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Estrogen levels in serum and urine of vegetarian and omnivore premenopausal women

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Abstract

Objective—Based on the hypothesis that high-meat diets may increase breast cancer risk through hormonal pathways, this analysis compared estrogens in serum and urine by meat-eating status.

Design—Intervention with repeated measures.

Setting—Two randomized soy trials (BEAN1 and BEAN2) among premenopausal healthy women.

Subjects—BEAN1 participants completed 7 unannounced 24-hour dietary recalls and donated 5 blood and urine samples over 2 years. BEAN2 women provided 7 recalls and 3 samples over 13 months. Serum samples were analyzed for estrone (E_1) and estradiol (E_2) using radioimmunoassays. Nine estrogen metabolites were measured in urine by liquid chromatography mass spectrometry. Semi-vegetarians included women who reported <30 g/day of red meat, poultry, and fish and pescatarians who consumed <20 g/day of meat/poultry but >10 g/day of fish. All other women were classified as non-vegetarians. We applied mixed models to compute least-square means by vegetarian status adjusted for potential confounders.

Results—The mean age of the 272 participants was 41.9±4.5 years. Serum E_1 (85 vs 100 pg/mL, p=0.04) and E_2 (140 vs 154 pg/mL, p=0.04) levels were lower in the 37 semi-vegetarians than in the 235 non-vegetarians. The sum of the 9 urinary estrogen metabolites (183 vs 200 pmol/mg creatinine, p=0.27) and the proportions of individual estrogens and pathways did not differ by meat-eating status. Restricting the models to the samples collected during the luteal phase strengthened the associations.

Conclusions—Given the limitations of this study, the lower levels of serum estrogens in semi-vegetarians than non-vegetarians need confirmation in larger populations.

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Conflicts of interest

None of the authors has a conflict of interest to declare.

Authors' contributions

G.M. and A.A.F. designed the studies and obtained funding. B.E.H. and G.M. planned the analyses. B.E.H., Y.M. and F.B. performed the statistical programming. B.E.H. and F.B. drafted the paper and prepared the tables. B.E.H. and Y.M. conducted the literature review. A.A.F. and F.Z.S. provided laboratory results and interpreted the results. All authors provided feedback on the initial draft of the manuscript and approved the final version.

Keywords

Breast cancer risk; estrogens; vegetarian; meat intake; repeated measures

Introduction

As our understanding of breast cancer risk factors increases, so does interest in the influence of lifestyle factors. Nutrients, foods, and dietary patterns have been explored in efforts to determine what dietary recommendations can be made to reduce breast cancer risk[1]. Several meta-analyses suggested a possible association between meat intake and breast cancer risk despite inconsistent results[2–4]. One way dietary components may influence breast cancer risk is through hormonal pathways. For example, dietary fiber[5] and dairy foods[6] were shown to influence endogenous sex hormone levels. More evidence that a low-meat diet may affect steroid hormones comes from dietary pattern studies. In the Nurses' Health Study[7], a better Alternative Healthy Eating Index score was associated with lower plasma levels of estradiol (E_2), and estrogen levels were higher among women with a food pattern high in meat[8]. In a study of postmenopausal women[9], vegetarians had lower plasma levels of estrogens than omnivores. Another comparison of vegetarians and omnivores found lower circulating free E_2 and testosterone in vegetarians even after controlling for body weight[10]. In a randomized dietary trial, women on a Mediterranean diet characterized by high vegetable intake, a significant decrease in total estrogens was observed[11].

To date, most studies of diet and estrogen metabolism were conducted in postmenopausal women due to fluctuations during the menstrual cycle that challenge steroid hormone assessment in premenopausal women. However, one study reported few differences in hormonal and dietary profiles by menopausal status[10]. The role of endogenous estrogens in breast cancer etiology among premenopausal women is less understood than in postmenopausal women; testosterone and progesterone appear to play an important role[12–14]. For premenopausal women within the Nurses' Health Study, higher urinary estrone (E_1) and E_2 levels were associated with a significant 50% lower risk suggesting that a higher urinary excretion of parent estrogens may be protective[15]. Differences in the major metabolic pathways for estrogens, i.e., the more carcinogenic 4-hydroxy (OH) and 16α-OH metabolites and the less harmful 2-OH metabolites, may also contribute to breast cancer risk[16].

