Asian-American Women

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Soy Intake is Associated with Increased 2-Hydroxylation and Decreased 16α-Hydroxylation of Estrogens in Asian-American Women

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Abstract

Introduction: In Asian and Asian-American women, soy consumption is associated with reduced breast cancer risk, perhaps due to its effects on estrogen production or metabolism. In a sample of Asian-American women, we investigated the associations of usual adult soy intake with the urinary concentrations of 15 estrogens and estrogen metabolites (EM) measured using liquid chromatography-tandem mass spectrometry.

Methods: Participants included 430 Chinese-American, Japanese-American, and Filipino-American women, ages 20 to 55 years, and living in San Francisco-Oakland (California), Los Angeles (California), or Oahu (Hawaii). They were postmenopausal (n = 167) or premenopausal in luteal phase (n = 263) when 12-hour urine samples were collected. Robust linear regression was used to assess soy intakes as predictors of log-transformed EM measures. Individual and grouped EM were considered as concentrations (pmol/mg creatinine) and as percentages of total EM (%EM).

Results: Factor analysis confirmed that EM groups defined by metabolic pathways appropriately captured covariation in EM profiles. Total EM concentrations were not significantly associated with soy in premenopausal or postmenopausal women. Among all women, %2-hydroxylated EM and %4-hydroxylation pathway EM were 16% higher (P_trend = 0.02) and 19% higher (P_trend = 0.03) in the highest versus lowest soy tertiles, respectively. In contrast, 16% hydroxylated EM were 11% lower (P_trend < 0.01). Results were consistent across ethnic and menopausal groups and after adjustment for westernization measured by birthplace (Asia or United States).

Discussion: Findings suggest that regular soy intake is associated with increased ratios of 2:16-pathway EM and with higher relative levels of 4-hydroxylated EM. The observed variations in estrogen metabolism might modify breast cancer risk.

(Cancer Epidemiol Biomarkers Prev 2009;18(10):2751–60)

Introduction

Estrogens play important roles in the pathophysiology of breast tumors and are also recognized as causal factors in the etiology of this disease (1, 2). It has long been known that estrogens exert mitogenic effects on cells in the breast via receptor-mediated signaling (3). More recently, it has been recognized that some estrogen metabolites could be converted into reactive oxidative species which can damage DNA directly (4). Thus, estrogens and estrogen metabolites (jointly referred to as EM) might contribute to cancer risk by acting as cancer-promoting growth factors, or as cancer-initiating mutagens. Although prospective studies have shown consistent associations between circulating estrogen levels and risk of subsequent breast cancer in postmenopausal women, the relative importance of proposed pathways in breast carcinogenesis is not yet understood.

Bradlow et al. suggested that variability among women in susceptibility to breast cancer may result from interindividual variations in estrogen metabolism (5). Metabolism of estrogens occurs in the liver and kidneys as well as in breast and other target tissues, and includes oxidative metabolism (hydroxylation) and conjugative metabolism (glucuronidation, sulfation, glutathionylation, and/or O-methylation; ref. 6). Hydroxylation of estrone and estradiol most commonly occurs at ring carbon positions 2, 4, or 16 to yield 2-hydroxylated EM, 4-hydroxylated EM, or 16-hydroxylated EM, respectively (7). EM may vary in binding affinity with estrogen receptors, susceptibility to oxidative conversion into reactive species, susceptibility to conjugative metabolism, and bioavailability in target tissues. Therefore, EM are likely to vary with respect to specific roles in cancer etiology or cancer prevention.

Soy foods are a dietary staple in East Asian countries and may play a role in this region’s historically low...
incidence of breast cancer. Epidemiologic studies conducted to date among Asian women have found weak to moderate reductions in breast cancer risk associated with soy intake in adulthood (8, 9). In three studies which included measures of childhood and/or adolescent soy intake, results suggest stronger protective effects of soy consumed early in life (10-12). Isoflavones found in soy and soy-derived foods have structural similarities to estrogens and have been observed to have both estrogenic and antiestrogenic effects in animal models and in humans (13, 14). The health effects of isoflavones may be mediated through modulation of estrogen receptor signaling (15), but could also be mediated through their effects on enzymes with roles in production and/or metabolism of estrogens (16).

Various studies have tried to assess the effects of soy intake on endogenous levels of EM, including observational studies (17-19), short-term feeding studies (20-23), and randomized controlled trials (24). However, the studies have produced conflicting results.

We therefore decided to study the association between usual adult soy intake and urinary concentrations of 15 EM among Asian-American women. Study participants were of Chinese, Japanese, and Filipino ancestry and were living in San Francisco-Oakland (California), Los Angeles (California), and Oahu (Hawaii) when they were enrolled as controls in the population-based Asian-American Breast Cancer Study. In that study, we observed statistically significant inverse trends in breast cancer risk across soy tertiles; risk was 58% lower and 29% lower, respectively, in women in the highest versus lowest tertiles of childhood soy intake and usual adult soy intake (12, 25). In the present analysis, we test the hypothesis that adult intake of soy foods is associated with creatinine-adjusted urinary EM concentrations and/or relative proportions of urinary EM.

