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Urinary Estrogen Metabolites in 2 Soy Trials with Premenopausal Women

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Abstract

Background—Soy consumption may protect against breast cancer through modification of estrogen metabolism.

Objective—We examined the effect of soy foods on urinary estrogens and the 2-hydroxy (OH)/16 α -OH estrone (E₁) ratio in 2 dietary interventions with premenopausal women.

Methods—BEAN1 was a 2-year randomized trial and BEAN2 a 13-month randomized crossover study. In both interventions, study participants consumed a high-soy diet with 2 soy food servings/day and a low-soy diet with <3 servings of soy/week. Urine samples were collected at baseline and at the end of the diet periods, analyzed for 9 estrogen metabolites by liquid chromatography mass spectrometry, and adjusted for creatinine levels. For BEAN1, 2 samples for 188 participants and for BEAN2, 3 samples for 79 women were analyzed. We applied mixed-effects regression models with log-transformed values of estrogen metabolites and soy intake as the exposure variable.

Results—In BEAN1, no effect of the high-soy diet on individual estrogen metabolites or hydroxylation pathways was observed. The median 2-OH/16 α -OH E₁ ratio decreased non-significantly in the intervention group from 6.2 to 5.2 as compared to 6.8 and 7.2 in the control group (p=0.63). In BEAN2, only 4-OHE₁ was significantly lower after the high-soy diet. Interaction terms of the high-soy diet with equol producer status, ethnicity, and weight status revealed no significant effect modification.

Conclusions—Contrary to our hypothesis and some previous reports, the results from 2 well controlled dietary interventions do not support an effect of a high-soy diet on a panel of urinary estrogen metabolites and the 2-OH/16 α -OHE₁ ratio.

Keywords

Soy foods; estrogen metabolites; isoflavones; breast cancer risk; dietary intervention; premenopausal women

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Conflict of Interest None of the authors has any conflicts of interest.

Introduction

Soy beans and isoflavones have been investigated as cancer protective agents for a long time; a meta-analysis described a 15% lower breast cancer risk associated with soy intake, but the association is primarily found among women of Asian background.¹ Although the estrogen-like isoflavones appear to exert, depending on the hormonal milieu, estrogenic or antiestrogenic effects through competitive binding to the estrogen receptors (ER),² there is little evidence for an effect of soy foods on circulating estrone (E₁) and estradiol (E₂) levels.³ The protective action of soy isoflavones against breast cancer may be due to the modulation of cytochrome P450 enzymes, including CYP1A1, CYP1A2, CYP1B1, and the 3A family, which play a role in estrogen hydroxylation and person-to-person differences in estrogen action.⁴ Three major competing pathways result in the more carcinogenic 4-hydroxy (OH) and 16 α -OH metabolites and the less harmful 2-OH metabolites.⁵ Thus, women who metabolize a larger proportion of endogenous estrogens as 16 α -OHE₁ and have a lower 2-OH/16 α -OHE₁ ratio may be at greater breast cancer risk,⁶ but a recent report from the Nurses' Health Study did not detect a lower breast cancer risk with a higher 2/16 α -OH metabolite ratio.⁷ An influence of isoflavones on several P450 enzymes has been shown in experimental settings^{8;9} and a number of intervention studies in women suggested beneficial changes in the 2-OH/16 α -OHE₁ ratio after administration of soy foods or supplements.¹⁰⁻¹² Whereas previous reports relied on ELISA assays and on gas chromatography mass spectrometry (GCMS) to assess 2-OHE₁ and 16 α -OHE₁, new liquid chromatography mass spectrometry (LCMS) methods allows for the detection of multiple estrogen metabolites in a faster, less expensive and more efficient manner.¹³⁻¹⁵ Based on the hypothesis that soy intake may result in a decrease of the more carcinogenic 4-OH and 16 α -OH metabolites and an increase in the 2-OH pathway, we explored the effect of 2 daily servings of soy on the formation of urinary, 2, 4, and 16 α -OH estrogen metabolites in 2 randomized soy trials with premenopausal women. One consisted of 93 intervention and 95 control participants¹⁶ and the other investigation used a cross-over design among 79 women.^{17;18}

Methods

Study design and recruitment

The first Breast, Estrogens, And Nutrition (BEAN1) study was designed as a randomized clinical trial to examine the effects of 2 daily soy servings on sex steroid hormones and mammographic densities.^{16;19} Overall, 10,022 women with normal mammograms were contacted, 975 (9.73%) replied, and 352 were found eligible. Women were excluded from the study due to pregnancy or breast-feeding, consumption of estrogen-containing oral contraceptives or supplements containing isoflavones, cancer diagnosis, breast implants, hysterectomy, lack of a regular menstrual period, or intake of >5 soy servings per week. After a run-in period, 220 women were randomized to a soy diet or to the control group and 189 subjects completed 2 years of intervention. The number of dropouts did not differ by group; 17 (15.6%) women in the intervention group and 14 (12.6%) controls dropped out of the study prematurely (p=0.53).

