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Endogenous Estrogens, Estrogen Metabolites, and Breast Cancer Risk in Postmenopausal Chinese Women

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Abstract

Background: The role of estrogen metabolism in determining breast cancer risk and differences in breast cancer rates between high-incidence and low-incidence nations is poorly understood.

Methods: We measured urinary concentrations of estradiol and estrone (parent estrogens) and 13 estrogen metabolites formed by irreversible hydroxylation at the C-2, C-4, or C-16 positions of the steroid ring in a nested case-control study of 399 postmenopausal invasive breast cancer case participants and 399 matched control participants from the population-based Shanghai Women's Health Study cohort. Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer by quartiles of metabolic pathway groups, pathway ratios, and individual estrogens/estrogen metabolites were estimated by multivariable conditional logistic regression. Urinary estrogen/estrogen metabolite measures were compared with those of postmenopausal non-hormone-using Asian Americans, a population with three-fold higher breast cancer incidence rates. All statistical tests were two-sided.

Results: Urinary concentrations of parent estrogens were strongly associated with breast cancer risk ($OR_{Q4vsQ1} = 1.94$, 95% CI = 1.21 to 3.12, $P_{trend} = .01$). Of the pathway ratios, the 2-pathway:total estrogens/estrogen metabolites and 2-pathway:parent estrogens were inversely associated with risk ($OR_{Q4vsQ1} = 0.57$, 95% CI = 0.35 to 0.91, $P_{trend} = .03$, and $OR_{Q4vsQ1} = 0.61$, 95% CI = 0.37 to 0.99, $P_{trend} = .04$, respectively). After adjusting for parent estrogens, these associations remained clearly inverse but lost statistical significance ($OR_{Q4vsQ1} = 0.65$, 95% CI = 0.39 to 1.06, $P_{trend} = .12$ and $OR_{Q4vsQ1} = 0.76$, 95% CI = 0.44 to 1.32, $P_{trend} = .28$). The urinary concentration of all estrogens/estrogen metabolites combined in Asian American women was triple that in Shanghai women.

Conclusions: Lower urinary parent estrogen concentrations and more extensive 2-hydroxylation were each associated with reduced postmenopausal breast cancer risk in a low-risk nation. Markedly higher total estrogen/estrogen metabolite concentrations in postmenopausal United States women (Asian Americans) than in Shanghai women may partly explain higher breast cancer rates in the United States.

Since the mid-1960s (1), it has been recognized that developed nations such as the United States have breast cancer incidence rates that greatly exceed those of developing nations such as China (2,3). When Chinese women migrate to the United States, their breast cancer rates rise in successive generations until they converge with those of US whites (4,5), suggesting that lifestyle and environment, not ethnicity and genetics, are the primary factors driving breast cancer disparities. While some specific lifestyle factors, such as later age at first birth, having fewer children, and reduced breast feeding, have been implicated in the rise in breast cancer incidence among Chinese migrants (6,7), the underlying biological basis remains elusive (8).

Since the late 1960s (9), estrogen metabolism has been hypothesized to underlie international breast cancer rate differences. Estrogen metabolism occurs when estrone and estradiol, the parent estrogens, are irreversibly hydroxylated at the 2-, 4-, or 16-position of the steroid ring (Figure 1), producing 13 known estrogen metabolites. Laboratory studies show that 16-hydroxylation metabolites can bind covalently to the estrogen receptor (10) with strong estrogenic effects (11) and, conversely, that 2-hydroxylation metabolites bind weakly to the estrogen receptor, with only modest proliferative effects (12), and are rapidly cleared from circulation (13). Additionally, the 2-hydroxylation and especially 4-hydroxylation catechols generate mutagenic quinones through redox cycling, resulting in DNA adducts (14). Methylation of catechol estrogens prevents quinone production (15).

These laboratory findings suggest intriguing mechanistic hypotheses regarding the competing estrogen metabolism pathway groups, but few epidemiologic studies have examined these hypotheses in humans. Seven cohort studies—

using prediagnostic serum, plasma, or urine—have examined two specific estrogen metabolites, 2-hydroxyestrone and 16 α -hydroxyestrone, and their ratio in relation to postmenopausal breast cancer risk (16–22). Findings, however, were inconsistent and generally not statistically significant, possibly because of questionable validity of direct immunoassays for steroid hormone measurement (23,24). Recently, prospective studies of premenopausal (25) and postmenopausal breast cancer (26–28) used a newly developed high-sensitivity, high-specificity liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (29) to examine serum or urinary concentrations of estrone, estradiol, and all 13 estrogen metabolites in relation to risk. In postmenopausal women, more extensive 2-hydroxylation, as measured by the ratio of 2-pathway metabolite concentration to parent estrogens' concentration, was consistently associated with lower breast cancer risk (26–28,30). Increased methylation of 4-hydroxylation catechols, as measured by the ratio of 4-pathway methylated catechol concentration to 4-pathway catechol concentration, was also associated with lower risk in one study (26).

These recent studies included only women of the United States, a nation that, on the global scale, has a relatively high breast cancer incidence (2). Whether estrogen metabolism risk associations are as evident in low-risk Asian populations is not known. Concentrations of estrogens are comparatively low in postmenopausal Asian women, particularly in China (31,32), and the concentrations of individual estrogens/estrogen metabolites (defined as estrone, estradiol, and derived estrogen metabolites) have not been comprehensively assessed with a state-of-the-art LC-MS/MS assay in low-risk populations.

Figure 1. Pathways of estrogen metabolism. The parent estrogens estrone and estradiol can be irreversibly hydroxylated at the 2-, 4-, or 16-position of the steroid ring. The structures are for the unconjugated forms of estrogens and estrogen metabolites.

We therefore examined the associations of estradiol, estrone, and 13 estrogen metabolites with breast cancer risk in prospectively stored urine samples from a population-based study of postmenopausal women in Shanghai, China. We also examined, on an exploratory basis, how concentrations of estrogens/estrogen metabolites and estrogen metabolism profiles differ between Shanghai and Asian American postmenopausal women to ascertain whether differences could explain higher breast cancer rates in the United States than in China.

Methods

Study Population

Incident invasive breast cancer case participants and control participants were selected from the Shanghai Women's Health Study, a population-based cohort of 74 942 women age 40 to 70 years and enrolled between 1997 and 2000. The study was approved by all relevant institutional review boards in the United States and China. All participants provided written consent. As previously described (33), trained interviewers assessed participant characteristics and 88% of participants donated a spot urine into a 100-mL cup containing 125 mg of ascorbic acid. The cup was kept cold, processed within six hours of collection, and stored at -80°C .