Materials and Methods

Study Population. Subjects for this cross-sectional analysis were drawn from a population-based breast cancer case-control study that has been described in detail previously (26). Briefly, cases in the parent study were Asian-American women of Chinese, Japanese, or Filipino ancestry, living in San Francisco-Oakland (California), Los Angeles (California), and Oahu (Hawaii) when they were diagnosed with breast cancer at 20 to 55 y of age during 1983 to 1987. Controls were frequency-matched to breast cancer cases in the parent study sample (26). During baseline interviews, participants were queried about their usual intake of approximately 60 food items/food groups, of which 10 to 15 were specific to their ethnic group. Frequencies of intake were recorded as times per day, week, month, or year, according to the most convenient time frame for the food item and respondent. Estimates of weekly soy intake for Chinese and Filipino women were based on reported frequency of intake of “a tofu dish made with any fresh, dried or deep-fried tofu product” whereas estimates for Japanese women were based on frequencies of intake of tofu, natto, and miso soup. Miso soup and natto were weighted as having 1/4 and 1/12, respectively, of the soy content of a tofu dish. Serving size was not queried. Participants were sorted into tertiles of soy intake using cutpoints generated from the distribution in the entire study sample (n = 430). Because soy intake was measured using more food items in Japanese compared with other ethnic groups, and because ethnic groups may differ both in EM profiles and in dietary patterns, all statistical models have been adjusted for ethnicity.

Because menopausal status is an important determinant of circulating and urinary endogenous estrogen levels and so many of the participants in this study were ages 45 to 55 y and potentially perimenopausal, we assessed menopausal status carefully using data from multiple sources, including self-reported histories of menopause and surgical procedures from the baseline interview, surgical procedures reported in interviews at the time of urine collection, a postcard mailed by participants to study investigators recording the day of the first menstrual period following urine collection, and levels of follicle-stimulating hormone, progesterone, and estradiol in serum collected on the same day as urine. Decision rules were designed to identify women who were clearly premenopausal (having regular menstrual cycles or evidence of continuing ovulation) and women who were clearly postmenopausal (questionnaire data indicating cessation of menstrual cycles due to natural menopause at least 1 y prior to urine collection or circulating hormone levels indicating cessation of ovarian estrogen production). Postcard data and circulating progesterone levels were used to assess the phase of the menstrual cycle. Participants who reported continuing periods were given urine collection appointments that coincided with the midluteal phase (days 19-26) of the menstrual cycle. Because Xu et al. found that estrogen metabolism changes over the course of the menstrual cycle (27), premenopausal women thought to be in other phases of the menstrual cycle at the time of urine collection were excluded from most analyses.

Based on our criteria, of the 569 participants available for analysis, 167 women were classified as postmenopausal, 263 were classified as premenopausal in luteal phase, 98 were classified as premenopausal but not in luteal phase, and 41 women were assigned unknown/perimenopausal status due to missing or ambiguous information. Thus, we included 430 women, utilizing both premenopausal women in luteal phase and postmenopausal women in this study. Analyses were conducted separately for the two menopausal groups as well as combined. In sensitivity analyses, we also considered whether inclusion of premenopausal women who were not in luteal phase would modify study findings.

Soy Measures. During baseline interviews, participants were queried about their usual intake of approximately 60 food items/food groups, of which 10 to 15 were specific to their ethnic group. Frequencies of intake were recorded as times per day, week, month, or year, according to the most convenient time frame for the food item and respondent. Estimates of weekly soy intake for Chinese and Filipino women were based on reported frequency of intake of “a tofu dish made with any fresh, dried or deep-fried tofu product” whereas estimates for Japanese women were based on frequencies of intake of tofu, natto, and miso soup. Miso soup and natto were weighted as having 1/4 and 1/12, respectively, of the soy content of a tofu dish. Serving size was not queried. Participants were sorted into tertiles of soy intake using cutpoints generated from the distribution in the entire study sample (n = 430). Because soy intake was measured using more food items in Japanese compared with other ethnic groups, and because ethnic groups may differ both in EM profiles and in dietary patterns, all statistical models have been adjusted for ethnicity.
Table 1. Absolute and relative concentrations of urinary EM in premenopausal-luteal (n = 263) and postmenopausal (n = 167) Asian-American women