BEAN2 used a crossover design; 82 participants completed a 6-month high-soy diet and a 6-month low-soy diet, which were separated by a 1-month washout period. The participants

were recruited through multiple sources, as described elsewhere.¹⁷ After mailing 16,306 invitations, 825 interested women replied, 310 women were screened, 96 women were randomized, and 14 women dropped out of the study. The exclusion criteria were the same as in BEAN1 except for the mammogram requirement.

In both studies, all subjects completed a baseline questionnaire asking for demographic, anthropometric, reproductive, and dietary information. The protocols of the two studies were approved by the University of Hawaii Committee on Human Studies and by the institutional review boards of the participating hospitals. 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. During the high-soy diet, women consumed 2 daily servings of soy containing approximately 25 mg aglycone equivalents of isoflavones per serving. Participants chose between tofu, soy milk, roasted soy nuts, soy bars, and soy protein powder. Dietitians provided dietary counseling on how to replace common dishes with soy foods. During the low-soy diet, the women were instructed to maintain their regular diet and to consume <3 soy food servings per week. Adherence to the study protocol as assessed by unannounced 24-hour dietary recalls and urinary isoflavonoid excretion was high in both studies.^{16;17}

Urine collection and analysis

In both studies, repeated overnight urine samples were collected during the luteal phase, aliquoted into 2 mL containers, and stored at -80°C. Ascorbic and boric acid were added to the urine collection containers to control bacterial growth. For the BEAN1 study, the baseline and the final samples were analyzed for 188 women after 8-11 years of storage. If no sample was available at month 24, the month 12 sample was used instead. For the 79 BEAN2 participants, 3 samples (baseline and at the end of low-soy [month 6] and high-soy [month 13] diets) were analyzed after 1-4 years of storage. For one woman, only months 0 and 13 samples were available for this analysis.

In both studies, the most predominant steroidal estrogens in premenopausal women,¹⁴ namely E₁, E₂, 2-OHE₁, 2-OHE₂, 2-MeOE₁, 4-OHE₁, E₃, 16keto-E₂, 16α-OHE₁ were measured by LCMS (model Exactive, Thermo Fisher Scientific, Waltham, MA) using 5 labeled internal standards as described in detail previously.¹⁵ Ascorbic acid was added during hydrolysis and during derivatization to prevent artificial oxidation of sensitive analytes.¹⁵ Analysis of an external urine pool from premenopausal women repeated on 9 different days revealed coefficients of variation of 4-21% depending on the analyte concentrations. Urinary creatinine concentrations were measured using a Roche-Cobas MiraPlus clinical chemistry autoanalyzer (Roche Diagnostics, Switzerland). All estrogen and isoflavonoid measurements were expressed per mg creatinine to adjust for urine volume.

Urinary isoflavonoids as a biomarker for soy intake were measured previously by high-pressure liquid chromatography in BEAN1¹⁶ and by LCMS in BEAN2.¹⁷ The isoflavonoid equol was assessed by LCMS in both studies, but in BEAN1, it was measured at the same time as the estrogen metabolites in 2 urine samples only, whereas in BEAN2 equol was measured together with genistein and daidzein in 8 urine samples per woman. Since equol

producers are thought to experience more protective effects of isoflavones than non-producers,²⁰ equol producer status was determined based on 2 criteria: urinary daidzein excretion ≥ 2 nmol/mg creatinine and a urinary equol to daidzein ratio ≥ 0.018 .^{21;22} In BEAN1, 23 women met the criteria at least once and were considered equol producers: 7 were of Asian ethnicity and 16 were non-Asian. In BEAN2, 41 women were equol producers, of which 10 were of Asian ethnicity.

Statistical analysis

The SAS statistical software package version 9.2 (SAS Institute Inc., Cary, NC) was used for the statistical analysis. We calculated the sum of the 9 urinary metabolites measured in both studies and the relative percentages for the 3 pathways (2, 4, and 16 α) based on molar concentrations. The 2/16 α -OHE₁ ratio was computed as the ratio of the 2 metabolites. Urinary estrogen metabolite variables were log-transformed due to non-normal distributions. Student's t-tests of the log-transformed values and chi-square tests were performed to assess differences in the baseline values of urinary estrogen metabolites and demographic characteristics between randomization groups. To examine the effect of the high-soy diet on urinary estrogen metabolites, we applied mixed-effects regression models with log-transformed values of estrogen metabolites;²³ the models also included the randomization group and the time of urine collection (baseline vs. end of diet period).