Participants were followed using biennial home visits, annual linkage to cancer incidence and mortality data collected by the Shanghai Cancer Registry, and death certificate data collected by the Shanghai Vital Statistics Unit. All cases were confirmed by home visit and hospital record review.

We selected all 399 incident invasive breast cancers diagnosed through December 2009 among postmenopausal women not using exogenous hormones at the time of urine collection. One control, breast cancer free at the time of diagnosis of the case (± 6 months), was individually matched to each case by date of urine sample (± 1 month), age on that date (± 2 years), time (morning/afternoon) of sample, time since last meal (± 2 hours), and past week antibiotic use (yes/no). Control participants were postmenopausal and not using exogenous hormones at the time of urine collection. Menopause occurred naturally for 89% of women and by surgery for 8% of women (generally age 55+ years).

Comparison Populations

Our primary comparison population consisted of postmenopausal control participants age 45 to 65 years not using exogenous hormones from the Asian American breast cancer study, a 1983 to 1987 population-based case control study of breast cancer in Chinese, Japanese, and Filipino women of San Francisco-Oakland CA, Los Angeles CA, or Oahu, HI (4). This population was selected based on participants' Asian ancestry and low body mass index (BMI) (median = 24.0 kg/m^2 vs 24.5 kg/m^2 for Shanghai control participants). All participants donated 12-hour overnight urines. To confirm findings, we also benchmarked urinary concentrations of total estrogens/estrogen metabolites against those of three US populations of white postmenopausal women not using exogenous hormones from Colorado (34), Maryland (35), and western New York state (36). The same LC-MS/MS assay and lab were used for all studies.

Laboratory Assay

Stable isotope dilution LC-MS/MS was used to measure estrone, estradiol, and 13 estrogen metabolites in 500 μL of urine (29,35,37,38).

In urine, parent estrogens and their metabolites are present primarily in conjugated form. We used a *Helix pomatia* preparation to remove glucuronide and sulfate moieties, allowing measurement of total amounts for each estrogen/estrogen metabolite. To estimate absolute concentrations, six stable isotopically labeled internal standards were used: ^{13}C -labeled estrone and estradiol and ^2H -labeled 2-hydroxyestradiol, 2-methoxyestradiol, estriol, and 16-epiestriol. Calibration curves were included in each assay batch and were constructed by plotting estrogen/estrogen metabolite: isotopically labeled standard peak area ratios vs known amount of each estrogen/estrogen metabolite. The quantity of estrogen/estrogen metabolite was then interpolated by a linear function. Molar quantities were standardized to creatinine.

Assay reliability was measured using 92 masked replicates from 11 individuals. Each estrogen/estrogen metabolite had a coefficient of variation of less than 2% and an intraclass correlation coefficient of greater than 99%. The laboratory was blinded to case vs control status.

Statistical Analysis

Estrogens/estrogen metabolites were evaluated using metabolic pathway groups and metabolic pathway ratios based on shared biochemistry and metabolism and prior etiologic hypotheses. The metabolites comprising the 2-, 4-, and 16-hydroxylation pathways are shown in Figure 1. The catechol metabolites were 2-hydroxyestrone, 2-hydroxyestradiol, and 4-hydroxyestrone. The methylated catechols were 2-methoxyestrone, 2-methoxyestradiol, 2-methoxyestrone-3-methyl-ester, 4-methoxyestrone, and 4-methoxyestradiol. All individual and grouped estrogen/estrogen metabolite concentrations (pmol/mg creatinine) and ratios were log-transformed for analysis. Inter-relationships between individual estrogens/estrogen metabolites, metabolic pathway groups, and ratios were evaluated using Pearson correlations among control participants.

Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer were estimated for each quartile (based on control distribution) of estrogens/estrogen metabolites, metabolic pathway groups, and ratios using conditional logistic regression. Covariates were selected based on well-established associations with breast cancer risk (shown in table footnotes). Data was complete for all covariates. Trend tests were done by modeling quartile median concentrations (log-scale) and calculating Wald statistics.

Parent estrogens are strongly and statistically significantly associated with increased risk of postmenopausal breast cancer (39–41). Therefore, as an approximate way of evaluating the independent contribution of each metabolic pathway and pathway ratio to risk, we added parent estrogens to models and reevaluated statistical significance. Alternative models, adjusted for age only or for all adjustment variables plus BMI, were examined in sensitivity analyses. In additional analyses, we evaluated heterogeneity of associations according to estrogen receptor (ER) and progesterone receptor (PR) status and selected anthropometric and socioeconomic characteristics (Supplementary Methods, available online).

To compare estrogen metabolism measures across the Shanghai and Asian American control populations, we used generalized linear models, with terms for age and study, to standardize levels for each estrogen metabolism measure in each population to the mean age of the combined control populations. Adjusted levels for each individual were estimated by the residual adjustment method (39), ie, by adding residuals of each individual to study-specific means. Interquartile ranges

(IQRs) were calculated based on these individually adjusted values. In sensitivity analyses, we similarly adjusted BMI to the mean BMI of the combined control participants. For each study, we estimated Pearson correlations of BMI with parent estrogens and total estrogens/estrogen metabolites.

For the benchmark analysis with the three additional US populations, we compared unadjusted median concentrations and IQRs from prior publications (34–36) to those of Shanghai women.

Analyses were conducted with SAS 9.2. All *P* values are two-sided and considered statistically significant if less than .05.

Results

The median time from cohort entry to breast cancer diagnosis for case participants was 6.0 years (IQR = 1.3–10.3 years). Risk factors for breast cancer, ie, history of benign breast disease, young age at menarche, late age at first birth, low parity, and few months of breast feeding, were more prevalent among breast cancer case participants than control participants (Table 1). The 16-pathway estrogen metabolites, 2-pathway metabolites, and parent estrogens were similarly abundant in urine (36%, 31%, and 29% of total estrogens/estrogen metabolites, respectively), with wide ranges in concentration (2- to 2.5-fold increases across the IQRs) (Table 2). Urinary estrogen metabolism pathway ratios, in contrast, varied little in their distribution, with approximately 1.2-fold differences over the IQRs (Table 3). Parent estrogens and the three hydroxylation pathway groups were all highly positively correlated ($r = 0.64$ – 0.99). The ratios of 2-, 4-, and 16-pathway metabolites to total estrogens/estrogen metabolites were inversely correlated with parent estrogen concentrations ($r = -0.33$, -0.24 , and -0.33 , respectively). The 2- and 4-pathway ratios were strongly correlated with one another ($r = 0.85$) and not at all correlated with the 16-pathway ratio ($r = -0.04$ and -0.01 , respectively).