<table>
<thead>
<tr>
<th>EM (pmol/mg creatinine)</th>
<th>%EM</th>
<th>Premenopausal-luteal</th>
<th>Median</th>
<th>IQR</th>
<th>Median</th>
<th>IQR</th>
<th>Median</th>
<th>IQR</th>
<th>Median</th>
<th>IQR</th>
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</thead>
<tbody>
<tr>
<td>Parent estrogens</td>
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<tr>
<td>Estrone (E1)</td>
<td>23.9</td>
<td>16.6-35.7</td>
<td>2.8</td>
<td>1.7-4.0</td>
<td>12.7%</td>
<td>10.0-15.8%</td>
<td>11.3%</td>
<td>7.3-15.2%</td>
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<tr>
<td>Estradiol (E2)</td>
<td>10.4</td>
<td>7.8-15.9</td>
<td>1.6</td>
<td>0.9-2.7</td>
<td>5.9%</td>
<td>4.3-8.1%</td>
<td>6.3%</td>
<td>4.2-10.0%</td>
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<tr>
<td>2-Hydroxylation pathway EM catechols</td>
<td></td>
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<tr>
<td>2-Hydroxyestrone (2-OHE1)</td>
<td>26.5</td>
<td>16.2-40.9</td>
<td>3.2</td>
<td>1.8-5.0</td>
<td>14.3%</td>
<td>10.0-19.2%</td>
<td>12.5%</td>
<td>8.3-18.7%</td>
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<tr>
<td>2-Hydroxyestradiol (2-OHE2)</td>
<td>2.8</td>
<td>1.6-4.8</td>
<td>0.6</td>
<td>0.3-1.3</td>
<td>1.5%</td>
<td>0.9-2.3%</td>
<td>2.3%</td>
<td>1.3-4.9%</td>
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<tr>
<td>Methylated catechols</td>
<td></td>
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<tr>
<td>2-Methoxyestrone (2-MeOE1)</td>
<td>5.2</td>
<td>3.3-7.9</td>
<td>0.7</td>
<td>0.4-1.0</td>
<td>2.7%</td>
<td>1.8-4.0%</td>
<td>2.7%</td>
<td>1.6-3.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methoxyestradiol (2-MeOE2)</td>
<td>0.5</td>
<td>0.3-0.8</td>
<td>0.1</td>
<td>0.1-0.2</td>
<td>0.3%</td>
<td>0.2-0.4%</td>
<td>0.5%</td>
<td>0.2-0.8%</td>
<td></td>
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<tr>
<td>2-Hydroxyestrone-3-methyl ether (3-MeOE1)</td>
<td>0.9</td>
<td>0.7-1.7</td>
<td>0.3</td>
<td>0.2-0.6</td>
<td>0.6%</td>
<td>0.3-0.9%</td>
<td>1.3%</td>
<td>0.7-2.0%</td>
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<tr>
<td>4-Hydroxylation pathway EM catechols</td>
<td></td>
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<tr>
<td>4-Hydroxyestrone (4-OHE1)</td>
<td>3.5</td>
<td>2.1-5.3</td>
<td>0.7</td>
<td>0.4-1.0</td>
<td>1.8%</td>
<td>1.2-2.9%</td>
<td>2.8%</td>
<td>1.7-3.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated catechols</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4-Methoxyestrone (4-MeOE1)</td>
<td>0.2</td>
<td>0.1-0.4</td>
<td>0.1</td>
<td>0.1-0.2</td>
<td>0.1%</td>
<td>0.1-0.2%</td>
<td>0.5%</td>
<td>0.2-0.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Methoxyestradiol (4-MeOE2)</td>
<td>0.1</td>
<td>0.0-0.2</td>
<td>0.04</td>
<td>0.02-0.09</td>
<td>0.04%</td>
<td>0.02-0.088%</td>
<td>0.2%</td>
<td>0.1-0.4%</td>
<td></td>
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<tr>
<td>16-Hydroxylation pathway EM</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>16α-Hydroxyestrone (16α-OHE1)</td>
<td>11.7</td>
<td>7.3-17.3</td>
<td>1.3</td>
<td>0.8-2.1</td>
<td>5.9%</td>
<td>4.6-8.0%</td>
<td>5.3%</td>
<td>3.7-7.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>62.3</td>
<td>36.2-90.7</td>
<td>5.6</td>
<td>3.6-9.4</td>
<td>32.2%</td>
<td>23.6-39.5%</td>
<td>23.6%</td>
<td>16.0-34.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Epiestriol (17-epiE3)</td>
<td>11.1</td>
<td>6.0-21.4</td>
<td>0.2</td>
<td>0.1-0.3</td>
<td>0.6%</td>
<td>0.3-1.2%</td>
<td>0.6%</td>
<td>0.3-1.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Ketoestradiol (16-ketoE2)</td>
<td>22.0</td>
<td>14.3-33.6</td>
<td>3.7</td>
<td>2.2-5.4</td>
<td>11.8%</td>
<td>9.1-15.0%</td>
<td>14.3%</td>
<td>10.5-20.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Epiestriol (16-epiE3)</td>
<td>9.0</td>
<td>5.5-12.3</td>
<td>0.9</td>
<td>0.6-1.3</td>
<td>4.6%</td>
<td>3.6-5.5%</td>
<td>3.6%</td>
<td>2.6-4.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total EM</td>
<td>194.9</td>
<td>138.5-267.9</td>
<td>25.3</td>
<td>17.7-34.8</td>
<td></td>
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</tbody>
</table>

Abbreviation: IQR, interquartile range.

Urine Samples and Laboratory Methods. Participants were instructed to collect 12-h overnight urine samples using half-gallon containers kept at 4°C on ice or in the refrigerator. Boric acid was added as a preservative (28). After delivery to the study center, urine was mixed and aliquoted into conical tubes and sent to a repository for long-term storage at −80°C.

Aliquots were sent to Science Applications International Corporation (Frederick, MD), where 15 urinary EM were measured, including estrone (E1), estradiol (E2), estriol (E3), 2-hydroxyestrone (2-OHE1), 2-methoxyestrone (2-MeOE1), 2-hydroxyestradiol (2-OHE2), 2-methoxyestradiol (2-MeOE2), 2-hydroxyestrone-3-methyl ether (3-MeOE1), 4-hydroxyestrone (4-OHE1), 4-methoxyestrone (4-MeOE1), 4-hydroxyestradiol (4-MeOE2), 16α-hydroxyestrone (16α-OHE1), 17-epiestradiol (17-epiE3), 16-ketoestradiol (16-ketoE2), and 16-epiestradiol (16-epiE3). The analytical method for measurement of urinary EM has been described previously (29). In brief, an internal standard solution containing four deuterium-labeled EM was added to each urine sample. Urinary EM and deuterium-labeled EM were hydrolyzed using β-glucuronidase/sulfatase from Helix pomatia, and then, following a 20-h incubation, extracted with dichloromethane. EM were quantitatively dansylated to improve their ionization efficiency. Liquid chromatography-tandem mass spectrometry analysis was done using a ThermoFinnigan TSQ Quantum-AM triple quadrupole mass spectrometer equipped with an electrospray ionization source and coupled directly to a Surveyor HPLC system (ThermoFinnigan). Both the chromatography system and the mass spectrometer were controlled using Xcalibur software (ThermoFinnigan).

