

# Urinary Estrogen Metabolites and Their Ratio among Asian American Women

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## Abstract

Controversy persists regarding the role of a low ratio of 2-hydroxyestrogen (2-OHE<sub>1</sub>)/16 $\alpha$ -hydroxyestrogen (16 $\alpha$ -OHE<sub>1</sub>) as a potential estrogen metabolism marker of increased risk for breast cancer. Most of the evidence has been provided by case-control studies, where tumor effects on hormone metabolism are not known. Studies in populations at various risk of breast cancer are not consistent, with some suggesting that levels of the ratio may be altered by changes in diet and exercise. We studied Asian American women participating as controls in a case-control study of breast cancer in which migration history—a composite of the subject's place of birth, type of residence in Asia (urban or rural), length of time living in the West, and grandparents' place of birth—was associated with a 6-fold risk gradient that paralleled the historical differences in incidence rates between the United States and Asian countries. This population offered the possibility to address whether the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> differs according to recognized breast cancer risk factors, including migration history. Overnight 12-hour urines were obtained from 368 premen-

opausal and 143 naturally postmenopausal women of Chinese, Japanese, or Filipino descent who donated urines between 1985 and 1988. The estrogen metabolites 2-OHE<sub>1</sub> and 16 $\alpha$ -OHE<sub>1</sub> were measured with an ELISA kit and adjusted for creatinine levels. In each ethnic group, the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> was consistently lower in women born in the West than in those who had migrated from Asia. For premenopausal women, the ratio declined 20% due to lower levels of 2-OHE<sub>1</sub>. Among postmenopausal women, the ratio was 23% lower in those born in the West, but no consistent patterns based on place of birth were observed for either 2-OHE<sub>1</sub> or 16 $\alpha$ -OHE<sub>1</sub>. The ratio did not vary with most recognized breast cancer risk factors, except for lower metabolite ratios in women with a younger age at first birth and more children, which runs contrary to the hypothesis, because both characteristics reduce breast cancer risk. Our study suggests that the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> may be a marker for lifestyle influences on estrogen metabolism associated with westernization. (Cancer Epidemiol Biomarkers Prev 2005;14(1):221–6)

## Introduction

A role for endogenous estrogens in the development of breast cancer is well established, yet neither the mechanism of estrogen activity nor the spectrum of metabolites involved is entirely clear. Estrogen metabolism is complex, and the role of individual metabolites in breast carcinogenesis is controversial. Several hydroxylation sites exist, but estrogens are for the most part metabolized at C2 (and to a lesser degree at C4), yielding the catechol estrogens 2-hydroxyestrogen (2-OHE<sub>1</sub>), 4-hydroxyestrogen, 2-hydroxyestradiol, and 4-hydroxyestradiol, or at C-16, producing 16 $\alpha$ -hydroxyestrogen (16 $\alpha$ -OHE<sub>1</sub>) and estriol. Evidence for a carcinogenic role for 4-hydroxyestradiol (1) and for estrogenic activity of 16 $\alpha$ -OHE<sub>1</sub> is considerable (2–6), but the activity of 2-OHE<sub>1</sub> is controversial (7–9).

Levels of serum and urinary estrogens are higher in breast cancer cases compared with controls (10), but clinical studies linking the ratio of estrogen metabolites to breast cancer risk are not entirely consistent. Most (11–16) but not all (17) studies have observed lower ratios of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> in breast cancer cases than controls. Some have found a lower ratio in breast cancer cases in either premenopausal or postmenopausal women but not both (11, 15, 16), and the interpretation of findings from case-control studies is complicated by samples

collected after breast cancer diagnosis (11, 12, 14, 17). Studies that have measured the metabolites in samples collected before breast cancer diagnosis have not found consistent associations when menopausal status was considered, with low levels of the ratio linked to breast cancer risk in premenopausal women in one study and postmenopausal women in the other (15, 16).

Studies of estrogen metabolites in populations at different risk of breast cancer are inconclusive. High levels of the 2-hydroxylated estrogens, but not 16 $\alpha$ -OHE<sub>1</sub>, have been observed in Caucasian compared with Asian women (18–20). Racial differences in the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> have been observed in some studies (20, 21), with higher levels in White women compared with Asians or Blacks. More recently, differences in the metabolites and the ratio were slight between Chinese in Singapore and Caucasian and African American women in the United States (22).