To better understand the relation between diet, especially those with low versus high meat intake, and estrogen among premenopausal women, we combined data from two previous studies that collected multiple serum and urine samples[17,18] and compared serum E_1 and E_2 and 9 urinary estrogen metabolites from 272 premenopausal women stratified by meat intake. Neither of the two interventions detected an effect of soy on serum estrogen levels[17,18]; a small change in urinary estrogens was seen in BEAN2[19] but not when both studies were analyzed together[20].

Methods

Study Population

The original Breast, Estrogen, and Nutrition (BEAN 1) study, conducted in 2000–2003, randomized 220 women to intervention and control groups; 190 women contributed at least 4 samples to the present analysis[17]. The BEAN2 trial was conducted in 2007–2010 as a cross-over study with 6 months on a high and a low soy diet each separated by a 1-month washout period[21]. Of the 96 randomized women, 82 participants completed both diet periods. Eligibility criteria for both studies included a normal mammogram, no breast implants, no oral contraceptives, not pregnant, no previous cancer diagnosis, intact uterus and ovaries, regular menstrual periods, and low soy intake. For BEAN2, the participants also had to produce at least 10 μL nipple aspirate fluid (NAF), one of the study outcomes[21]. The same dietary intervention protocol was used in both studies; the high soy diet consisted of 2 servings of soy foods providing approximately 50 mg of isoflavones per day. During the low soy diet, participants continued their regular diet and were counseled to minimize soy intake. The protocols of both studies were approved by the University of Hawaii Committee on Human Studies and by the Institutional Review Boards of the participating clinics. All women signed an informed consent form before entry into the trial and gave written permission to use frozen samples for future analyses. A Data Safety Monitoring Committee reviewed the progress of the studies, reasons for dropouts, and any reported symptoms annually.

Data Collection

All participants completed a baseline food frequency questionnaire (FFQ) validated for a multiethnic population[22]; in a calibration study, the correlations between FFQ and 24-hour recall data were 0.57–0.74 for nutrient densities. The questionnaire also included information on demographic characteristics, anthropometric measures, and reproductive health. To assess adherence to the study protocol, all participants completed 7 unannounced 24-hour dietary recalls. In BEAN1, all recalls during the 2-year period were conducted by telephone[17], whereas in BEAN2, trained staff collected the first recall in person during the screening visit and 3 recalls by telephone during each diet period[21]. The 24-hour recalls were scheduled randomly in intervals of a few weeks or months, used standardized protocols, standard probes, and a 3-pass method to obtain a detailed account of all foods and beverages consumed during the previous day. The dietitian inquired about preparation methods and additions and probed about easily forgotten foods. Both weekdays and weekend days were captured. Recalls were conducted at multiple points during the study years to reflect seasonal variation in food selection. The FFQs and the 24-hour recalls were analyzed utilizing the Food Composition Table maintained by the Nutrition Support Shared Resource at our Center[23]; the databases represent an extensive list of local foods consumed by the various ethnic populations of Hawaii and the Pacific.

Estrogens in Blood and Urine

Collection of serum and urine samples was attempted to occur during the midluteal phase (3–11 days before the next menstruation). However, due to scheduling problems, 14% and 24% of visits in BEAN1 and BEAN2, respectively, occurred outside the luteal phase. Based

on the available information, it was not possible to estimate the exact cycle day. In BEAN1, timing was determined using ovulation kits and confirmed retrospectively by serum progesterone levels[17], while in BEAN2 the cycle day was estimated based on the last menstruation date and confirmed by the onset date of the next menstruation obtained via telephone contact with participants[21]. All specimens were stored at −80°C after aliquoting. Using validated radioimmunoassays, 5 repeated serum samples for BEAN1 and 3 samples for BEAN2 were analyzed for E_1 and E_2 in 0.5 mL serum[24]. Based on blinded samples, the interassay coefficients of variations (CV) were 17.7% for E_1 and 11.2% for E_2 in BEAN1 and 15.0% for both E_1 and E_2 in BEAN2[17,21].