The mass spectrometry conditions used in this study were as follows: source, positive mode ESI; spray voltage, 4,600 V; sheath and auxiliary gas, nitrogen; sheath gas pressure, 49 arbitrary units; auxiliary gas pressure, 23 arbitrary units; ion transfer capillary temperature, 350°C; scan type, selected reaction monitoring; collision gas, argon; collision gas pressure, 1.5 mTorr. The selected reaction monitoring conditions for the protonated molecules (MH⁺) of EM-dansyl and deuterium-labeled EM-dansyl were as follows: E1 m/z 504 → 171 collision energy, 42 eV; E2 m/z 506 → 171 collision energy, 43 eV; E3, 16-epi E3, and 17-epi E3 m/z 522 → 171 collision energy, 43 eV; E2-16-ketoE2 and 16α-OHE1 m/z 520 → 171 collision energy, 43 eV; 2-MeOE1, 4-MeOE2, and 3-MeOE1 m/z 534 → 171 collision energy, 42 eV; 2-MeOE2 and 4-MeOE2 m/z 536 → 171 collision energy, 43 eV; 2-OHE1 and 4-OHE1 m/z 753 → 170 collision energy, 44 eV; 2-OHE1 m/z 755 → 170 collision energy, 43 eV; δ2-E2 m/z 510 → 171 collision energy, 43 eV; δ2-E3 and δ16-epiE3 m/z 525 → 171 collision energy, 43 eV; δ2-2-MeOE2 m/z 541 → 171 collision energy, 43 eV; δ2-2-OHE2 m/z 760 → 170 collision energy, 43 eV. The following mass spectrometry variables were used for all experiments: scan width, 0.7 u scan time, 0.50 s; Q1 peak width, 0.70 u full width at half maximum; Q3 peak width, 0.70 u full width at half maximum.

Calibration curves for the 15 EM were constructed by plotting EM-dansyl/deuterium-labeled EM-dansyl peak area ratios obtained from calibration standards versus amounts of EM and fitting these data using linear regression with 1/X weighting. The amount of each EM in a urine sample was then interpolated using this linear function. Based on structural similarity and retention times, δ2-E2 was used as the internal standard for E2 and E3; δ16-E3 for 16α-ketoE2 and 16α-OHE1; δ16-epiE3 for 16-epiE3 and 17-epiE3; δ2-2-MeOE2 for 2-MeOE2, 4-MeOE2, 2-MeOE1, 4-MeOE1, and 3-MeOE1; δ3-2-OHE2 for 2-OHE2, 2-OHE1, and 4-OHE1.

Quality control samples at three concentrations (0.12, 0.96, and 6.4 ng of each EM/mL) were included in each
Soy Intake and Estrogen Metabolism in Asian-American Women

Table 2. Participant characteristics by menopausal status and tertiles of usual adult soy intake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Premenopausal women in luteal phase (n = 263)</th>
<th>Postmenopausal women (n = 167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy tertiles*</td>
<td>Prevalence (%)</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td></td>
<td>Low (n=109)</td>
<td>Medium (n=75)</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>41.4 ± 4.3</td>
<td>42.4 ± 4.8</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>20.8 ± 2.4</td>
<td>20.9 ± 2.6</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td>Filipino (40.4)</td>
<td>16.0</td>
</tr>
<tr>
<td>Study center (%)</td>
<td>Chinese (33.9)</td>
<td>44.0</td>
</tr>
<tr>
<td>Place of birth (%)</td>
<td>Hawaii (41.3)</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>Los Angeles (28.4)</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>San Francisco (30.3)</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td>United States (55.6)</td>
<td>45.3</td>
</tr>
<tr>
<td></td>
<td>Asia (44.4)</td>
<td>54.7</td>
</tr>
</tbody>
</table>

*Cutpoints for soy tertiles were determined based on the distribution of soy intake in the combined sample (n = 430) of premenopausal-luteal and postmenopausal women.

Results

In Table 1, medians and interquartile ranges are presented according to menopausal status for each urinary EM, batch of assays. In addition, urine samples from two premenopausal and two postmenopausal women were used as blinded quality control samples. Four quality control samples, including two from the same subject, were randomly included in every batch of ~40 samples. Total laboratory coefficients of variation were <10% for all EM except 4-methoxyestradiol (15%), and were ≤4% for estrone, estradiol, and estriol, and ~1% for total EM. In general, laboratory coefficients of variation decreased as urinary concentrations increased. Creatinine levels were obtained to adjust EM levels for differences in urinary volume (30).

Statistical Methods. Statistical analyses were conducted using SAS v. 9.1 (SAS Institute, Cary, NC). Absolute levels of each urinary EM were expressed in picomoles per milligram of creatinine. Relative EM levels (%EM) were expressed as a percentage of total EM. Each of these measures was log-transformed to better approximate normal distributions.