Most of the well-established risk factors for breast cancer concern the timing of hormonal and/or reproductive events, and hormonal factors have figured prominently in hypotheses attempting to explain international differences in breast cancer incidence rates, particularly the low rates in Asian women. We had the opportunity to measure urinary hormones in Asian American controls participating in a population-based, case-control study of breast cancer (23). In this study, a composite measure of several aspects of migration showed a pattern of breast cancer risk that paralleled the historical 6-fold difference in incidence rates between the United States and Asian countries (23). Studies of migrant groups such as this offer a unique opportunity to investigate whether differences in breast cancer risk factors, including migration patterns, are

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associated with measurable differences in endogenous hormones and their metabolites. Previously, we reported that plasma estrogens did not differ significantly between Asian-born and Western-born controls in this study (24). In this report, we present findings for the urinary metabolites 2-OHE<sub>1</sub>, 16 $\alpha$ -OHE<sub>1</sub>, and their ratio.

## Materials and Methods

**Study Population.** The design of this population-based study has been reported (23). Women of Chinese, Japanese, or Filipino descent, ages 25 to 55 years, with incident breast cancer diagnosed between April 1983 and June 1987 in the San Francisco-Oakland metropolitan area, Los Angeles, and Oahu, Hawaii were identified. Population controls were frequency matched to cases in their region by ethnicity and year of birth by 5-year age groups. Controls from San Francisco-Oakland and Los Angeles were obtained by random digit dialing; in Hawaii, controls were obtained through the Health Surveillance Program, which annually samples 2% of households in the state. Where possible, a 2:1 ratio of controls to cases was obtained.

Interviews were conducted at the participant's home by trained interviewers. The questionnaire obtained information on the subject's residential history, place of birth of parents and grandparents, reproductive and menstrual history, medical history and cancer in family members, anthropometry, and diet. Women were asked to identify each country they had lived in for at least 1 year, and for each, the length of time at that residence and whether the community was urban or rural. Countries of birth considered "East" included China, Taiwan, Hong Kong, Macao, Japan, the Philippines, countries in Southeast Asia and the Malaysian Peninsula, Singapore, India, and countries in the southwest Pacific Ocean, except Australia and New Zealand. "West" included countries in North America, western Europe, central Europe, the former USSR, Australia, and New Zealand.

In the case-control study, women born in the West had a near 2-fold risk of breast cancer compared with those who had migrated from Asia and a 6-fold spread in risk when additional aspects of migration history were considered. For those born in the East, these included the type of community in which they lived (urban or rural) and the number of years living in the United States, whereas for those born in the West the number of grandparents also born in the West predicted risk.

Fasting blood samples and 12-hour overnight urines were obtained from all subjects willing to participate. Among menstruating women, the timing of the urine collection was scheduled to coincide with the midluteal phase, days 19 to 26 of the menstrual cycle. To facilitate this, women were telephoned and asked to report the start date of their current menstrual cycle, and following urine collection, women were instructed to return a postcard indicating the starting date of their next menses. Women were considered postmenopausal if their last menses occurred >1 year before urine collection.

**Laboratory Methods.** Overnight 12-hour urines were collected and stored in half-gallon containers with boric acid as preservative. Containers were kept at 4°C on ice or in a refrigerator during the overnight collection, and the following day, urines were mixed and aliquoted into conical tubes and sent to a repository for long-term storage at -70°C. The urinary metabolites 2-OHE<sub>1</sub> and 16 $\alpha$ -OHE<sub>1</sub> were measured in the Strang Cancer Research Laboratory (New York, NY) using a commercially available competitive immunoassay kit (EIA, Immunacare, Inc., Bethlehem, PA) that measured the metabolites directly. This kit has been described in detail elsewhere (25). For each subject, results were obtained in triplicate and averaged. Creatinine levels were obtained to adjust metabolite

results for differences in urine volumes and metabolite values are expressed in nanogram per milligram creatinine (26). Although the case-control study elucidated cases diagnosed between 1983 and 1987, urine collections occurred between 1985 and 1988. Quality-control samples used for the assay were obtained in 1993 and the assays were conducted in 1997. The intra-assay and interassay coefficients of variation obtained from duplicate blinded quality-control samples were as follows: 2-OHE<sub>1</sub>, within-batch and between-batch coefficients of variation were 6.7% and 7.4%, respectively; 16 $\alpha$ -OHE<sub>1</sub>, within-batch and between-batch coefficients of variation were 7.4% and 11.6%, respectively. The sensitivity of each assay was 0.65 ng/mL.

**Statistical Methods.** The metabolites 2-OHE<sub>1</sub> and 16 $\alpha$ -OHE<sub>1</sub> were analyzed on the natural logarithmic scale, with results from premenopausal and postmenopausal women analyzed separately. The ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> was analyzed without transformation. Adjusted least squares estimates of hormone means according to levels of migration variables and menstrual, reproductive, and anthropometric characteristics were obtained using standard ANOVA models and adjusted for age at urine collection (continuous) and ethnicity. For dichotomous variables, two-sided *P*s were obtained from ANOVA models. To test for trends in hormones across levels of categorical variables, *P*s were obtained by ranking the categories and considering the ranked variable as a continuous variable in standard regression models. Analyses were conducted using SYSTAT software (27).