All metabolic pathway groups were positively associated with breast cancer risk (Table 4). The urinary concentrations of total estrogens/estrogen metabolites, parent estrogens, and 16-pathway metabolites were strongly and statistically significantly associated with breast cancer risk, with a 90% to 100% higher risk of breast cancer comparing top vs bottom quartiles. For parent estrogens, for example, risk was increased by 94% in the top vs bottom quartile ($OR_{Q4vsQ1} = 1.94$, 95% CI = 1.21 to 3.12, $P_{trend} = .01$); for 16-pathway metabolites, risk was increased by 101% ($OR_{Q4vsQ1} = 2.01$, 95% CI = 1.23 to 3.27, $P_{trend} = .003$). Trends in risk were statistically significant but less pronounced for the 2-pathway and 4-pathway metabolites, with a 46% higher risk of breast cancer for the top as compared with bottom quartile for 2-pathway metabolites ($OR_{Q4vsQ1} = 1.46$, 95% CI = 0.91 to 2.36, $P_{trend} = .02$) and a 64% higher risk for 4-pathway metabolites ($OR_{Q4vsQ1} = 1.64$, 95% CI = 1.02 to 2.63, $P_{trend} = .01$). Associations for catechols, whether in the 2- or 4-pathway, were positive (60%–70% increase across quartiles), but results for methylated catechols were statistically nonsignificant (20%–40% increase across quartiles). Except for four of the five methylated catechols, the individual estrogen metabolites that comprise metabolic pathway groups were positively associated with risk (Supplementary Table 1, available online).

Because metabolic pathway groups are moderately positively correlated with parent estrogens, we further adjusted models for parent estrogens. The associations became attenuated and were no longer statistically significant, including the 2- and 4-pathway catechols (Table 4). In sensitivity analyses, adding BMI to multivariable models modestly reduced positive

associations for estrone, estradiol, and nearly all the metabolic pathway groups, with the most substantial attenuation noted for estradiol (HR_{Q4vsQ1} reduced by 9% to 1.57, 95% CI = 0.99 to 2.50, $P_{trend} = .21$) (Supplementary Table 2, available online).

Next, to explicitly assess the role of competing estrogen metabolism pathway groups, we evaluated metabolic pathway ratios in relation to breast cancer. Statistically significant inverse associations with breast cancer were found for two metabolic pathway ratios—2-pathway metabolites: total estrogens/estrogen metabolites (HR_{Q4vsQ1} = 0.57, 95% CI = 0.35 to 0.91, $P_{trend} = .03$) and 2-pathway metabolites: parent estrogens (HR_{Q4vsQ1} = 0.61, 95% CI = 0.37 to 0.99, $P_{trend} = .04$) (Table 5). Adjustment for BMI in sensitivity analyses modestly attenuated associations for the 2-pathway metabolites: total estrogens/estrogen metabolites ratio (HR_{Q4vsQ1} = 0.62, 95% CI = 0.38 to 1.00, $P_{trend} = .06$) and 2-pathway metabolites: parent estrogens ratio (HR_{Q4vsQ1} = 0.69, 95% CI = 0.41 to 1.15, $P_{trend} = .13$) (Supplementary Table 3, available online). Other metabolic pathway ratios were not associated with breast cancer risk.

We added parent estrogens to the metabolic pathway ratio models to eliminate confounding by parent estrogens (Table 5). Adjustment for parent estrogens attenuated the associations for the 2-pathway metabolites: total estrogens/estrogen metabolites ratio (HR_{Q4vsQ1} = 0.65, 95% CI = 0.39 to 1.06, $P_{trend} = .12$) and 2-pathway metabolites: parent estrogens ratio (HR_{Q4vsQ1} = 0.76, 95% CI = 0.44 to 1.32, $P_{trend} = .28$), and associations were no longer statistically significant. Nonetheless, point estimates were still substantially below 1.

Associations for metabolic pathway groups and pathway ratios did not have statistically significant differences by steroid hormone receptor status though associations with total estrogens/estrogen metabolites, parent estrogens, and the three hydroxylation pathway groups were substantially stronger for ER+/PR+ (ORs = 2.3–2.6 comparing risk at the fourth quartile midpoint vs first quartile midpoint) than ER-/PR- tumors (ORs = 1.2–1.3) (Supplementary Table 4, available online). Comparable differences by receptor status were not evident for metabolic pathway ratios. There was no statistically significant effect modification by BMI at cohort entry, BMI at age 20 years, height, income, or education.

Population Comparisons

Compared with Shanghai women, Asian American women had more than triple the concentration of estradiol ($P = 1.7 \times 10^{-38}$) and 16-pathway estrogen metabolites ($P = 4.9 \times 10^{-69}$), more than double the concentration of total estrogens/estrogen metabolites ($P = 3.6 \times 10^{-48}$) and 4-pathway metabolites ($P = 2.0 \times 10^{-42}$), and a smaller increase—70% to 80% higher—for total parent estrogens ($P = 2.6 \times 10^{-12}$) and 2-pathway estrogen metabolites ($P = 1.1 \times 10^{-16}$) (Table 6). The ratios of 2-pathway metabolites: total estrogens/estrogen metabolites and parent estrogens: total estrogens/estrogen metabolites were 35–40% lower ($P = 7.0 \times 10^{-30}$ and $P = 5.1 \times 10^{-41}$, respectively) and the ratio of 16-pathway metabolites: total estrogens/estrogen metabolites was 40% higher in Asian American women than Shanghai women ($P = 7.6 \times 10^{-51}$). Adjustment for BMI did not substantively affect observed population differences (Supplementary Table 5, available online), perhaps reflecting weak correlations of BMI with total estrogens/estrogen metabolites and parent estrogens in Shanghai women ($r = 0.15$ and 0.17 , respectively) and Asian American women ($r = 0.08$ and 0.18).

