04**
	25.1 – 38.3	43.9		*32*	42.2		*24*	46.5	
	38.3 – 49.5	45.4		*30*	42.2		*12*	51.1	
	49.5 – 91.1	45.6		*37*	44.2		*11*	46.5	
2-OHE_1 (%)	0.0 – 13.5	40.8	0.06	*16*	37.6	0.08	*26*	44.4	0.32
	13.6 – 22.8	45.0		*27*	43.8		*21*	47.2	
	22.8 – 32.8	44.3		*34*	42.3		*15*	48.3	
	32.9 – 71.2	44.8		*36*	43.1		*11*	47.2	
2-OHE_2 (%)	0.0 – 1.1	43.2	0.92	*21*	40.6	0.58	*25*	45.9	0.83
	1.1 – 1.9	43.8		*26*	42.3		*24*	46.5	
	1.9 – 3.2	43.6		*36*	41.7		*10*	47.6	
	3.2 – 17.2	44.4		*30*	43.2		*14*	46.3	

Estrogen Metabolites	Quartile range[a]	All women			Non-Asian			Asian	
		%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]
2-$MeOE_1$ (%)	0.0 – 5.4	41.8	0.07	*20*	40.1	0.43	*23*	43.8	**0.03**
	5.4 – 8.5	44.9		*30*	43.7		*15*	46.4	
	8.5 – 12.2	43.4		*30*	41.3		*20*	47.2	
	12.3 – 79.5	45.0		*33*	42.7		*15*	49.1	
2-OHE_1-3-methyl ether (%)	0.0 – 1.1	42.3	0.18	*21*	39.9	0.24	*18*	45.7	0.48
	1.1 – 1.6	43.1		*32*	42.2		*22*	44.6	
	1.6 – 2.1	45.1		*35*	43.5		*15*	47.3	
	2.2 – 18.3	44.4		*25*	42.0		*18*	48.2	
4-OH pathway[e] (%)	0.0 – 2.9	44.7	0.97	*17*	43.0	0.50	*14*	47.1	0.47
	2.9 – 4.3	42.4		*35*	40.7		*18*	45.3	
	4.4 – 6.4	42.9		*34*	41.6		*19*	44.7	
	6.4 – 32.9	44.9		*27*	43.1		*22*	47.8	
4-OHE_1 (%)	0.0 – 2.6	44.7	0.99	*17*	43.2	0.53	*14*	46.8	0.50
	2.9 – 3.9	42.3		*34*	40.3		*18*	45.7	
	4.0 – 6.0	43.7		*33*	42.5		*22*	45.4	
	6.0 – 32.8	44.3		*29*	42.4		*19*	47.6	
4-OHE_2 (%)	0.0 – 0.2	44.6	0.36	*25*	41.8	0.88	*11*	49.4	0.25
	0.2 – 0.4	43.7		*35*	41.5		*17*	46.8	
	0.4 – 0.5	44.1		*28*	44.4		*26*	44.5	
	0.5 – 2.3	42.6		*25*	40.6		*19*	46.1	
16α-OH pathway[f] (%)	2.8 – 18.1	46.1	**0.01**	*32*	44.2	0.07	*11*	49.5	**0.04**
	18.2 – 26.1	44.9		*29*	43.6		*19*	46.9	
	26.4 – 40.5	42.9		*29*	40.5		*20*	47.4	
	40.5 – 81.4	41.1		*23*	39.0		*23*	43.2	
16α-OHE_1 (%)	0.0 – 2.0	43.1	0.82	*28*	41.3	0.41	*15*	45.6	0.72
	2.0 – 3.5	45.1		*25*	42.9		*23*	48.2	
	3.5 – 5.6	43.0		*32*	42.4		*16*	44.7	