## Results

In total, 966 women participated as controls in the interview study, with participation rates among eligible controls as follows: Chinese 72%, Japanese 78%, and Filipino 73%. Overnight 12-hour urines were obtained from 682 women, including 195 Chinese (68% of those interviewed), 292 Japanese (74% of those interviewed), and 195 Filipino (69% of those interviewed) women. Participation in the urine collection was slightly higher among more Westernized women, with urines obtained from 65% born in the East compared with 70% born in the West. Compared with women for whom we did not obtain a urine sample, participants were older (45.2 versus 44; *P* = 0.02), had fewer children (2.1 versus 2.4; *P* = 0.02), and were older at menarche (13.2 versus 13; *P* = 0.03), but these differences were slight. They were similar with respect to height, weight, and other reproductive characteristics.

Excluding women who reported using exogenous hormones at the time of urine collection, urinary assays were done on samples from a total of 595 women, including 369 premenopausal and 143 naturally postmenopausal women, 23 women with surgical removal of both ovaries, 17 women with surgical removal of one ovary, 32 women with surgical removal of the uterus only, and 11 women with cessation of menses during the 12 months preceding urine collection. We present results for premenopausal or naturally postmenopausal women not currently using exogenous hormones. Excluding one premenopausal woman whose place of birth was not known, this includes 368 premenopausal and 143 naturally postmenopausal women.

**Migration Indicators.** Table 1 presents age-adjusted geometric mean urinary estrogen metabolite levels according to place of birth and ethnicity. In premenopausal women, levels of 2-OHE<sub>1</sub> and the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> were significantly lower in Filipino women than in women of Chinese or Japanese descent (*P* < 0.01), but in all ethnic groups, levels were lower in women born in the West than in Asian-born women (for 2-OHE<sub>1</sub>, *P* = 0.07; for the ratio 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub>, *P* < 0.01). On average, the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> was 20%

**Table 1. Geometric mean urinary hormone levels adjusted for age according to place of birth, ethnicity, and menopausal status**

Ethnicity	Premenopausal				Premenopausal, luteal phase				Postmenopausal			
	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio
Chinese												
East	82	14.10	8.02	1.96	68	14.64	8.14	2.00	28	6.57	4.31	1.77
West	32	12.48	8.20	1.70	23	12.27	8.11	1.66	13	5.09	3.63	1.58
Japanese												
East	33	16.49	8.86	2.05	28	16.91	8.62	2.17	15	6.03	4.02	1.82
West	120	12.35	8.95	1.57	98	12.71	9.16	1.59	43	6.79	4.91	1.63
Filipino												
East	89	8.85	7.52	1.35	70	9.84	8.29	1.34	36	5.20	3.85	1.88
West	12	7.93	7.95	1.02	11	8.56	8.31	1.04	8	5.16	3.90	1.43
<i>P</i> (birthplace)		0.07	0.68	<0.01		0.04	0.79	<0.01		0.72	0.86	0.36
<i>P</i> (ethnicity)		<0.01	0.20	<0.01		<0.01	0.46	<0.01		0.43	0.30	0.98

lower in Western-born women. Premenopausal women (298 of 368, 81%) were considered in luteal phase at the time of sample collection, with specimens collected between days 19 and 26 of the menstrual cycle or plasma progesterone values >300 ng/dL, indicating ovulation have occurred. Restricting analyses to these women showed similar patterns, with levels of 2-OHE<sub>1</sub> and the ratio of 2-OHE<sub>1</sub>:16α-OHE<sub>1</sub> continuing to be significantly lower in Filipino women and in Western-born compared with Asian-born women in all ethnic groups (Table 1).

In postmenopausal women, levels of the ratio of 2-OHE<sub>1</sub>:16α-OHE<sub>1</sub> and the metabolites were somewhat lower in Filipino compared with Chinese or Japanese women but not significantly (Table 1). As observed in premenopausal women, the ratio of 2-OHE<sub>1</sub>:16α-OHE<sub>1</sub> was lower in women born in the West in each ethnic group although not significantly, with an overall difference of 23%. No consistent differences according to place of birth were observed for the metabolites.

Table 2 presents the estrogen metabolites and their ratio according to additional aspects of migration history that contributed to the 6-fold spread in risk in the case-control study. For those born and raised in the East, estrogen metabolite levels were estimated according to the number of years the migrants had lived in the United States and the type

of community in which they had lived in the East (urban or rural); for women born and raised in the West, metabolites and their ratio were evaluated according to whether their grandparents were also born in the West.