In both studies, repeated overnight urine samples were collected in containers with added ascorbic and boric acid to control bacterial growth[20]. For BEAN1, the baseline and the final samples (24 months) were analyzed for 173 women after 7–10 years of storage. For the 79 BEAN2 participants, 3 samples (baseline, end of low soy, and end of high soy diet at 6 or 13 months) were analyzed after 0–3 years of storage. The samples were divided into 3 sets and analyzed during 2010. Consistency across rounds was checked by including external urines. The predominant steroidal estrogens in premenopausal women[25], namely E_1, E_2, 2-OHE_1, 2-OHE_2, 2-$MeOE_1$, 4-OHE_1, E_3, 16keto-E_2, 16α-OHE_1, were measured by liquid chromatography mass spectrometry (LCMS) (model Exactive, Thermo Fisher Scientific, Waltham, MA) using 5 labeled internal standards as described previously[26]. Six less common metabolites that constituted 6.5% of all metabolites in an analysis among premenopausal women were not assessed[25]. Ascorbic acid was added during hydrolysis and derivatization to prevent artificial oxidation of sensitive analytes. This urine pool from premenopausal women repeated on 9 different days revealed CVs of 4–21% depending on the analyte concentrations. Urinary creatinine concentrations were measured using a Roche-Cobas MiraPlus clinical chemistry autoanalyzer (Roche Diagnostics, Switzerland). Urinary isoflavonoids as a biomarker for soy intake were assessed by high-pressure liquid chromatography in BEAN1[17] and by LCMS in BEAN2[21]. All urinary measurements were expressed per mg creatinine to adjust for urine volume.

Statistical Analysis

The SAS statistical software package version 9.2 (SAS Institute Inc., Cary, NC) was used for the statistical analysis. Based on the literature[27] and on mean values for the seven 24-hour recalls, we defined vegetarians as women who ate less than 30 g/day in the combined categories of red meat, poultry, and fish, and pescatarians as those consuming less than 20 g/day of red meat and poultry but greater than 10 g/day of fish. All other women were considered non-vegetarians. Because of the small numbers, the 18 vegetarians and 19 pescatarians were combined into one group of semi-vegetarians for statistical analysis. We calculated the sum of the 9 urinary metabolites measured in both studies (total urinary estrogens), the relative percentages for the 3 metabolic pathways (2,4, and 16α-OH) based on molar concentrations, and the 2/16α-OHE_1 ratio. We applied mixed models to incorporate the covariance structure of the repeated measures within subjects and to allow for the varied lengths and sample collection times of the two studies and the inclusion of women with partially-missing data to compute least-square means adjusted for age, body mass index (BMI), ethnicity, total energy intake, parity, study status (BEAN1 or BEAN2),

dietary assignment (low vs. high soy), time of sample collection (study month), and menstrual cycle phase (within or outside the luteal phase). With one exception (2-OH pathway), serum and urinary measures were log-transformed due to non-normal distributions prior to assessing the significance of the difference between groups. Because soy food consumption did not significantly modify estrogen levels in serum[17,18] or urine[20], we included all time points into the current analysis. However, we performed sensitivity analyses to examine the same models after excluding samples collected during the high soy period and samples not taken during the luteal period.

Results

The ethnic distribution of the 272 participants, aged 41.9±4.5 years at randomization, was 41% White (N=112), 36% Asian (N=98), primarily Japanese, and 23% Other (N=62), primarily Native Hawaiian (Table 1). Differences in dietary intake between BEAN1 and BEAN2 participants were seen at baseline and persisted throughout the study period. Using dietary recall data, nearly 14% of participants (N=37) were categorized as semi-vegetarians consisting of 60% Whites (N=22), 32% Asians (N=12), and 8% other ethnic groups (N=3). The two groups were similar in age (p=0.78), but semi-vegetarians had a lower BMI (23.9±5.1 vs 26.3±5.3 kg/m^2; p=0.01) and non-significantly lower urinary creatinine (868±372 vs. 1140±152 mg/L; p=0.50) than non-vegetarians.

Mean intake levels according to the dietary recalls during the study period were in agreement with most FFQ-based values (Table 2). Both the FFQ analysis and the recalls showed that semi-vegetarians consumed less meat, poultry, and fish than non-vegetarians; these differences were significant except for fish according to the recalls. According to both assessments, semi-vegetarians consumed more vegetables, whole grains, and dietary fiber than non-vegetarians although not all differences were statistically significant. At the same time, semi-vegetarians reported lower total energy intakes according to both methods and lower total grain intake based on the FFQ values. Adjustment for BMI attenuated the differences in total energy intake but did not eliminate them. Intakes of dairy and fruit did not differ much by dietary group.