To assess the effect of soy intake on EM measures, we first fit standard linear regression models and then robust linear regression models (PROC ROBUSTREG with option MM, which are less sensitive to outliers), to each EM measure as a dependent variable. We tested our hypotheses of linear trend across soy tertiles by assigning median frequency of soy intake to each tertile and treating the variable as a continuous covariate. We used the regression coefficient associated with the highest soy tertile to estimate the percent difference based on the formula: 100 [exp (β) – 1]. This represents a covariate-adjusted estimate of the difference between EM measures for the highest vs. the lowest soy tertile, expressed as a percent change.

Models of EM and %EM measures were fit separately for premenopausal and postmenopausal strata and were adjusted for age and ethnicity. In additional models we adjusted, in turn, for body mass index (BMI) and birthplace (Asia/West) to assess whether observed associations were independent of other aspects of acculturation. To assess effect modification, we fit models for EM grouped by metabolic pathways and expressed as a percentage of total, to estimate normal distributions.
expressed first as an absolute concentration (pmol/mg creatinine) and then as a proportion of total EM. Even within menopausal groups, urinary EM concentrations varied by factors of 10 to 100. Median EM concentrations were 2 to 10 times higher in premenopausal-luteal women compared with postmenopausal women. In contrast, interquartile ranges for %EM in premenopausal and postmenopausal women overlapped. Most median %EM differed by <2 percentage points between premenopausal-luteal and postmenopausal women. One exception is estriol, which had a markedly lower percentage of total EM in postmenopausal women compared with their premenopausal-luteal counterparts (23.6% versus 32.2%, respectively).

Participant characteristics by tertiles of soy intake are presented in Table 2 for premenopausal-luteal and postmenopausal women. Soy intake was nonsignificantly higher in postmenopausal compared with premenopausal-luteal women ($P = 0.09$), with postmenopausal women making up 32.7% of the lowest tertile, but 44% of the highest tertile. Soy intake was associated with ethnicity in both premenopausal and postmenopausal women. Filipino women had the lowest frequency of soy intake (with median intakes of 0.2 and 0.5 servings per week in premenopausal-luteal and postmenopausal women, respectively) and so were overrepresented in the lowest soy tertile. Soy intake was associated with ethnicity making up 32.7% of the lowest tertile, but 44% of the highest tertile. Soy intake was nonsignificantly associated with tertile estimate.

For menopause-specific strata, regression models were fitted separately for premenopausal-luteal and postmenopausal women, no significant associations were observed for absolute concentrations of any individual EM with soy intake. We tested for associations of urinary concentrations of individual EM with soy intake by modeling each EM measure using robust linear regression and adjusting for age and ethnicity (Table 3). No statistically significant associations were seen between soy intake tertiles and urinary concentrations of E$_1$, E$_2$, E$_3$, or total EM in either menopausal group. In postmenopausal women, urinary levels of 2-MeOE$_1$ were directly associated with soy intake ($P_{\text{trend}} = 0.02$). Also among postmenopausal women, urinary levels of 16-ketoE$_2$ declined significantly across soy tertiles ($P_{\text{trend}} = 0.009$). These associations remained statistically significant when birthplace or BMI were added to the model. Among premenopausal-luteal women, no significant associations were observed for absolute levels of any individual EM with soy intake.

In Table 3, we also present the results of robust linear regression models for individual EM expressed as a percentage of total EM, in a combined sample of premenopausal-luteal and postmenopausal women. 4%-OHE$_1$, 2%-MeOE$_1$, and 2%-MeOE$_2$ increased significantly, and 2%-MeOE$_1$ was directly associated with soy intake ($P_{\text{trend}} = 0.009$). These associations remained statistically significant when birthplace or BMI were added to the model. Among premenopausal-luteal women, no significant associations were observed for absolute levels of any individual EM with soy intake.

### Table 3. Percent difference in concentrations of urinary EM among Asian-American women in the highest vs. lowest tertiles of soy intake, by menopausal status

<table>
<thead>
<tr>
<th>EM measure</th>
<th>EM (pmol/mg creatinine)</th>
<th>Percent difference*</th>
<th>EM (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Highest vs. lowest tertile</td>
<td>$P_{\text{trend}}$</td>
</tr>
<tr>
<td>Premenopausal-luteal women ($n = 263$)</td>
<td>Postmenopausal women ($n = 167$)</td>
<td>Combined premenopausal-luteal and postmenopausal women ($n = 430$)</td>
<td></td>
</tr>
</tbody>
</table>

- Parent EM
- Estrone (E$_1$)
- Estradiol (E$_2$)
- 2-Hydroxylated pathway EM catechols
- 2-Hydroxyestrone (2-OHE$_1$)
- 2-Hydroxyestradiol (2-OHE$_2$)
- Methylenated catechols
- 2-Methoxyestrone (2-MeOE$_1$)
- 2-Methoxyestradiol (2-MeOE$_2$)
- 4-Hydroxylation pathway EM catechols
- 4-Hydroxyestrone (4-OHE$_1$)
- Methylenated catechols
- 4-Methoxyestrone (4-MeOE$_1$)
- 4-Methoxyestradiol (4-MeOE$_2$)
- 16-Hydroxylated pathway EM
- 16a-Hydroxyestrone (16a-OHE$_1$)
- Estriol (E$_3$)
- 17-Epiestriol (17-epiE$_3$)
- 16-Ketoestradiol (16-ketoE$_2$)
- 16-Epiestradiol (16-epiE$_2$)
- Total EM

$^*$Percent difference = 100[exp(β) − 1], where β is the coefficient from robust linear regression corresponding to women in the highest compared with the lowest tertile of soy on log-transformed EM measures (pmol/mg creatinine or mol% of total EM). For menopause-specific strata, regression models were adjusted for continuous age and ethnicity (Chinese, Japanese, and Filipino); for the combined sample, regression models were also adjusted for menopausal status.