Among premenopausal women born in the East, levels of the ratio of 2-OHE<sub>1</sub>:16α-OHE<sub>1</sub> did not vary according to the number of years lived in the West. 2-OHE<sub>1</sub> was significantly higher in those who had lived in the West for ≥18 years, whereas 16α-OHE<sub>1</sub> increased nonsignificantly. Levels of the ratio and the metabolites did not depend on the type of community in which the subject lived in the East. For those born in the West, levels of the ratio and the metabolites did not differ based on the place of birth of the subject's grandparents.

For postmenopausal women born in the East, no significant differences were observed in the ratio or the metabolites according to the number of years lived in the West (Table 2). In women who had lived in an urban environment in the East, levels of the ratio and 2-OHE<sub>1</sub> tended to be lower, whereas 16α-OHE<sub>1</sub> levels were higher than those coming from a rural environment, although these differences were not significant. For women born in the West, levels of the ratio and the metabolites were lower in women who also had at least one grandparent born in the West, but differences were not significant.

**Table 2. Geometric mean hormone levels adjusted for age and ethnicity according to migration factors**

	Premenopausal				Postmenopausal			
	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio
Years living in the West*								
<5	45	11.58	7.74	1.62	14	4.85	4.27	1.44
5-11	48	12.38	7.72	1.90	19	7.92	4.68	2.15
12-17	56	10.70	7.89	1.66	13	5.79	4.55	1.39
≥18	55	15.53	8.96	1.87	33	5.78	3.73	1.98
<i>P</i>		0.05	0.11	0.35		0.97	0.16	0.48
Type of residence in the East*								
Rural	55	12.27	7.89	1.78	21	6.32	4.08	2.05
Urban	121	12.47	8.22	1.74	43	5.94	4.14	1.78
Both	29	13.22	8.04	1.84	15	5.84	4.22	1.70
<i>P</i>		0.95	0.62	0.71		0.77	0.94	0.56
Grandparents' place of birth†								
All born East	115	9.92	8.13	1.38	21	5.36	4.08	1.53
One or more born East	25	10.25	8.03	1.44	43	4.58	3.16	1.38
Unknown	24	12.65	9.37	1.46	15	7.60	4.93	1.73
<i>P</i>		0.24	0.28	0.93		0.07	0.10	0.62

\*Hormone levels in women born West not presented.

†Hormone levels in women born East not presented.

**Table 3. Geometric mean hormone levels adjusted for age and ethnicity according to breast cancer risk factors**

	Premenopausal				Postmenopausal			
	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio	<i>n</i>	2-OHE <sub>1</sub> (ng/mg creatinine)	16α-OHE <sub>1</sub> (ng/mg creatinine)	Ratio
Benign breast disease								
Yes	39	12.64	9.04	1.64	14	7.74	4.40	1.71
No	329	11.50	8.08	1.62	129	5.77	4.17	1.94
<i>P</i>		0.40	0.18	0.91		0.12	0.67	0.59
Family history of breast cancer								
Yes	29	13.25	9.41	1.60	17	4.81	3.60	1.52
No	339	11.47	8.08	1.62	126	6.09	4.27	1.75
<i>P</i>		0.25	0.10	0.90		0.17	0.12	0.55
No. live births								
0	55	11.70	7.60	1.72	14	5.06	4.06	1.54
1	62	13.76	8.58	1.87	15	8.32	5.27	2.08
2	135	11.92	8.79	1.51	35	5.59	3.87	1.69
3	72	11.29	8.10	1.62	32	6.36	4.40	1.75
4	24	10.49	7.94	1.53	19	7.01	4.98	1.66
5	20	7.51	6.04	1.45	28	4.77	3.57	1.71
<i>P</i> *		<0.01	0.01	0.10		0.12	0.19	0.60
Age at first live birth (y)								
0	55	11.49	7.52	1.71	14	4.98	4.01	1.54
<20	34	10.97	8.52	1.54	18	5.33	3.97	1.48
21-25	104	11.12	7.79	1.57	56	6.37	4.62	1.74
26-30	113	11.42	8.36	1.59	35	5.56	3.73	1.75
31-35	47	13.28	9.23	1.68	20	6.64	4.18	2.03
35	15	12.79	7.88	2.01				
<i>P</i> *,†		0.13	0.35	0.12		0.58	0.57	0.37
Age at menarche (y)								
≤11	72	10.79	7.70	1.57	16	4.30	3.70	1.39
12	102	12.27	8.62	1.61	30	6.23	3.94	1.80
13	78	11.43	7.93	1.64	35	7.19	5.17	1.71
14	54	11.10	8.13	1.66	19	5.52	4.28	1.34
15	34	13.24	8.65	1.70	10	5.19	4.65	1.20
16	28	10.93	7.92	1.59	33	5.88	3.65	2.22
<i>P</i>		0.78	0.83	0.64		0.70	0.73	0.24
BMI (kg/m <sup>2</sup> , quartiles)‡								
1	93	12.88	8.66	1.75	35	5.95	4.25	1.64
2	89	11.54	8.04	1.63	34	6.33	4.06	2.02
3	91	13.53	8.63	1.78	37	5.65	4.47	1.41
4	95	9.08	7.45	1.34	37	5.80	4.00	1.86
<i>P</i>		<0.01	0.09	<0.01		0.69	0.80	0.98
Height (in)								
≤60	169	12.39	8.86	1.57	53	5.35	4.36	1.46
61	82	14.37	8.93	1.87	3	6.45	4.35	1.69
62	112	11.67	8.45	1.54	25	5.64	3.80	1.78
≥63	137	13.04	8.65	1.70	30	7.22	4.30	2.18
<i>P</i>		0.31	0.88	0.12		0.19	0.57	0.20
Years since menopause§								
1-2					35	6.06	4.42	1.66
3-4					38	6.14	4.24	1.80
5-8					43	6.26	4.13	1.84
9					24	5.14	4.19	1.57
<i>P</i>						0.76	0.97	0.89
Age at menopause (y)§								
<46					30	5.88	4.34	1.95
46-49					30	4.70	4.25	1.30
50					25	7.23	4.68	1.79
51-52					26	6.42	4.05	2.16
53					29	6.17	4.54	1.53
<i>P</i>						0.20	0.86	0.21