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Estrogen Metabolites	Quartile range[a]	All women			Non-Asian			Asian	
		%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]	*N*	%density[b]	P_{trend}[c]
	5.7 – 36.4	43.7		*28*	41.9		*19*	46.8	
16-ketoE$_2$ (%)	0.3 - 2.4	44.2	0.54	*32*	41.3	0.99	*15*	49.6	0.32
	2.4 – 3.6	43.8		*31*	42.7		*16*	45.9	
	3.6 – 5.1	43.6		*21*	41.5		*22*	46.6	
	5.1 – 11.4	43.4		*29*	42.7		*20*	44.5	
Estriol (E$_3$) (%)	2.4 – 12.5	46.3	**0.002**	*37*	43.9	**0.01**	*11*	51.7	**0.02**
	12.5 – 19.4	45.0		*31*	44.9		*17*	46.1	
	19.5 – 28.4	43.2		*21*	41.0		*23*	46.0	
	28.6 – 72.4	40.5		*24*	37.3		*22*	44.3	
2/16α-OH ratio	0.0 – 2.7	41.4	0.24	*21*	40.0	0.83	*23*	43.6	0.08
	2.8 – 6.3	43.8		*32*	41.8		*17*	46.9	
	6.4 – 13.9	45.7		*28*	43.7		*22*	48.6	
	13.9 – 408.9	44.1		*32*	42.3		*10*	47.4	
Testosterone (pmol/mg creatinine)	0.0 – 1.6	43.8	0.25	*14*	41.6	0.07	*32*	46.8	0.91
	1.6 – 6.1	45.4		*28*	44.3		*20*	46.9	
	6.1 – 11.3	42.6		*26*	41.5		*14*	43.4	
	11.3 – 234.2	43.2		*45*	41.1		*7*	48.9	
Progesterone (pmol/mg creatinine)	0.0 – 0.8	43.4	0.61	*30*	40.8	0.35	*16*	48.2	0.64
	0.8 – 1.5	44.1		*27*	43.1		*17*	44.7	
	1.5 – 2.2	43.7		*30*	41.8		*12*	47.6	
	2.2 – 16.7	43.8		*26*	42.6		*28*	45.6	

[a] Minimum and maximum for each quartile; individual metabolites are expressed as % of total estrogen metabolites.

[b] Adjusted means were obtained from least square means in mixed-effects regression models adjusted for randomization group, time, age, body mass index, Asian ethnicity, number of children, age at first live birth, alcohol intake, age at menarche, and family history of breast cancer.

[c] P values for trend were calculated from mixed-effects regression models using log-transformed values of urinary estrogen metabolites (except for E_1, 2-OHE_1, and the 2-OH metabolites, which were normally distributed.

[d] (2-OHE_1 + 2-OHE_2 + 2-$MeOE_1$ + 2-OHE_1-3-methyl ether)

[e] (4-OHE_2 + 4-OHE_1)

[f] (E_3 + 16α-OHE_1 + 16-ketoE2)

Table 3

Associations for Dense Area and Non-dense Area with Urinary Metabolites[a]