To corroborate these population differences, we compared total estrogens/estrogen metabolite concentrations of three

Table 1. Participant characteristics at cohort entry according to case/control status

Characteristic	Cases, % (n = 399)	Controls, % (n = 399)	P _{difference} *
Age, y			Matched, N/A
<55	22.1	22.3	
55-59	27.6	25.3	
60-64	25.3	26.3	
65+	25.1	26.1	
Family history of breast cancer, %	3.0	2.5	.66
History of benign breast disease, %	14.8	8.5	.005
Age at menarche, y			.10
<13	6.5	6.3	
13-14	37.6	30.1	
15-16	38.9	41.9	
17+	17.0	21.8	
Age at birth of first child, y			.03
Nulliparous	4.8	4.0	
<20	8.5	14.3	
20-24	35.3	37.3	
25-29	37.1	34.3	
30+	14.3	10.0	
Parity			.05
Nulliparous	4.8	4.0	
1 live birth	24.3	19.1	
2 live births	37.1	35.6	
3+ live births	33.8	41.4	
Lifetime duration of breastfeeding, mo			.01
Never	16.0	12.5	
<12	25.8	24.6	
13-24	31.8	26.1	
25+	26.3	36.8	
Ever regular use of oral contraceptives	24.3	24.8	.87
Ever drank alcohol regularly	3.0	2.0	.37
Ever participated in competitive sports	47.4	47.9	.88
BMI, kg/m ²			.40
<18.5	3.3	3.3	
18.5-20.9	9.3	11.5	
21.0-22.9	13.0	17.3	
23.0-24.9	24.1	23.8	
25.0-29.9	40.6	35.3	
30.0+	9.8	8.8	

*Two-sided P_{difference} value by case-control status was estimated by conditional logistic regression and accounted for case-control matching. BMI = body mass index.

additional US populations with those of Shanghai women. In each study, the median concentration of total estrogens/estrogen metabolites was higher, by 2.5 to six times, than in Shanghai women (Figure 2).

Discussion

Our nested case-control study of postmenopausal Shanghai women found that urinary concentrations of parent estrogens were strongly associated with breast cancer risk, with a doubling of risk for the highest as compared with the lowest quartile. These results are in line with prior findings for circulating and urinary parent estrogens (39–41). In addition, our study found that the ratios of 2-pathway metabolites to total estrogens/estrogen metabolites and 2-pathway metabolites to parent estrogens—ratios indicative of increased 2-hydroxylation—were associated with a 40% lower breast cancer risk. Similar associations had been observed previously in higher-risk developed nations (26–28) but not in low-risk populations. Finally, our results contradict the laboratory-based hypothesis that mutagenic catechols of the 2- and 4-pathway groups increase

breast cancer risk. When adjusted for concentrations of parent estrogens, these metabolites had no independent associations with breast cancer risk.

The 2-pathway estrogen metabolites have been shown by laboratory studies to have low affinity with the estrogen receptor and rapid clearance from circulation. It has therefore been previously hypothesized that 2-pathway estrogen metabolites may be related to lower breast cancer risk through decreasing bioavailable estrogens (26). In our study, enhanced metabolism through the 2-pathway, as measured by 2-pathway ratios, was associated with reduced breast cancer risk while 2-pathway metabolites themselves were not independently associated with breast cancer after adjusting for parent estrogens. One explanation consistent with these lines of biological and epidemiological evidence is that 2-pathway metabolites may have no independent carcinogenic effect, but may be associated with lower breast cancer risk in so far as they act to mark accelerated estrogen clearance.

Our study findings for preferential 2-hydroxylation were consistent with those of prior US studies of postmenopausal women (26–28,30). Three serum-based studies found that a high vs low level, based on quartiles or quintiles of the ratio of 2-pathway

Table 2. Concentration (pmol/mg creatinine) of urinary estrogens/estrogen metabolites and metabolic pathway groups among control participants in the Shanghai Women's Health Study

Estrogen/estrogen metabolite measure	Median (25th, 75th)	% of total
Total estrogens/estrogen metabolites	8.84 (5.78, 12.96)	100.0
Parent estrogens	2.49 (1.57, 3.88)	28.2
Estrone	2.04 (1.29, 3.20)	23.1
Estradiol	0.42 (0.24, 0.66)	4.8
2-Hydroxylation pathway	2.65 (1.87, 4.15)	30.0
2-Hydroxyestrone	1.62 (1.10, 2.46)	18.3
2-Hydroxyestradiol	0.38 (0.27, 0.59)	4.3
2-Methoxyestrone	0.36 (0.23, 0.57)	4.1
2-Methoxyestradiol	0.16 (0.09, 0.27)	1.8
2-Hydroxyestrone-3-methyl ether	0.09 (0.05, 0.14)	1.0
4-Hydroxylation pathway	0.30 (0.21, 0.47)	3.4
4-Hydroxyestrone	0.22 (0.16, 0.34)	2.5
4-Methoxyestrone	0.05 (0.03, 0.08)	0.6
4-Methoxyestradiol	0.02 (0.01, 0.04)	0.2
16-Hydroxylation pathway	3.18 (2.15, 4.78)	36.0
16 α -Hydroxyestrone	0.49 (0.33, 0.70)	5.5
Estriol	1.83 (1.23, 2.77)	20.7
17-Epiestriol	0.12 (0.07, 0.20)	1.4
16-Ketoestradiol	0.53 (0.37, 0.84)	6.0
16-Epiestriol	0.20 (0.13, 0.31)	2.3

Table 3. Levels of urinary estrogen metabolism pathway ratios among control participants in the Shanghai Women's Health Study

Estrogen/estrogen metabolite measure	Median (25th, 75th)
Pathway ratios	
Parent estrogens: total estrogens/estrogen metabolites	0.29 (0.25, 0.31)
2-pathway: total estrogens/estrogen metabolites	0.31 (0.28, 0.33)
4-pathway: total estrogens/estrogen metabolites	0.04 (0.03, 0.04)
16-pathway: total estrogens/estrogen metabolites	0.36 (0.34, 0.39)
2-pathway: parent estrogens	1.11 (0.92, 1.31)
4-pathway: parent estrogens	0.13 (0.10, 0.15)
16-pathway: parent estrogens	1.34 (1.14, 1.50)
2-pathway: 4-pathway	8.80 (8.11, 9.40)
2-pathway: 16-pathway	0.84 (0.76, 0.94)
4-pathway: 16-pathway	0.10 (0.08, 0.11)
Catechol: methylated catechol ratios	
Catechols*: methylated catechols†	3.29 (2.32, 4.24)
2-catechols*: 2-methylated catechols†	3.30 (2.33, 4.30)
4-catechols*: 4-methylated catechols†	3.08 (2.11, 4.24)

*Catechols comprise both the 2-catechols and 4-catechols. The 2-catechols consist of 2-hydroxyestrone and 2-hydroxyestradiol. The 4-catechol is 4-hydroxyestrone.