\**P* for age at first pregnancy and number of births tests for trend in hormones among parous women only.

†Postmenopausal women ages >35 years at first birth grouped with women ages 31-35 years at first birth.

‡Quartiles for BMI categories based on following cut points (kg/m<sup>2</sup>): premenopause: 20.5, 22.3, and 24.6 and postmenopause: 20.9, 23, and 24.9.

§Age at and time since menopause unknown for three women.

**Breast Cancer Risk Factors.** Among premenopausal women, neither the ratio nor the metabolites varied significantly according to personal history of benign breast disease, family history of breast cancer, or age at menarche (Table 3). Levels did not decline with body mass index (BMI), although 2-OHE<sub>1</sub> and the ratio were significantly lower in women with a relatively high BMI (>24.6 kg/m<sup>2</sup>) and even lower among the few obese women with BMI >29 kg/m<sup>2</sup> in this population (data not shown). Patterns were, however, observed for parity and age at first birth. Among parous women, levels of the ratio

of 2-OHE<sub>1</sub>:16α-OHE<sub>1</sub> tended to be lower among women with more children and higher in those with a later age at first birth, but these trends were not significant. Levels of 2-OHE<sub>1</sub> and 16α-OHE<sub>1</sub> in parous women declined significantly with increasing numbers of live births, whereas 2-OHE<sub>1</sub> levels tended to increase with later age at first live birth although not significantly.

Levels of the ratio did not vary consistently according to breast cancer risk factors among postmenopausal women (Table 3), with the exception of nonsignificant increases with

later age at first live birth and height. No consistent patterns were observed for the metabolites alone. Metabolite levels did not decline over the first several years after menopause, and only after 8 years was there a suggestion of a nonsignificant decline in 2-OHE<sub>1</sub> and the ratio. Estrogen metabolites did not vary with BMI, even among women  $\geq 5$  years after menopause, where ovarian production of estradiol is minimal (data not shown).

The pattern of results for the ratio and breast cancer risk factors did not change with adjustment for place of birth; similarly, the differences in the 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> ratio across place of birth remained after controlling for parity, age at first birth, and BMI for both premenopausal and postmenopausal women (data not shown).

## Discussion

We found a lower ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> in both premenopausal and postmenopausal Chinese, Japanese, and Filipino women born in the West compared with those who had migrated from Asia. Further, among postmenopausal women born in the East, levels of the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> tended to be lower in women who had migrated from an urban rather than rural environment. These observations are consistent with the hypothesis first suggested >20 years ago that low values of the ratio of estrogen metabolites may be a marker of elevated breast cancer risk.

The influence of breast cancer risk factors on the ratio of estrogen metabolites has not been studied extensively in many women, and results from the few epidemiologic studies are unclear. Most have not found significant differences in the ratio according to anthropometric indicators and menstrual or reproductive risk factors (11, 16, 17), with the exception of higher ratios of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> (16) or levels of 2-OHE<sub>1</sub> (17) in women reporting an early age at menarche. A slight decline in the metabolite ratio with increasing BMI was observed in one study (11) but not in others (16, 17). In our study, the metabolite ratio did not vary with age at menarche or BMI, although levels were low in overweight and obese premenopausal women. Among postmenopausal women, the ratio tended to increase with height. In both premenopausal and postmenopausal women, there was the suggestion that age at first live birth may influence levels of the ratio, with higher levels observed in women having children at a later age. Conversely, in parous women, levels of the ratio declined with increasing number of children. These findings are contrary to the speculation that lower ratios are associated with an elevated risk, because height is associated with breast cancer risk, whereas parity and having a first birth at an early age are factors known to reduce breast cancer risk.