$^*$Statistical significance of linear trend was tested by assigning median frequency of soy intake to each tertile and treating the variable as a continuous covariate.

**Cancer Epidemiology, Biomarkers & Prevention**

Published OnlineFirst September 29, 2009; DOI:10.1158/1055-9965.EPI-09-0388
whereas 16%-keto E2 and 16%-epiE2 each had statistically significant inverse trends across tertiles of soy intake. Other %EM in these pathways had similar but nonsignificant associations with soy. This observation led us to examine covariation among EM.

Factor analysis with rotation resulted in the extraction of four independent factors in each menopausal group (Fig. 1). The composition of factors was observed to be similar in premenopausal-luteal and postmenopausal women, in spite of the large differences between these groups in absolute levels of total EM. The predominant factor in both premenopausal and postmenopausal women, termed “catechols” and accounting for 46% and 30% of the variance in EM profiles, respectively, included all three catechol estrogens (2-OHE1, 2-OHE2, and 4-OHE1) and some of their five methylated metabolites (2-MeOE1 and 3-MeOE1 in both groups, and 2-MeOE2 in premenopausal women only). The second factor in both groups (termed “16-hydroxylated pathway EM”) included all five EM that were initially hydroxylated at the 16-C position (16-keto E2, 16a-OHE1, 16-epiE3, E3, and 17-epiE3), and accounted for 18% and 16%, respectively, of the variance in EM profiles in the two menopausal groups.

The next factor, termed “parent estrogens”, was the third factor in premenopausal women, accounting for 7% of variation in EM profiles, and the fourth in postmenopausal women, accounting for 8% of variation in EM profiles. This factor included high loadings for E1 and E2 in both menopausal groups; in postmenopausal women only, two EM from the 16-pathway also contributed in a moderate fashion (E3 and 16-EpiE3). A fourth factor termed “methylated catechols” (4-MeOE1 and 4-MeOE2 in both groups, and 2-MeOE2 in postmenopausal women only) accounted for 6% and 14% of variation in premenopausal and postmenopausal women, respectively. The fact that methylated catechols in the 4-hydroxylation pathway did not cluster with those in the 2-hydroxylation pathway led us to define groups that examined these metabolites separately.

In Table 4, we present the results of robust linear regression models, fitted in each menopausal stratum and then in a combined sample, for EM grouped by metabolic pathway and expressed as a percentage of total EM. Patterns were similar in premenopausal-luteal and postmenopausal strata; accordingly, there was no statistically significant effect modification of the associations by menopausal status. In the combined sample, %2-hydroxylation pathway EM and %4-pathway EM each significantly increased across increasing tertiles of soy intake; these pathways were 16% higher ($P_{\text{trend}} = 0.02$) and 19% higher ($P_{\text{trend}} = 0.03$), respectively, in the highest compared with the lowest tertile of soy intake. The 16%-hydroxylated pathway EM were 11% lower in the highest versus the lowest tertile of soy intake ($P_{\text{trend}} < 0.01$).
Increased across the range of soy intake (4-pathway EM to 16-hydroxylation pathway) showed no linear trend (P_trend < 0.01). Although %catechol estrogens in the 2-hydroxylation and 4-hydroxylation pathways each increased significantly with soy intake, trends in %methylated catechols differed by pathway. Methylated catechols of the 2-pathway increased as a proportion of total EM across soy tertiles (P_trend < 0.01), whereas %methylated catechols in the 4-hydroxylation pathway showed no linear trend (P_trend = 0.80).

Results for the ratios of EM were consistent with findings for EM groups. Ratios of 2-pathway EM and 4-pathway EM to 16α-pathway metabolites significantly increased across the range of soy intake (P_trend = 0.01 for each). Among all women, the ratio of catechols/methylated catechols in the 4-pathway was 20% higher for each tertile. Among all women, the ratio of catechols/methylated catechols in the 2-pathway was 20% higher in the highest tertile compared with the lowest (P_trend = 0.11); no trend was noted in the same ratio for the 2-pathway.

Finally, we considered several potential confounders (data not shown) of the EM/soy relationships. Birthplace is considered a marker of acculturation and of breast cancer risk (26). BMI might play a causal role in breast cancer etiology and has sometimes been observed to be inversely associated with soy intake (31). Additional adjustment of all models for birthplace and for BMI did not modify the direction or magnitude of observed associations.

Figure 2 shows the percentage of difference between the highest and lowest soy tertiles in relative levels of urinary EM for each stratum defined, in turn, by menopausal status, ethnicity, birthplace, and BMI. Results suggest that associations of %EM groups with soy intake were not significantly modified by these factors. One statistically significant interaction was noted: in women with BMI below the median, the %2-hydroxylation pathway showed a significant increasing trend across soy tertiles and was 16.6% higher in the highest versus lowest soy tertile; among heavier women, the %2-hydroxylation pathway also increased with soy intake but the magnitude of the effect was smaller, with a 10% increase seen in the highest versus lowest tertile (P for interaction = 0.04). This statistically significant finding may reflect the large number of statistical tests done.

Inclusion of premenopausal women in other phases of the menstrual cycle also did not modify the direction or magnitude of the observed associations; however, it did result in wider confidence limits for many estimates.