†Methylated catechols comprise both the 2-methylated catechols and 4-methylated catechols. The 2-methylated catechols consist of 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether. The 4-methylated catechols consist of 4-methoxyestrone and 4-methoxyestradiol.

metabolites to parent estrogens, was associated with a 28% (28), 31% (27), and 34% (26) reduction in breast cancer risk, similar to the 39% reduction we observed. The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) study specifically reported that this ratio was independently associated with lower breast cancer even after adjusting for circulating unconjugated estradiol (26). After adjusting for parent estrogens, our results for

this ratio were of a similarly strong magnitude (24% compared with 28%) but no longer statistically significant. Our adjusted estimate may, however, underestimate the reduction in risk because urinary parent estrogens are imperfect proxies for circulating estradiol, the bioactive form of estrogen.

Similar to several prior studies (26,42), we did not adjust our primary models for BMI because of evidence suggesting there is no association between BMI and breast cancer independent of estrogen (43,44). Recent studies, however, suggest that BMI may have some estrogen-independent role in breast carcinogenesis (45,46) and further studies may be needed to clarify BMI's role in estrogen metabolism–breast cancer associations.

For decades, scientists have speculated that differences in endogenous estrogens contribute to the higher breast cancer rates of developed populations in the West as compared with developing populations in Asia (31,32,47,48). In our analysis, postmenopausal urinary concentrations of each estrogen metabolic pathway were markedly higher in Asian American women than in Shanghai women and urinary estradiol concentrations were 3.4 times higher in Asian American than in Shanghai women. In the two published studies involving comparable population comparisons, Key et al. (32) examined circulating estradiol concentrations in the United Kingdom and China, and Ursin et al. (31) examined urinary estradiol concentrations in the United States and China. Each study reported that estradiol concentrations in postmenopausal women were 2.5 times higher in the higher-risk country than in China, a result remarkably consistent with our own. Additional studies have reported moderately higher postmenopausal estradiol concentrations in the West than in Japan, a nation of intermediate breast cancer risk (48,49).

It is commonly held that peripheral aromatization of testosterone and androstenedione in adipose tissue is the primary source of estrogens in postmenopausal women. However, BMI was not a major reason for the higher estrogen/estrogen metabolite concentrations in Asian American women as compared with Shanghai women. The Asian American women had BMIs similar to those of Shanghai women, results did not

Table 4. Multivariable ORs and 95% CIs of postmenopausal breast cancer according to urinary concentrations of metabolic pathway groups and individual parent estrogens in the Shanghai Women's Health Study

Metabolic pathway groups	Quartile 1 OR (95% CI)	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	P _{trend} *
Total estrogens/estrogen metabolites					
Cases	71	99	111	118	
Multivariable adjusted†	1.00 (Referent)	1.38 (0.87 to 2.17)	1.66 (1.05 to 2.63)	1.86 (1.16 to 2.97)	.01
Parent estrogen adjusted‡	1.00 (Referent)	1.23 (0.61 to 2.47)	1.65 (0.68 to 3.98)	1.61 (0.56 to 4.68)	.45
Parent estrogens					
Cases	74	108	97	120	
Multivariable adjusted†	1.00 (Referent)	1.50 (0.96 to 2.37)	1.48 (0.93 to 2.34)	1.94 (1.21 to 3.12)	.01
Parent estrogen adjusted‡	N/A	N/A	N/A	N/A	N/A
Estrone					
Cases	76	103	104	116	
Multivariable adjusted†	1.00 (Referent)	1.27 (0.81 to 2.01)	1.46 (0.93 to 2.28)	1.71 (1.07 to 2.73)	.02
Parent estrogen adjusted‡	N/A	N/A	N/A	N/A	N/A
Estradiol					
Cases	67	121	101	110	
Multivariable adjusted†	1.00 (Referent)	1.79 (1.15 to 2.80)	1.58 (1.00 to 2.49)	1.73 (1.10 to 2.73)	.07
Parent estrogen adjusted‡	N/A	N/A	N/A	N/A	N/A
2-Hydroxylation pathway					
Cases	81	79	135	104	
Multivariable adjusted†	1.00 (Referent)	0.96 (0.61 to 1.49)	1.87 (1.20 to 2.92)	1.46 (0.91 to 2.36)	.02
Parent estrogen adjusted‡	1.00 (Referent)	0.80 (0.46 to 1.40)	1.54 (0.79 to 3.03)	0.98 (0.41 to 2.32)	.85
4-Hydroxylation pathway					
Cases	78	89	121	111	
Multivariable adjusted†	1.00 (Referent)	1.11 (0.71 to 1.74)	1.68 (1.07 to 2.62)	1.64 (1.02 to 2.63)	.01
Parent estrogen adjusted‡	1.00 (Referent)	0.94 (0.55 to 1.60)	1.36 (0.72 to 2.56)	1.15 (0.52 to 2.55)	.60
16-Hydroxylation pathway					
Cases	73	93	115	118	
Multivariable adjusted†	1.00 (Referent)	1.36 (0.84 to 2.21)	1.71 (1.08 to 2.69)	2.01 (1.23 to 3.27)	.003
Parent estrogen adjusted‡	1.00 (Referent)	1.26 (0.65 to 2.45)	1.75 (0.82 to 3.71)	1.94 (0.76 to 4.96)	.15
Catechols§					
Cases	80	84	117	118	
Multivariable adjusted†	1.00 (Referent)	1.06 (0.67 to 1.69)	1.59 (1.01 to 2.52)	1.69 (1.06 to 2.70)	.01
Parent estrogen adjusted‡	1.00 (Referent)	0.87 (0.48 to 1.60)	1.36 (0.65 to 2.85)	1.31 (0.55 to 3.11)	.47
Methylated catechols					
Cases	90	87	120	102	
Multivariable adjusted†	1.00 (Referent)	1.09 (0.71 to 1.68)	1.35 (0.89 to 2.05)	1.33 (0.86 to 2.08)	.17
Parent estrogen adjusted‡	1.00 (Referent)	0.91 (0.57 to 1.46)	1.03 (0.63 to 1.68)	0.86 (0.48 to 1.54)	.62
2-catechols§					
Cases	82	83	116	118	
Multivariable adjusted†	1.00 (Referent)	0.98 (0.61 to 1.57)	1.52 (0.97 to 2.39)	1.63 (1.03 to 2.59)	.01
Parent estrogen adjusted‡	1.00 (Referent)	0.75 (0.41 to 1.40)	1.20 (0.58 to 2.49)	1.14 (0.48 to 2.72)	.62
2-methylated catechols					
Cases	87	90	119	103	
Multivariable adjusted†	1.00 (Referent)	1.19 (0.78 to 1.83)	1.42 (0.93 to 2.16)	1.39 (0.89 to 2.17)	.13
Parent estrogen adjusted‡	1.00 (Referent)	0.99 (0.62 to 1.59)	1.10 (0.67 to 1.80)	0.91 (0.50 to 1.64)	.77
4-catechols§					
Cases	86	76	117	120	
Multivariable adjusted†	1.00 (Referent)	0.79 (0.50 to 1.27)	1.43 (0.92 to 2.23)	1.67 (1.05 to 2.66)	.01
Parent estrogen adjusted‡	1.00 (Referent)	0.64 (0.36 to 1.14)	1.23 (0.63 to 2.42)	1.39 (0.61 to 3.19)	.50
4-methylated catechols					
Cases	82	115	109	93	
Multivariable adjusted†	1.00 (Referent)	1.43 (0.94 to 2.19)	1.37 (0.89 to 2.11)	1.23 (0.78 to 1.94)	.37
Parent estrogen adjusted‡	1.00 (Referent)	1.24 (0.79 to 1.94)	1.06 (0.66 to 1.72)	0.75 (0.42 to 1.33)	.38