Several explanations for the lack of association between levels of the ratio and well-established breast cancer risk factors must be considered, including chance and the relatively small study size. Selection bias may play a role, because the overall response rate was relatively low ( $\sim 50\%$ ), and the willingness to participate in this study may have been influenced by cultural beliefs and degree of acculturation. Breast cancer risk factors, such as parity, anthropometry, and age at menarche, are known to change in migrant populations, but it is unlikely that the observed differences for Asian-born versus Western-born women operate through these risk factors. The ratio tended to decrease with parity and early age at first childbirth, factors more prevalent in our Asian-born women, and although levels were significantly lower in overweight premenopausal women, BMI did not vary with the ratio in postmenopausal women or in thinner premenopausal women. Moreover, patterns for the ratio according to breast cancer risk factors were similar in the crude analysis and with adjustment for age, ethnicity, and place of birth. Stratified

analyses by place of birth did not clarify these patterns, with the possible exception of BMI where, among premenopausal women, levels of the ratio continued to be low only in overweight Asian-born and Western-born women. Among postmenopausal women, a lower ratio was observed for heavier women born in the West only.

Interest in estrogen metabolism continues in part because low levels of the ratio may be a marker for breast cancer risk and because factors speculated to play a role in breast cancer etiology, such as diet, smoking, and exercise, may alter it. Some small clinical studies have shown differences in 2-OHE<sub>1</sub> related to diet, cigarette smoking, and physical activity. Levels of 2-OHE<sub>1</sub> are high in athletes (28); moreover, they increase following exercise (29), soy intake (30), cigarette smoking (31), and *Brassica* vegetable consumption or intake of indole-3-carbinol, a constituent of these vegetables (32, 33). By contrast, these factors do not seem to modify the extent of 16 $\alpha$ -hydroxylation activity. Few large studies have investigated these effects; in one that did, the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> was not altered by dietary modification, exercise, or weight reduction (34). In our study, no participant reported smoking during the 2 weeks before urine collection, and no information regarding diet at the time of specimen collection was available; thus, we were unable to evaluate their impact, if any, on the ratio of these estrogen metabolites. Only 24% of the participating women reported ever smoking during their adult life, but neither the amount nor the duration of smoking influenced the ratio (data not shown).

Studies of endogenous hormones have long been plagued with concerns about assay performance. We used an improved version of the immunoassay kit for the 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> ratio developed specifically to measure the low estrogen concentrations in postmenopausal women, which has been shown to yield results that correlate highly with measurements by gas chromatography-mass spectroscopy (25). Based on our blinded quality-control materials, coefficients of variation were <12% for both metabolites. Sample collection and handling of specimens have also been problematic in studies of endogenous hormones, because the time of day, the day within menstrual cycle, and, for urines in particular, the duration of specimen collection may lead to variable results for any given woman. Indeed, in the studies reported to date, urine collection procedures varied, with most relying on a single early morning sample, although at least one obtained urines over a 72-hour period (18). Recently, several methodologic studies have examined some of these concerns with promising results. In one, values of the ratio obtained from a first morning void and a 24-hour collection were comparable; whereas the absolute values of the metabolites differed between premenopausal and postmenopausal women, levels of the ratio did not (35). In another study, the ratio did not vary considerably in urines collected weekly over a 2-month period in premenopausal women (36). Taken together, these findings suggest the ratio is relatively constant concerning type of collection (first morning void versus 24-hour collection), menopausal status, and, in premenopausal women, time in menstrual cycle, so that reliance on a single measure of the 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> ratio in a 12-hour urine may be justified. Finally, the stability of the metabolites following long-term storage has not been extensively studied. However, in our assessment of the performance of the assay (25), we found no significant differences in the metabolite measurements after three freeze-thaw cycles on the same sample.

It has been proposed that efforts to shift the metabolism away from 16 $\alpha$ -hydroxylation to 2-hydroxylation may prevent breast cancer (37), but this may not be without risk, because carcinogenic activity has been shown for at least one catechol estrogen, 4-hydroxyestradiol. The cytochrome P450 estrogen metabolic pathway is complex, and although the enzymes responsible for 2-hydroxylation and 4-hydroxylation differ

(i.e., cytochrome P450 1A2 and 3A4 for the C2 metabolites and cytochrome P450 1B1 for the C4 compounds), some studies suggest that the lack of specificity of the 2-hydroxylases may play a role in 4-hydroxylation (38, 39). In a study using gas chromatography-mass spectroscopy methods (data not shown), we found that levels of the 4-hydroxylated metabolites correlated highly with 2-OHE<sub>1</sub>, suggesting that high levels of 2-OHE<sub>1</sub> detected by immunoassay methods may indicate relatively high levels of the 4-hydroxyestradiol.