### Discussion

In this study of premenopausal and postmenopausal Asian-American women with varying diets, lifestyles, and risks of breast cancer, we found no significant trends in absolute levels of estrone, estradiol, or total EM across tertiles of soy intake. This is consistent with the findings of a long-term intervention trial in which 220 premenopausal women were randomized to a soy intervention or control arm; in this study, investigators found no differences between groups in circulating estrone, estradiol, or free estradiol at four time points up to 24 months (24). In contrast, in two observational studies, investigators found that high soy intake was associated with reduced serum estrone in postmenopausal Chinese women (18) and reduced serum estradiol in Japanese premenopausal women (17). Evidence from short-term feeding trials (<6 months) has been inconsistent (16, 32, 33).

Although our findings do not support the hypothesis that soy intake influences endogenous production of estrogens, they do suggest that regular soy consumption modifies estrogen metabolism, perhaps through effects on breast cancer etiology and has sometimes been observed to be inversely associated with soy intake (26).

### Table 4. Percent difference in urinary EM grouped by metabolic pathway in highest vs. lowest tertiles of soy intake among premenopausal (luteal) and postmenopausal Asian-American women

<table>
<thead>
<tr>
<th>EM Ratio by Pathway (% of total EM)</th>
<th>Percent Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Highest vs. lowest tertile</td>
</tr>
<tr>
<td></td>
<td>P trend†</td>
</tr>
<tr>
<td>2-Hydroxylation pathway EM</td>
<td>13.5 0.06</td>
</tr>
<tr>
<td>4-Hydroxylation pathway EM</td>
<td>16.7 0.11</td>
</tr>
<tr>
<td>16-Hydroxylation pathway EM</td>
<td>−7.1 0.05</td>
</tr>
<tr>
<td>Parent estrogens</td>
<td>1.6 0.70</td>
</tr>
<tr>
<td>Catechol estrogens</td>
<td>10.9 0.10</td>
</tr>
<tr>
<td>Methylated catechols</td>
<td>22.8‡ 0.06</td>
</tr>
<tr>
<td>Methylated catechols of the 2-hydroxylation pathway</td>
<td>25.8‡ 0.04</td>
</tr>
<tr>
<td>Methylated catechols of the 4-hydroxylation pathway</td>
<td>4.3 0.90</td>
</tr>
</tbody>
</table>

*Percent difference = 100[exp(β)−1], where β is the coefficient from robust linear regression corresponding to women in the highest compared to the lowest tertile of soy on log-transformed EM measures (pmol/mg creatinine or mol% of total EM). For menopause-specific strata, regression models were adjusted for continuous age and ethnicity (Chinese, Japanese, and Filipino); for the combined sample, regression models were also adjusted for menopausal status. For linear trend was tested by assigning median frequency of soy intake to each tertile and treating the variable as a continuous covariate. †P for linear trend was tested by assigning median frequency of soy intake to each tertile and treating the variable as a continuous covariate. ‡P < 0.05, for tertile estimate.
on enzymes involved in the hydroxylation of estrogens. In our study, women who reported more soy intake had relatively higher levels of 2- and 4-hydroxylated EM and relatively lower levels of 16-hydroxylated EM.

There are more than 10 human cytochrome P450 isoforms capable of hydroxylating estrone and estradiol to produce a wide range of potential metabolites (34). These phase I enzymes vary in distribution across target tissues, catalytic activity, and regiospecificity. The activities of several specific hepatic enzymes likely influence EM profiles; for example, CYP1A2 preferentially hydroxylates estrogens at the second carbon, whereas CYP3A4 and CYP3A5 have relatively higher activity for the production of 16-hydroxylated EM (35). The effects of polymorphic variants in extrahepatic enzymes, CYP1A1, which preferentially produces catechol estrogens, and CYP1B1, which selectively produces 4-hydroxylated EM, could also be detected in women’s profiles of circulating or excreted EM (36, 37). Isoflavones can affect enzyme activity directly through competitive inhibition, or indirectly, through interactions with receptors that induce enzyme expression (38).

In vitro studies suggest that exposure to soy isoflavones could modulate both the expression and activity of several of these enzymes; however, the net effects of soy exposure on estrogen metabolism in vivo remain unclear (39, 40).

Several observational studies have identified dietary or lifestyle components associated with levels of 2-OHE1, 16α-OHE1, or with their ratio (41, 42). The evidence regarding soy intake and levels of these EM is not conclusive. Of four intervention trials conducted in premenopausal women, two found an increased ratio of 2-hydroxyestrone/16α-hydroxyestrone during periods of exposure to high levels of soy isoflavones (16, 43) whereas two did not (44, 45). A feeding trial conducted with postmenopausal participants (33) showed a decreased ratio of 2-hydroxyestrone/16α-hydroxyestrone during a period of daily supplementation with 65 mg of soy isoflavones in comparison to measures at baseline and during use of the control supplement; but found no significant effect of 135 mg of soy isoflavones per day on the same parameter. This same study showed decreased levels of 4-OHE1 during use of isoflavone supplements at both doses. A more recent trial showed no effects of supplementation with soy flour on the ratio of

Figure 2. Percentage of difference between the highest and lowest tertiles of soy intake in relative levels of urinary EM (percentage of total) by menopausal status, ethnicity, and BMI. Percentage of difference was calculated based on regression coefficients associated with the highest tertile of soy intake in robust regression models fitted to predict log-transformed EM measures, using the formula $100^{[\exp(\hat{\beta}) - 1]}$. For menopause-specific strata, regression models were adjusted for continuous age and ethnicity (Chinese, Japanese, and Filipino); for ethnic-specific strata, regression models were adjusted for continuous age and menopausal status; for birthplace and BMI, models were adjusted for continuous age, ethnicity, and menopausal status. There was no statistically significant effect modification of the associations between soy and grouped %EM by menopausal status, by ethnicity or by birthplace. *, $P < 0.05$, for interaction.