When E_1 and E_2 levels were compared by meat-eating status, serum but not urinary levels were significantly lower in semi-vegetarians than non-vegetarians (Table 3). Whereas the respective differences for serum E_1 and E_2 were 85 vs 100 pg/mL and 140 vs 154 pg/mL (p=0.04 for both), the urinary E_1 and E_2 values were similar in both groups. The small difference in total urinary estrogens (183 vs 200 pmol/mg creatinine) was not statistically significant (p=0.27), nor were any differences for the other metabolites or the 2/16α-OHE1 ratio (10.9 vs 11.6; p=0.36) observed. Repeating the analyses for urinary E_1, E_2, and E_3 using absolute values instead of percentages did also not reveal any differences by dietary pattern (data not shown). Dividing non-vegetarians into low- and high meat consumers did not indicate any trend with increasing meat intake (data not shown).

When the analysis was restricted to the 943 serum or 461 urine samples collected during the luteal phase of the menstrual cycle, the associations by dietary pattern were strengthened. The differences between non-vegetarians and semi-vegetarians were greater for serum E_1

(78 vs 95 pg/mL; p=0.003), serum E_2 (126 vs 146 pg/mL; p=0.003), total urinary estrogens (171 vs 193 pmol/mg; p=0.15), and urinary E_2 (7.0 vs 8.1%; p=0.09). Similarly, the differences between semi-vegetarians and non-vegetarians persisted after excluding all samples collected during the high soy diet period and limiting the analysis to the remaining 703 serum or 411 urine specimens; the respective values for serum E_1 were (82 vs 100 pg/mL; p=0.02), for serum E_2 (131 vs 156 pg/mL; p=0.003), and for total urinary estrogens (187 vs 201 pmol/mg; p=0.21). The results for individual urinary estrogens, metabolic pathways, and the 2/16α-OH ratio remained non-significant in these sensitivity analyses.

Discussion

In this comparison among premenopausal women, those with minimal meat intake (vegetarians and pescatarians) had lower levels of circulating E_1 and E_2 than non-vegetarians, but no significant differences in the amount or the relative proportion of urinary estrogen metabolites were seen. The fact that limiting the analysis to luteal samples strengthened the associations for serum estrogen levels confirms the importance of controlling for timing within the menstrual cycle when studying sex hormones among premenopausal women. The dietary analysis indicated that the semi-vegetarian group consumed less food and total energy than non-vegetarians with higher intakes of whole grains, fiber, and vegetables. These findings agree with other reports showing that vegetarians consume less saturated fat by replacing animal-based foods with lower fat and energy-dense plant-based foods and tend to weigh 3%–20% less[28].

To date, the research on meat intake and breast cancer risk has been inconsistent[2–4]. Several meta-analyses reported relatively weak associations primarily from case-control studies; however, hormone receptor status and timing of meat consumption in early versus later life were suggested as areas that need additional research[4,29]. Dietary patterns and their assessment of overall intake versus a single food or nutrient appear to be a promising avenue for gaining new insight into the relation between high meat intake and breast cancer risk[7–11]. These studies indicate that patterns high in meat and low in plant-based foods may be associated with higher circulating sex steroid levels in women, but other studies reported little difference in estrogen levels in relation to meat consumption[6,30]. Given the difficulties of assessing dietary intake and hormone status accurately, observational studies may not be able to provide a conclusive answer whether diet affect sex steroid levels. It may take dietary trials, such as the Mediterranean diet intervention described above[11], to further explore this question.

The current analysis was limited by the relatively low proportion of women who maintained a vegetarian or pescatarian diet. As in all studies among premenopausal women, fluctuations of hormone levels during the menstrual cycle challenge the interpretation of hormonal measurements. However, standardization of specimen collection during the luteal phase and the ability to exclude values not considered luteal allowed us to control for cyclical variations in hormone levels. It is also well known that estrogen levels in serum do not necessarily reflect concentrations in the breast. Confounding by physical activity[31] and other lifestyle factors may have affected our results, but physical activity was not assessed in our studies. The major strength of this analysis is the repeated measurement design, both for

24-hour dietary recalls and for hormonal measures. This approach allowed us to capture long-term dietary behavior and hormonal exposure better than a single measurement at one point in time. As indicated by the lack of an effect of the soy diet on hormonal outcomes[17,18,20] and the results of the sensitivity analysis, the intervention design did not affect our findings. Although the classification of dietary status was based on intake as estimated by 24-hour dietary recalls and not self-defined eating patterns, past research indicates that even self-defined vegetarians eat some meat; one study reported that 2 out of 3 self-defined vegetarians consumed some meat and often more than 10 g/day[27]. The agreement between food intake assessed by 24-hour recalls and FFQs provides additional validity to our classification despite the well documented shortcomings of both methods; we found consistent differences in reported dietary patterns of non-vegetarians vs vegetarians/pescatarians. Small differences were to be expected because the baseline FFQ and the 24-hour recalls covered different time periods and because some change in food consumption occurred as a result of the dietary intervention.