Results

For BEAN1, 188 participants were included in the analysis (Table 1); 95 women were in the control group and 93 women in the intervention group. In both groups, nearly half of the women were Asians. At baseline, the 2 groups did not differ in age, body mass index, dietary isoflavone intake, urinary isoflavonoid excretion, total estrogen metabolites, and the 2/16 α -OHE₁ ratio. The number of equol producers was low in both groups (7 in the control and 17 in the intervention group; $p=0.29$). The median total estrogen metabolite levels were 53.9 and 54.8 ng/mg creatinine for the control and the intervention group, while the median 2/16 α -OHE₁ ratios were 6.8 and 6.2. BEAN2 included 79 participants (Table 1); 40 women were in group A and 39 women in group B. Women in group B were significantly younger than women in group A with mean age of 37.6 ± 5.8 versus 41.3 ± 5.6 ($p=0.01$). The mean urinary isoflavonoid excretion was lower in group B than in group A (3.0 ± 6.3 vs. 7.3 ± 11.3 ; $p=0.04$). Except for 4-OHE₁ ($p=0.03$), levels of estrogen metabolites (43.4 vs. 48.7 ng/mg creatinine) and the 2/16 α -OHE₁ ratio (3.8 vs. 5.3) were similar across randomization groups. In both studies, 2-OH metabolites constituted the largest proportion and were closely followed by 16-OH metabolites, but the 4-OH pathway constituted less than 10% (Figure 1).

In BEAN1, no significant effect of the high-soy diet on any estrogen metabolite was observed (Table 2); there was only a tendency for lower 4-OHE₁ ($p=0.06$). For example, the respective medians of total estrogen metabolites in the control versus the intervention group were 53.9 and 54.8 and 45.9 and 49.7 ng/mg creatinine at baseline and at the end of the study ($p=0.81$). The decrease over time in median total estrogen metabolites of 8 ng/mg creatinine for the controls and 5 ng/mg creatinine for the intervention group was not

statistically significant ($p=0.15$). For the $2/16\alpha\text{-OHE}_1$ ratio, the median was similar between the 2 groups at baseline (6.2 and 6.8), whereas at the end of the study the ratio decreased in the intervention but not in the control group (5.2 vs. 7.5). However, neither the dietary intervention effect ($p=0.63$) or its interaction with time were significant ($p=0.93$).

In BEAN2, the high-soy diet significantly affected 4-OHE_1 but not any other metabolite (Table 2); 4-OHE_1 was lower at the end of the high-soy diet than at baseline or at the end of the low-soy diet ($p<0.01$). The respective medians for total estrogen metabolite levels were 47.1, 55.8, and 53.2 ng/mg creatinine at baseline, low-soy and high-soy ($p=0.99$). For the $2/16\alpha\text{-OHE}_1$ ratio, the respective medians were 5.1, 4.5, and 4.4 ($p=0.24$). No significant time effects were found for total estrogen metabolites ($p=0.31$) and the $2/16\alpha\text{-OHE}_1$ ratio ($p=0.47$). Interaction terms of the dietary assignment with ethnicity, equol producer status, and overweight indicated no significant effect modification.

Grouping the metabolites by pathway confirmed the lack of an intervention effect on the relative proportions of 2, 4, and 16-OH metabolites in both studies (Figure 1); only the 4-OH pathway in BEAN2 reached statistical significance ($p=0.01$).

Discussion

Contrary to our hypothesis that soy may increase the $2/16\alpha\text{-OHE}_1$ ratio, the current analysis showed little effect of long-term, daily soy food consumption on urinary estrogen metabolite excretion. In both trials, estrogen metabolites, individually or grouped by pathway, and the $2/16\alpha\text{-OHE}_1$ ratio, did not change significantly. Only 4-OHE_1 , a metabolite with a very low concentration, decreased at the end of the high-soy diet in BEAN2, probably a chance finding given the multiple testing. In BEAN1, total estrogen metabolites decreased over time in both groups and the $2/16\alpha\text{-OHE}_1$ ratio was slightly lower at the end in the intervention than in the control group without reaching statistical significance. Furthermore, the data on equol producer status need to be interpreted with caution. Equol production was not adequately assessed in BEAN1, especially in the control group, because it was only measured in 2 urine samples and not all participants were actually exposed to isoflavones. As shown before, a soy challenge is needed to assess the ability to produce equol.²⁴