*P_{trend} (two-sided) was calculated using the Wald statistic for the quartile median concentrations (log-scale) modeled on a continuous basis. CI = confidence interval; OR = odds ratio.

†Odds ratios were estimated with conditional logistic regression and adjusted for age at cohort entry (<55, 55-59, 60-64, 65+ years), family history of breast cancer (yes/no), history of benign breast disease (yes/no), age at menarche (<13, 13-14, 15-16, 17+ years), age at first birth and parity (nulliparous, age <25 years with 1+ children, age > 25 years with 1+ children), duration of breast feeding (never, 1-12, 13-24, 25+ months), ever regularly used oral contraceptives (yes, no), ever drank alcohol regularly (yes, no), and ever participated in competitive sports (yes, no).

‡ORs adjusted for covariates above and additionally adjusted for total concentration of parent estrogens (in quartiles). Values of N/A are indicated for parent estrogens, estrone, and estradiol given this adjustment.

§Catechols comprise both the 2-catechols and 4-catechols. The 2-catechols consist of 2-hydroxyestrone and 2-hydroxyestradiol. The 4-catechol is 4-hydroxyestrone.

||Methylated catechols comprise both the 2-methylated catechols and 4-methylated catechols. The 2-methylated catechols consist of 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether. The 4-methylated catechols consist of 4-methoxyestrone and 4-methoxyestradiol.

Table 5. Multivariable ORs and 95% CIs of postmenopausal breast cancer according to urinary estrogen metabolism pathway ratios in the Shanghai Women's Health Study

Metabolic pathway ratio	Quartile 1 OR (95% CI)	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	P _{trend} *
Pathway ratios					
Parent estrogens: total estrogens/estrogen metabolites					
Cases	84	103	109	103	
Multivariable adjusted†	1.00 (Referent)	1.23 (0.78 to 1.93)	1.34 (0.85 to 2.12)	1.36 (0.81 to 2.30)	.24
Parent estrogen adjusted‡	1.00 (Referent)	1.06 (0.66 to 1.70)	1.13 (0.70 to 1.82)	1.02 (0.57 to 1.81)	.94
2-pathway: total estrogens/estrogen metabolites					
Cases	123	101	100	75	
Multivariable adjusted†	1.00 (Referent)	0.72 (0.47 to 1.12)	0.78 (0.50 to 1.20)	0.57 (0.35 to 0.91)	.03
Parent estrogen adjusted‡	1.00 (Referent)	0.76 (0.49 to 1.19)	0.86 (0.55 to 1.34)	0.65 (0.39 to 1.06)	.12
4-pathway: total estrogens/estrogen metabolites					
Cases	113	98	97	91	
Multivariable adjusted†	1.00 (Referent)	0.81 (0.51 to 1.30)	0.77 (0.48 to 1.22)	0.73 (0.45 to 1.18)	.21
Parent estrogen adjusted‡	1.00 (Referent)	0.85 (0.53 to 1.35)	0.86 (0.54 to 1.37)	0.85 (0.51 to 1.40)	.55
16-pathway: total estrogens/estrogen metabolites					
Cases	104	74	92	129	
Multivariable adjusted†	1.00 (Referent)	0.72 (0.46 to 1.15)	0.93 (0.59 to 1.48)	1.26 (0.79 to 1.99)	.19
Parent estrogen adjusted‡	1.00 (Referent)	0.71 (0.44 to 1.13)	0.89 (0.56 to 1.43)	1.41 (0.88 to 2.27)	.09
2-pathway: parent estrogens					
Cases	116	105	98	80	
Multivariable adjusted†	1.00 (Referent)	0.88 (0.56 to 1.37)	0.72 (0.45 to 1.14)	0.61 (0.37 to 0.99)	.04
Parent estrogen adjusted‡	1.00 (Referent)	0.95 (0.60 to 1.51)	0.81 (0.50 to 1.32)	0.76 (0.44 to 1.32)	.28
4-pathway: parent estrogens					
Cases	105	111	91	92	
Multivariable adjusted†	1.00 (Referent)	1.08 (0.69 to 1.69)	0.79 (0.48 to 1.30)	0.85 (0.51 to 1.41)	.41
Parent estrogen adjusted‡	1.00 (Referent)	1.20 (0.76 to 1.90)	0.96 (0.57 to 1.62)	1.11 (0.64 to 1.92)	.81
16-pathway: parent estrogens					
Cases	98	101	88	112	
Multivariable adjusted†	1.00 (Referent)	1.01 (0.63 to 1.61)	0.88 (0.53 to 1.47)	1.08 (0.66 to 1.77)	.78
Parent estrogen adjusted‡	1.00 (Referent)	1.19 (0.73 to 1.93)	1.05 (0.62 to 1.79)	1.48 (0.86 to 2.56)	.17
2-pathway: 4-pathway					
Cases	95	115	96	93	
Multivariable adjusted†	1.00 (Referent)	1.29 (0.81 to 2.06)	1.02 (0.63 to 1.65)	1.04 (0.63 to 1.71)	.82
Parent estrogen adjusted‡	1.00 (Referent)	1.23 (0.76 to 1.97)	0.98 (0.60 to 1.59)	1.00 (0.61 to 1.65)	.73
2-pathway: 16-pathway					
Cases	128	87	98	86	
Multivariable adjusted†	1.00 (Referent)	0.75 (0.49 to 1.15)	0.77 (0.51 to 1.16)	0.69 (0.44 to 1.07)	.11
Parent estrogen adjusted‡	1.00 (Referent)	0.72 (0.47 to 1.11)	0.78 (0.51 to 1.18)	0.72 (0.46 to 1.14)	.17
4-pathway: 16-pathway					
Cases	118	94	99	88	
Multivariable adjusted†	1.00 (Referent)	0.75 (0.49 to 1.15)	0.73 (0.47 to 1.15)	0.75 (0.48 to 1.18)	.19
Parent estrogen adjusted‡	1.00 (Referent)	0.75 (0.49 to 1.16)	0.75 (0.48 to 1.19)	0.80 (0.50 to 1.26)	.30
Catechol: methylated catechol ratios					
Catechols§: methylated catechols 					
Cases	116	71	123	89	
Multivariable adjusted†	1.00 (Referent)	0.63 (0.41 to 0.98)	1.08 (0.71 to 1.66)	0.74 (0.48 to 1.16)	.41
Parent estrogen adjusted‡	1.00 (Referent)	0.59 (0.37 to 0.92)	1.02 (0.66 to 1.59)	0.66 (0.42 to 1.04)	.21
2-catechols: 2-methylated catechols					
Cases	110	82	119	88	
Multivariable adjusted†	1.00 (Referent)	0.78 (0.51 to 1.20)	1.11 (0.73 to 1.69)	0.77 (0.49 to 1.21)	.50
Parent estrogen adjusted‡	1.00 (Referent)	0.76 (0.49 to 1.17)	1.06 (0.69 to 1.64)	0.70 (0.44 to 1.11)	.28
4-catechols: 4-methylated catechols					
Cases	107	89	105	98	
Multivariable adjusted†	1.00 (Referent)	0.85 (0.55 to 1.31)	0.99 (0.64 to 1.54)	0.97 (0.63 to 1.47)	.98
Parent estrogen adjusted‡	1.00 (Referent)	0.80 (0.51 to 1.25)	0.92 (0.58 to 1.45)	0.90 (0.59 to 1.39)	.75