Our findings suggest that semi-vegetarians have lower serum estrogen levels than non-vegetarians and agree with current dietary recommendations for cancer prevention published by the American Institute for Cancer Research, “To choose mostly plant foods, limit red meat, and avoid processed meat[1].” Given that an estimated 30%–35% of all cancers may be due to dietary factors[28], such advice may have a strong potential for cancer-preventive effects. However, considering the inconsistent literature related to meat consumption as a risk factor for breast cancer, the relatively small number of participants in the semi-vegetarian group, the null findings for urinary estrogen concentrations, and the wide variability of estrogen values, this study has to be interpreted with caution. Future investigations need to look at a larger population of premenopausal women who maintain a vegetarian or pescatarian diet and donated specimens at well-defined times during the menstrual cycle and/or conduct randomized dietary modification trials.

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Abbreviations

BEAN1	Breast, Estrogen, And Nutrition study 1
BEAN2	Breast, Estrogen, And Nutrition study 2
CV	Coefficient of variation
E_1	Estrone
E_2	Estradiol
E_3	Estriol
FFQ	Food frequency questionnaire
LCMS	Liquid chromatography mass spectrometry

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Table 1

Characteristics of BEAN1 and BEAN2 participants at baseline (N [%] or mean±SD)[1]

Characteristic		BEAN1 (N=190)	BEAN2 (N=82)	All (N=272)
Ethnicity, *N (%)*	*White*	70 (37%)	42 (51%)	112 (41)
	Asian	76 (40%)	22 (27%)	98 (36)
	Other	44 (23%)	18 (22%)	62 (23)
Age, *years*		43.1±2.9	39.2±6.1	41.9±4.5
Parous, *N (%)*		139 (73)	59 (72)	198 (73)
First live birth <30, *N (%)*		89(47)	30 (37)	119 (44)
Body mass index, kg/m^2		26.1±5.7	25.7±5.1	26.0±5.6
Vegetarian, *N (%)*		30 (16)	7 (9)	37 (14)
Total energy intake, *kJ/day*		7623±3314	8786±4515	7975±3745
Red meat intake, *servings/day*		1.2±0.9	1.5±1.3	1.3±1.1
Poultry intake, *servings/day*		1.3±1.2	1.4±1.4	1.3±1.3
Fish intake, *servings/day*		0.7±0.7	0.9±1.0	0.8±0.8
Dairy intake, *servings/day*		1.5±1.0	1.5±1.1	1.5±1.0
Vegetable intake, *servings/day*		2.7±1.6	4.7±3.2	3.3±2.4
Fruit intake, *servings/day*		1.2±1.0	2.4±2.2	1.5±1.6
Total grain intake, *servings/day*		6.6±3.5	7.6±4.3	6.9±3.8
Whole grain intake, *servings/day*		1.7±1.2	2.3±2.0	1.9±1.5
Dietary fiber intake, *g/day*		18.3±9.5	24.1±14.8	20.1±11.6
Isoflavone intake, *mg/day*[1]		4.7±6.3	21.2±39.7	9.7±23.6
Urinary isoflavonoids, *nmol/mg creatinine*		6.7±16.9	5.0±9.5	6.2±15.0
Ever equol producer, *N (%)*[2]		21 (11)	29 (36)	50 (18)
Serum estrone (E_1), *pg/mL*		94±53	106±52	97±53
Serum estradiol (E_2), *pg/mL*		144±77	152±90	147±81
Total urinary estrogens[3], *ng/mg creatinine*		188±113	197±147	190±124
Urinary 2:16α-OH E_1 ratio		10.5±12.6	8.2±10.0	9.8±11.9

[1] Dietary intakes are estimated from a 1-year food frequency questionnaire; isoflavone intake is estimated from a 24-hr recall.