Cancer Epidemiol Biomarkers Prev 2009;18(10). October 2009

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2-hydroxyestrone/16α-hydroxyestrone (46). Two studies found that soy interventions modified the ratio of 2-OHE1/16α-OHE1 only in a metabolic or ethnic subgroup (22, 47).

Methylation is an important pathway for detoxification and excretion of catechol estrogens (48). The enzyme catechol-O-methyl transferase is present in liver, kidney, and in breast tissue. Several in vitro studies have found that exposure of breast cells to soy isoflavones results in reduced expression and/or activity of catechol-O-methyl transferase (49, 50). Our findings suggest no significant difference in ratios of catechols/methylated catechols in the 2-hydroxylation pathway, but do show nonsignificant increases in this ratio for EM in the 4-pathway. Results of studies in human liver cells and in hamster kidney cells suggest that methylation by catechol-O-methyl transferase is more efficient for 2-hydroxylated EM than for 4-hydroxylated EM (51). Methylated 2-hydroxylated EM might actually inhibit the methylation of 4-hydroxylated catechols (52). Our findings suggest that soy isoflavones may directly or indirectly reduce the rate at which 4-hydroxyestrone is methylated.

Our study has several strengths. It includes a large population-based sample of Asian-American women with varying levels of acculturation, corresponding to a wide range of dietary and lifestyle choices, and a 6-fold gradient in breast cancer risk (26). Measures of usual adult soy intake represent long-term exposures at doses that are relevant to levels and patterns of intake in free-living populations. Another major strength of this study is the use of a highly sensitive, specific, and reliable assay to measure 15 of the most prevalent EM in urine. Many studies on the role of estrogen metabolism in breast cancer risk have used genetic variants as surrogates because these are easy to measure on a large scale; however, simulations suggest that genetic variants across metabolic pathways contribute in a complex fashion to the levels of EM (53). The EM profile is a phenotypic measure and thus provides a direct way to test hypotheses about the effects of dietary and lifestyle factors on estrogen metabolism and to study the effects of estrogen metabolism on disease risk.

The primary weakness of this observational study is the potential for confounding. We assessed two potential confounders and found that the observed associations were independent of birthplace (Asia/West), and of BMI, which are each associated with acculturation and breast cancer risk. Findings were also relatively consistent across ethnic subgroups, and are thus less likely to result from confounding by unmeasured dietary factors because dietary patterns are somewhat different in each ethnic group. Another study weakness is that assessment of soy intake was based on a limited set of queried soy foods, and on frequency of intake without reference to portion size. Results of a study of Asian-American women living in Los Angeles county (54), suggest that the queried foods may account for most of the variation in soy intake among Japanese participants, but only half of the variability among Chinese and Filipino participants.

Pooled analyses of prospective studies in postmenopausal women have provided definitive evidence that high levels of circulating estrogens (estrone, estradiol, and free estradiol) are associated with increased breast cancer risk (2). Our data suggests that if soy decreases breast cancer risk, its effects are not mediated by effects on absolute levels of estrogens or total EM. Increasing soy intake was associated with increased levels of 2-hydroxylated and 4-hydroxylated metabolites, and reduced levels of 16-hydroxylated metabolites. These variations in estrogen metabolism are consistent with a protective effect of soy on breast cancer risk based on Bradlow’s theory of estrogen-mediated carcinogenesis (5). However, the “genotoxic metabolites” hypothesis, which posits oxidized products of catechol estrogens as primary causal agents in breast cancer (4), suggests that observed variations in estrogen metabolism could increase risk.

Methylated catechol estrogens have reduced estrogenicity, are readily cleared from circulation, and unlike the catechol estrogens from which they are derived, are not readily oxidized to form DNA-damaging quinones (6). The metabolite 2-methoxyestradiol has antiproliferative and antiangiogenic effects and therefore may play a role as an endogenous anticancer agent (55). Thus, observed trends of increasing methylated catechols in the 2-pathway across tertiles of soy intake could be consistent with the anticancer effects of soy.

Soy may also have effects on breast cancer risk mediated through other causal pathways; for example, through interactions of isoflavones with estrogen receptors. Further study of estrogen metabolism and EM phenotypes in relation to cancer risk will provide more information about the mechanisms by which soy can modify breast cancer risk.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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We thank David Check of the Biostatistics Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute for assistance with figures.

References
Soy Intake and Estrogen Metabolism in Asian-American Women


Correction

Correction: Soy Intake Is Associated with Increased 2-Hydroxylation and Decreased 16-Hydroxylation of Estrogens in Asian-American Women

In this article (1), which was published in the October 2009 issue of Cancer Epidemiology, Biomarkers & Prevention, there were errors in some abbreviations used for estrogens and estrogen metabolites (EM) when considered as a proportion of total EM. For example, "16%-hydroxylation pathway EM" is incorrect, and should be "%16-hydroxylation pathway EM." Similarly, "%4-OHE1," "%2-MeOE1," "%2-MeOE2," "%16-ketoE2," and "%16-epiE3" are the correct abbreviations.

Reference

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doi:10.1158/1055-9965.EPI-09-0388