Our finding of a lower ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> among Western-born Asian women is intriguing in light of the 2-fold risk observed for these women in the case-control study and supports the suggestion that the ratio may be a marker of lifestyle changes that occur over one or two generations in a migrant population. These changes, however, do not seem to be due to alterations in established breast cancer risk factors, because levels of the ratio of 2-OHE<sub>1</sub>:16 $\alpha$ -OHE<sub>1</sub> tended to be lower in women with a low risk profile. In particular, levels were low among women who had a first birth at a young age and had more children. Explanations for this inconsistency include the possibility that the metabolites do not reflect breast cancer risk, that levels of 2-OHE<sub>1</sub> and 16 $\alpha$ -OHE<sub>1</sub> in urine may not reflect breast tissue levels of the unconjugated forms of these metabolites, or that the ratio is not a unifying hypothesis underlying these hormonally related breast cancer risk factors. Although a role for hormones in breast carcinogenesis is well recognized, the conflicting results from this study highlight the lack of certainty about the range of hormones and the metabolites involved. Future studies exploring the hormonal mechanisms underlying breast cancer risk should focus their efforts on a variety of hormones and their metabolites in urine, blood, and breast tissue. Efforts to develop and validate reliable high-throughput assay methodology that can measure a broad spectrum of estrogens, androgens, and their metabolites would be particularly useful in this process.