Estrogen Metabolites		Dense Area (cm^2)[b]			Non-dense Area (cm^2)[c]		
		All women	Non-Asian	Asian	All women	Non-Asian	Asian
Total E metabolites (pmol/mg creatinine)	β	0.09	−0.002	0.29	−0.02	0.02	−0.11
	p	*0.20*	*0.98*	***0.01***	*0.42*	*0.42*	***0.01***
Estrone (E_1) (%)	β	−0.01	−0.02	−0.005	−0.003	−0.003	−0.001
	p	*0.42*	*0.32*	*0.80*	*0.56*	*0.58*	*0.90*
Estradiol (E_2) (%)	β	−0.11	−0.12	−0.19	0.05	0.02	0.13
	p	*0.58*	*0.65*	*0.65*	*0.50*	*0.79*	*0.26*
2-OH pathway[d] (%)	β	0.008	0.01	0.005	−0.004	−0.003	−0.007
	p	*0.10*	*0.08*	*0.52*	***0.03***	*0.15*	***0.02***
2-OHE_1 (%)	β	0.005	0.01	−0.001	−0.004	−0.004	−0.005
	p	*0.37*	*0.18*	*0.89*	***0.04***	*0.08*	*0.20*
2-OHE_2 (%)	β	−0.17	−0.15	−0.15	−0.004	−0.06	0.02
	p	*0.27*	*0.50*	*0.50*	*0.94*	*0.42*	*0.84*
2-$MeOE_1$ (%)	β	0.12	0.14	0.12	−0.08	−0.02	−0.19
	p	*0.28*	*0.39*	*0.47*	***0.04***	*0.66*	***0.002***
2-OHE_1-3-methyl ether (%)	β	0.10	0.04	0.15	−0.08	−0.05	−0.19
	p	*0.61*	*0.88*	*0.64*	*0.26*	*0.64*	*0.11*
4-OH pathway[e] (%)	β	−0.04	−0.11	−0.02	−0.05	0.02	−0.10
	p	*0.72*	*0.51*	*0.88*	*0.16*	*0.66*	***0.05***
4-OHE_1 (%)	β	−0.04	−0.10	−0.02	−0.05	0.03	−0.09
	p	*0.71*	*0.53*	*0.87*	*0.18*	*0.61*	***0.05***
4-OHE_2 (%)	β	−0.16	−0.23	−0.21	0.04	−0.05	0.15
	p	*0.59*	*0.60*	*0.64*	*0.73*	*0.70*	*0.37*

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Estrogen Metabolites		Dense Area (cm^2)[b]			Non-dense Area (cm^2)[c]		
		All women	Non-Asian	Asian	All women	Non-Asian	Asian
16α-OH pathway[f] (%)	β	−0.27	−0.42	−0.10	0.13	0.09	0.24
	p	*0.06*	***0.03***	*0.64*	***0.008***	*0.15*	***0.003***
16α-OHE_1 (%)	β	0.13	0.20	0.03	0.02	−0.02	0.10
	p	*0.26*	*0.19*	*0.88*	*0.58*	*0.76*	*0.16*
16-ketoE$_2$ (%)	β	−0.16	−0.19	−0.13	0.02	−0.009	0.11
	p	*0.29*	*0.35*	*0.58*	*0.71*	*0.89*	*0.22*
Estriol (E_3) (%)	β	−0.33	−0.53	−0.11	0.16	0.14	0.24
	p	***0.02***	***0.007***	*0.59*	***0.0007***	***0.02***	***0.002***
2/16α-OH ratio	β	−0.06	−0.10	0.04	−0.04	−0.01	−0.13
	p	*0.36*	*0.22*	*0.76*	*0.07*	*0.61*	***0.003***
Testosterone (pmol/mg creatinine)	β	−0.06	−0.10	−0.02	0.03	0.05	−0.004
	p	*0.49*	*0.38*	*0.89*	*0.40*	*0.21*	*0.93*
Progesterone (pmol/mg creatinine)	β	−0.04	−0.01	−0.08	−0.01	−0.04	0.03
	p	*0.74*	*0.94*	*0.67*	*0.79*	*0.46*	*0.68*

[a] Individual metabolites are expressed as % of total estrogen metabolites; β and *p* values were calculated from mixed-effects regression models using log-transformed values of urinary estrogen metabolites (except for E_1, 2-OHE_1, and the 2-OH metabolites) and adjusted for randomization group, time, age, body mass index, Asian ethnicity, number of children, age at first live birth, alcohol intake, age at menarche, and family history of breast cancer.

[b] Dense was square-root transformed

[c] Non-dense area was log transformed.

[d] (2-OHE_1 + 2-OHE_2 + 2-$MeOE_1$ + 2-OHE_1-3-methyl ether)

[e] (4-OHE_2 + 4-OHE_1)

[f] (E3 + 16α-OHE_1 + 16-ketoE$_2$)