*P_{trend} (two-sided) was calculated using the Wald statistic for the quartile median level of the ratio (log-scale) modeled on a continuous basis. CI = confidence interval; OR = odds ratio.

†Odds ratios were estimated with conditional logistic regression and adjusted for age at cohort entry (<55, 55-59, 60-64, 65+ years), family history of breast cancer (yes/no), history of benign breast disease (yes/no), age at menarche (<13, 13-14, 15-16, 17+ years), age at first birth and parity (nulliparous, age <25 years with 1+ children, age > 25 years with 1+ children), duration of breast feeding (never, 1-12, 13-24, 25+ months), ever regularly used oral contraceptives (yes, no), ever drank alcohol regularly (yes, no), and ever participated in competitive sports (yes, no).

‡ORs adjusted for covariates above and additionally adjusted for total concentration of parent estrogens (in quartiles).

§Catechols comprise both the 2-catechols and 4-catechols. The 2-catechols consist of 2-hydroxyestrone and 2-hydroxyestradiol. The 4-catechol is 4-hydroxyestrone.

||Methylated catechols comprise both the 2-methylated catechols and 4-methylated catechols. The 2-methylated catechols consist of 2-methoxyestrone, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether. The 4-methylated catechols consist of 4-methoxyestrone and 4-methoxyestradiol.

Table 6. Urinary estrogen metabolism measures in postmenopausal Shanghai women and Asian American women

Estrogen/estrogen metabolite measure	Age-adjusted geometric mean in Shanghai women (pmol/mg creatinine)*	IQR†, Shanghai women (pmol/mg creatinine)	Age-adjusted geometric mean in Asian American women (pmol/mg creatinine)*	IQR†, Asian American women (pmol/mg creatinine)	% difference in mean: Asian American vs Shanghai women	P population difference‡
Metabolic pathway group						
Total estrogens/estrogen metabolites	9.06	5.78–12.92	24.84	17.83–34.59	+174	3.6*10 ⁻⁴⁸
Parent estrogens	2.55	1.58–3.85	4.29	3.12–6.89	+68	2.6*10 ⁻¹²
Estrone	2.07	1.29–3.18	2.57	1.67–4.04	+24	.003
Estradiol	0.43	0.24–0.66	1.45	0.92–2.69	+237	1.7*10 ⁻³⁸
2-pathway	2.81	1.87–4.15	4.99	3.43–7.91	+78	1.1*10 ⁻¹⁶
4-pathway	0.32	0.21–0.47	0.86	0.58–1.24	+169	2.0*10 ⁻⁴²
16-pathway	3.28	2.16–4.77	12.58	8.32–19.08	+284	4.9*10 ⁻⁶⁹
Total catechols	2.34	1.54–3.37	4.37	2.92–7.38	+87	1.6*10 ⁻¹⁷
Total methylated catechols	0.73	0.50–1.13	1.31	0.86–2.00	+78	2.8*10 ⁻¹⁶
Metabolic pathway ratio						
Parent estrogens: total estrogens/estrogen metabolites	0.28	0.25–0.31	0.17	0.13–0.26	–39	5.1*10 ⁻⁴¹
2-pathway: total estrogens/estrogen metabolites	0.31	0.29–0.34	0.20	0.15–0.30	–35	7.0*10 ⁻³⁰
4-pathway: total estrogens/estrogen metabolites	0.04	0.03–0.04	0.03	0.03–0.05	–1	.68
16-pathway: total estrogens/estrogen metabolites	0.36	0.34–0.39	0.51	0.42–0.65	+40	7.6*10 ⁻⁵¹
2-pathway: parent estrogens	1.10	0.94–1.34	1.16	0.75–1.95	+6	.30
4-pathway: parent estrogens	0.13	0.10–0.16	0.20	0.11–0.33	+60	2.2*10 ⁻¹⁷
16-pathway: parent estrogens	1.29	1.13–1.48	2.93	1.78–4.28	+128	5.7*10 ⁻⁵⁷
2-pathway: 4-pathway	8.80	8.09–9.44	5.80	4.84–7.26	–34	1.1*10 ⁻⁵⁵
2-pathway: 16-pathway	0.86	0.77–0.95	0.40	0.23–0.68	–54	1.4*10 ⁻⁴³
4-pathway: 16-pathway	0.10	0.09–0.11	0.07	0.04–0.12	–30	9.9*10 ⁻¹²
Catechols: methylated catechols	3.20	2.37–4.31	3.35	2.30–5.15	+5	.37