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## References

- Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. *Annu Rev Pharmacol Toxicol* 1996;36:203–32.
- Osborne MP, Bradlow HL, Wong GYC, Telang NT. Upregulation of estradiol C16 $\alpha$ -hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. *J Natl Cancer Inst* 1993;85:1917–20.
- Telang NT, Axelrod DM, Bradlow HL. Metabolic biotransformation of estradiol in human mammary explant cultures. *Ann N Y Acad Sci* 1990; 586:70–8.
- Bradlow HL, Hershcopf R, Martucci C, Fishman J. 16 $\alpha$ -Hydroxylation of estradiol: a possible risk marker for breast cancer. *Ann N Y Acad Sci* 1986; 464:1338–51.
- Schneider J, Kinne D, Fracchia A, et al. Abnormal oxidative metabolism of estradiol in women with breast cancer. *Proc Natl Acad Sci U S A* 1982; 79:3047–51.
- Fishman J, Osborne MP, Telang NT. The role of estrogen in mammary carcinogenesis. *Ann N Y Acad Sci* 1995;768:91–100.
- Schneider J, Huh MM, Bradlow HL, Fishman J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. *J Biol Chem* 1984; 259:4840–5.
- Martucci CP, Fishman J. Impact of continuously administered catechol estrogens in uterine growth and luteinizing hormone secretion. *Endocrinology* 1979;105:1288–92.
- Vandewalle B, Lefebvre J. Opposite effects of estrogen and catechol estrogen on hormone-sensitive breast cancer cell growth and differentiation. *Mol Cell Endocrinol* 1989;61:239–46.
- Key TJ, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur J Cancer Clin Oncol* 1988;24:29–43.
- Kabat GC, Chang CJ, Sparano JA, et al. Urinary estrogen metabolites and breast cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 1997; 6:505–9.
- Zheng W, Dunning L, Jin F, Holtzman J. Correspondence re: Kabat GC, et al. Urinary estrogen metabolites and breast cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 1997;7:85–6.
- Coker AL, Crane MM, Sticca RP, Sepkovic DW. Re: Ethnic differences in estrogen metabolism in healthy women. *J Natl Cancer Inst* 1997;89:89–90.
- Ho GH, Luo XW, Ji CY, Foo SC, Ng EH. Urinary 2/16 $\alpha$ -hydroxyestrone ratio: correlation with serum insulin-like growth factor binding protein-3 and a potential biomarker of breast cancer risk. *Ann Acad Med Singapore* 1998;27:294–9.
- Muti P, Bradlow HL, Micheli A, et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16 $\alpha$ -hydroxyestrone ratio in premenopausal and postmenopausal women. *Epidemiology* 2000;11:635–40.
- Meilahn EN, De Stavola B, Allen DS, et al. Do urinary oestrogen metabolites predict cancer? Guernsey III cohort follow-up. *Br J Cancer* 1998;78:1250–5.
- Ursin G, London S, Stanczyk FZ, et al. Urinary 2-hydroxyestrone/16 $\alpha$ -hydroxyestrone ratio and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1999;91:1067–72.
- Adlercreutz H, Gorbach SL, Goldin BR, Woods MN, Dwyer JT, Hamalainen E. Estrogen metabolism and excretion in Oriental and Caucasian women. *J Natl Cancer Inst* 1994;86:1076–82.
- Lemon HM, Rodriguez-Sierra JF. Re: Ethnic differences in estrogen metabolism in healthy women. *J Natl Cancer Inst* 1997;89:1626–8.
- Jernstrom J, Klug T, Sepkovic DW, Bradlow HL, Narod SA. Predictors of the plasma ratio of 2-hydroxyestrone to 16 $\alpha$ -hydroxyestrone among premenopausal, nulliparous women from four ethnic groups. *Carcinogenesis* 2003; 24:991–1005.
- Taioli E, Garte SJ, Trachman J, et al. Ethnic differences in estrogen metabolism in healthy women. *J Natl Cancer Inst* 1996;88:617.
- Ursin G, Wilson M, Henderson BE, et al. Do urinary estrogen metabolites reflect the differences in breast cancer risk between Singapore Chinese and U.S. African American and White women? *Cancer Res* 2001;61:3326–9.
- Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst* 1993;85:1819–27.
- Falk RT, Fears TR, Hoover RN, et al. Does place of birth influence endogenous hormone levels in Asian-American women? *Br J Cancer* 2002; 87:54–60.
- Falk RT, Rossi SC, Fears TR, et al. A new ELISA kit for measuring urinary 2-hydroxyestrone, 16 $\alpha$ -hydroxyestrone, and their ratio: reproducibility, validity, and assay performance after freeze-thaw cycling and preservation by boric acid. *Cancer Epidemiol Biomarkers Prev* 2001;9:81–7.
- Flores OR, Sun L, Vaziri ND, Miyada DS. Colorimetric rate method for the determination of creatinine as implemented by the Beckman creatinine analyzer 2. *Am J Med Technol* 1980;46:792–8.
- Wilkinson L. SYSTAT: the system for statistics. Evanston (IL): SYSTAT, Inc.; 1986.
- Snow RC, Barbieri RL, Frisch RE. Estrogen 2-hydroxylase oxidation and menstrual function among elite oarswomen. *J Clin Endocrinol Metab* 1989; 69:369–76.
- De Cree C, Ball P, Seidlitz B, Van Kranenburg G, Geurten P, Keizer HA. Effects of a training program on resting plasma 2-hydroxycatecholestrogen levels in eumenorrheic women. *J Appl Physiol* 1997;83:1551–6.
- Lu LW, Cree M, Josyula S, Nagamani M, Grady JJ, Anderson KE. Increased urinary excretion of 2-hydroxyestrone but not 16 $\alpha$ -hydroxyestrone in premenopausal women during a soya diet containing isoflavones. *Cancer Res* 2000;60:1299–305.
- Michnovicz JJ, Naganuma H, Hershcopf RJ, Bradlow HL, Fishman J. Increased urinary catechol estrogen excretion in female smokers. *Steroids* 1988;52:69–83.
- Bradlow HL, Sepkovic DW, Telang NT, Osborne MP. Indole-3-carbinol. A novel approach to breast cancer prevention. *Ann N Y Acad Sci* 1995; 68:180–200.
- Fowke JH, Longcope C, Hebert JR. *Brassica* vegetable consumption shifts estrogen metabolism in healthy postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2000;9:773–9.
- Pasagian-Macaulay A, Meilahn EN, Bradlow HL, et al. Urinary markers of estrogen metabolism 2- and 16 $\alpha$ -hydroxylation in premenopausal women. *Steroids* 1996;61:461–7.
- Westerlind KC, Gibson KJ, Wolfe P. The effect of diurnal and menstrual cyclicity and menopausal status on estrogen metabolites: implications for disease-risk assessment. *Steroids* 1999;64:233–43.
- Chen Z, Zheng W, Dunning LM, Anderson KG, Parrish RS, Holtzman JL. Within-person variability of the ratios of urinary 2-hydroxyestrone to 16 $\alpha$ -hydroxyestrone in Caucasian women. *Steroids* 1999;64:856–9.
- Bradlow HL, Telang NY, Sepkovic DW, Osborne MP. 2-Hydroxyestrone: the “good” estrogen. *J Endocrinol* 1996;150:S259–65.
- Liehr JG. Dual role of oestrogens as hormones and pro-carcinogens: tumour initiation by metabolic activation of oestrogens. *Eur J Cancer Prev* 1997;6:3–10.
- Badawi AF, Cavalieri EL, Rogan EG. Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16 $\alpha$ -hydroxylation of 17 $\beta$ -estradiol. *Metabolism* 2001;50:1001–3.