[2] Equol producer status is based on detecting urinary daidzein excretion of ≥2 nmol/mg and urinary equol to daidzein ratio of ≥0.018 in at least one of the urine samples collected throughout the study.

[3] Sum of E_1, E_2, 2-OHE_1, 2-OHE_2, 2-$MeOE_1$, 4-OHE_1, E_3, 16keto-E_2, and 16α-OHE_1

Table 2

Dietary intakes among 272 study participants

Characteristic	Diet from food frequency questionnaire			Diet from 24-hour dietary recalls[2]		
	Semi-vegetarians	Non-vegetarians	P-value[1]	Semi-vegetarians	Non-vegetarians	P-value[1]
Total energy intake, *kJ/day*	6732±2778	8171±3845	0.01	6493±1469	7122±1590	0.01
Red meat intake[3], *servings/day*	0.5±0.5	1.4±1.1	<0.0001	0.1±0.2	1.3±1.0	<0.0001
Poultry intake[3], *servings/day*	0.7±1.0	1.5±1.3	<0.0001	0.2±0.2	1.4±1.0	<0.0001
Fish intake[3], *servings/day*	0.5±0.4	0.8±0.8	<0.0001	0.6±0.7	0.7±0.7	0.58
Dairy intake, *servings/day*	1.5±1.2	1.5±1.0	0.83	1.0±0.8	0.9±0.7	0.46
Vegetable intake, *servings/day*	3.8±2.3	3.2±2.4	0.18	3.2±1.6	2.4±1.1	<0.0001
Fruit intake, *servings/day*	1.5±1.0	1.5±1.6	0.76	2.0±1.5	1.4±0.9	0.001
Total grain intake, *servings/day*	6.0±2.5	7.0±4.0	0.04	5.5±2.0	5.5±1.6	0.96
Whole grain intake, *servings/day*	2.1±1.3	1.8±1.6	0.15	1.8±1.3	1.1±0.8	<0.0001
Dietary fiber intake, *g/day*	22.1±9.3	19.8±12.0	0.18	20.4±6.9	15.6±5.0	<0.0001

[1] Obtained from Student t-tests

[2] Mean of 7 unannounced 24-hour dietary recalls over 2 years (BEAN1) and 13 months (BEAN2)

[3] 1 serving ≈ 30 g

Table 3

Serum and urinary estrogen levels by meat-eating status

Analyte	Semi-vegetarians	Non-vegetarians	All samples	Luteal phase only	Low soy only
	Means ± standard error[1]		p-values[2]		
Number of serum samples[3]	*163*	*963*	*1126*	*943*	*703*
Serum E_1, *pg/mL*	85±8	100±3	0.04	0.003	0.02
Serum E_2, *pg/mL*	140±12	154±5	0.04	0.003	0.003
Number of urine samples[3]	*73*	*495*	*568*	*461*	*411*
Total urinary estrogens[3], *pmol/mg creatinine*	183±21	200±9	0.27	0.15	0.21
Estrone (E_1), %	22.0±1.1	21.9±0.5	0.62	0.75	0.97
Estradiol (E_2), %	7.6±0.6	8.2±0.3	0.34	0.09	0.09
Estriol (E_3), %	23.4±2.5	26.2±1.1	0.63	0.50	0.51
2-OH pathway (2-OHE_1, 2-OHE_2, 2-$MeOE_1$), %	35.8±2.7	34.6±1.2	0.64	0.43	0.68
4-OH pathway (4-OHE_1), %	4.3±0.5	3.9±0.2	0.97	0.99	0.60
16α-OH pathway (16keto-E_2, 16α-OHE_1), %	33.6±3.2	36.7±1.4	0.67	0.57	0.60
2/16α-OHE_1 ratio	10.9±3.5	11.6±1.5	0.36	0.48	0.39

[1] Results are least-square means ± standard error obtained from mixed models and adjusted for ethnicity, body mass index, age, total energy intake, parity, study month, luteal phase (yes or no), study (BEAN1 or BEAN2), dietary assignment (low or high soy)

[2] p-values were computed on log-transformed variables except for the 2-OH pathway

[3] Number of samples analyzed differ between serum and urine because more serum samples were measured

[4] Sum of E_1, E_2, 2-OHE_1, 2-OHE_2, 2-$MeOE_1$, 4-OHE_1, E_3, 16keto-E_2, and 16α-OHE_1