*Adjusted to the mean age of the combined control populations. Age-adjusted means were calculated by generalized linear models including terms for age (continuous) and study using a dataset combining the postmenopausal control participants of the Shanghai and Asian American breast cancer studies. Models were based on log-transformed concentrations (pmol/mg creatinine), and means given here are geometric means, ie, means on the log scale were exponentiated. Because geometric means were used, metabolic pathway subgroups may not add up to the total. IQR = interquartile range.

†Age-adjusted interquartile ranges for each study were calculated based on the distribution of the individual adjusted values.

‡P population difference (two-sided) was calculated using the Wald statistic for the coefficient of study population in a generalized linear model.

Figure 2. Median and interquartile range of urinary concentration of total estrogens/estrogen metabolites (pmol/mg creatinine) in postmenopausal women: Chinese women of Shanghai, Asian women of the United States, and US white women. **Box plots** above show the median (middle line) and interquartile range (top and bottom lines) of urinary concentrations of total estrogens/estrogen metabolites in five prior studies of postmenopausal women who were not using exogenous hormones. All five studies used the same estrogen metabolism assay, lab, and technician. Concentrations are not adjusted for age differences between populations, although age explained little variance in estrogen concentrations in our own data, possibly because all women were postmenopausal. Details for each study are as follows: 1) Chinese women from Shanghai: Concentrations based on the 399 control participants from the current study, with spot urines collected and kept on ice for a maximum of six hours until long term storage at -80°C . 2) US white women from Colorado: Concentrations are based on 60 women from a random selection of Kaiser Permanente Colorado members (85% of whom were white), with spot urines collected and kept on ice for a maximum of 24 hours until long term storage at -80°C (34). 3) Asian American women from California and Hawaii: Concentrations are based on 168 study control participants from San Francisco-Oakland CA, Los Angeles CA, or Oahu HI, with 12 hour overnight urines collected in a jug kept on ice for a maximum of 18 hours (from start of collection) until long term storage at -70°C (4). 4) US white women from Maryland: Concentrations are based on 15 women, with overnight urines collected in a jug that was kept on ice until morning, then decanted, aliquoted, and frozen at -70°C (35). 5) US white women from western New York state: Concentrations are based on 194 study control participants, 98% of whom were white, with first morning urines collected and kept on ice for approximately four hours until long term storage at -80°C (36).

substantively change after adjusting for BMI, and BMI was only weakly correlated with estrogen/estrogen metabolite concentrations. In prior population studies of postmenopausal women, BMI and circulating estradiol had Pearson correlations of 0.25 to 0.40 (39,50,51), implying that BMI explains 5% to 16% of population variance in circulating estradiol. In a radiolabeled hormone study (52), peripheral aromatization (including in adipose tissue) produced 24% of the estradiol and 28% of the estrone in the circulating estrogen pool of postmenopausal women. Since BMI explains only a small to moderate proportion of variance in the estrogen/estrogen metabolite concentrations of postmenopausal women, other factors must be implicated in population estrogen/estrogen metabolite differences. What such factors may be remains unclear but could include lifestyle factors not yet known to be estrogen related.

Our study has considerable strengths. The Shanghai Women's Health Study is a population-based cohort with prospectively collected urine samples and standardized specimen collection and handling. This study is one of the largest breast cancer studies to comprehensively evaluate estrogen metabolism. The LC-MS/MS

assay used to measure estrogens and estrogen metabolites is highly sensitive, specific, and reproducible. Additionally, the same lab and assay were used for all comparison populations, and the assay is robust to the sample processing and storage conditions commonly used in epidemiologic studies (37).

A limitation is that the urine samples from the different populations were assayed at different times, thus laboratory drift could partly explain between-population differences. This assay variation should be minimal, however, because the same standards were used throughout for unlabeled and labeled metabolites. An additional limitation of population comparisons is that the Asian American study used 12-hour overnight urines while the Shanghai study used spot urines. However, no compelling evidence indicates that total estrogen/estrogen metabolite concentrations vary diurnally, and the Colorado population had spot urines (34) and their total estrogen/estrogen metabolite concentrations were still elevated relative to Shanghai women. Another limitation was that, although the Asian American control participants were population based, the other three comparison populations were not population representative and sample sizes were small.

Our LC-MS/MS method permitted exploration of urinary levels of 15 individual estrogens/estrogen metabolites for the first time in a breast cancer study in a low-risk population, and we therefore systematically examined risk associations for each, as well as for derived measures, including metabolic pathway groups and pathway ratios. We did not adjust for multiple comparisons because our aim was to identify promising markers of risk and to compare results with previous studies. Some of our findings could therefore be because of chance. We recognize that our findings require independent confirmation in additional well-designed studies.

In conclusion, our findings suggest that estrogen metabolism, specifically enhanced 2-hydroxylation, plays an important role in breast cancer etiology and that preferential 2-hydroxylation is associated with reduced risk in low-risk nations, as well as developed nations at higher risk. In addition, our results show that total estrogen/estrogen metabolite concentrations are markedly higher in the United States than in Shanghai. This may help explain why breast cancer rates are at least three times higher in the United States than in China (3).

Funding

This work was supported by the Intramural Research Program of the National Cancer Institute, National Institutes of Health (NIH), Department of Health and Human Services. The Shanghai Women's Health Study was supported primarily by NIH grants R37CA70867 and UM1CA182910.

Notes

The study sponsors had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication. The authors disclose no potential conflicts of interest related to this study.

We thank Micah Ziegler for preparing Figure 1 on the pathways of estrogen metabolism.

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