The current study conflicts with a cross-sectional study among 430 Asian American women that found no association of 15 estrogen metabolites assessed by LCMS with soy intake but reported a higher $2/16\alpha\text{-OHE}_1$ ratio among women with high soy intake.²⁵ The association was of similar size for pre- and postmenopausal women but only statistically significant in the entire population. Interventions among premenopausal women reported discrepant findings. An investigation with a soy beverage²⁶ and with an isoflavone supplement²⁷ detected no change in urinary estrogen metabolites and the $2/16\alpha\text{-OH}$ ratio. However, a GCMS-based crossover trial in 12 women consuming 10, 65, and 129 mg of isoflavones from soy protein powder for 3 months each described significantly lower $16\alpha\text{-OHE}_1$, 4-OHE_1 , and 4-OHE_2 and a higher $2/16\alpha\text{-OHE}_1$ ratio after supplement intake.¹⁰ In a similar investigation with 8 women, soy milk with high isoflavone content (113-202 mg/day) was associated with a higher urinary excretion of 2-OHE_1 and a higher $2/16\alpha\text{-OHE}_1$ ratio.¹¹ Our previous analysis using GCMS in 82 BEAN2 participants also found a higher $2/16\alpha\text{-$

OHE₁ ratio at the end of the high-soy diet (p=0.05), but the individual metabolites did not differ significantly.²⁸

Findings in postmenopausal women are of interest because the weak estrogenic effects of isoflavones are more likely to be physiologically important in women with low endogenous production,^{2,29} but conflicting results have also been reported. In a trial with >100 mg isoflavones from protein isolates, soy and seaweed plus soy significantly increased 2-OHE₁ and the 2/16 α -OHE₁ ratio,¹² but the 2/16 α -OHE₁ ratio only increased among women with detectable serum equol levels in another investigation.³⁰ No association between urinary isoflavonoid excretion and the 2/16 α -OH ratio was detected in a cross-sectional study³¹ and in 46 women consuming a soy flour or flaxseed muffin.³²

Possible explanations for the conflicting literature include the relatively long-term duration, the larger sample size, and the moderate isoflavone dosages in the BEAN studies as compared to short trials with high doses of isoflavones.¹⁰⁻¹² There is also a possibility that soy drinks as administered in some trials lead to higher isoflavone uptake than soy foods.³³ The discrepancy with the previously published BEAN2 results based on GCMS measurements of 2-OHE₁ and 16 α -OHE₁²⁸ may be due to analytical issues. As reported previously, 2-OHE₁ levels measured by LCMS were approximately twice as high as the GCMS results.¹⁵ This may be a result of different internal standards that were used for the 2 methods; the GCMS procedure included only one internal standard (5- α -Androstan-3- α) that was not well related to 2-OHE₁, whereas 5 labeled estrogen metabolites were used as internal standards in the LCMS assay.¹⁵ Nevertheless, using 4-OHE₂-d5 as internal standard for 2-OHE₁ in the LCMS assay was suboptimal, as most other methods are, for 2-OHE₁ determination and may have led to less than perfect values. For future studies, it may be advisable to use exact matches of internal standards for each measured estrogen metabolite. Alternatively, a loss of the relatively unstable analyte 2-OHE₁ might have occurred during derivatization in the GCMS procedure that did not include ascorbic acid for preservation.¹⁵

The current analysis had a number of limitations. The multiple testing for many different urinary estrogen measures may have resulted in false positive findings. The possible selection bias due to the strict participant eligibility criteria for the intervention studies likely limited the generalizability of the observed findings to all women. In BEAN2, a major weakness of the study is that the randomization did not lead to perfectly balanced groups as indicated by the baseline difference in age. On the other hand, this study had several strengths. The exposure to isoflavones by traditional soy foods represented soy intake in Asian countries more closely than the administration of oral supplements used in other studies. In our studies, urine collection was timed according to menstrual cycle and most samples were collected during the midluteal phase as confirmed by progesterone testing in BEAN1¹⁶ or subsequent menstruation dates in BEAN2.¹⁷ The repeated measures for urinary estrogens reduced concerns about intra-individual variability over time. The use of LCMS provided excellent assessments of multiple estrogen metabolites.^{13;15} Adherence to the dietary intervention was carefully monitored by 2 methods (dietary recalls and urinary isoflavonoid excretion) and remained high throughout the trial.¹⁷

In conclusion, no beneficial influence of a high-soy diet on the 2-OH/16 α -OHE₁ ratio and a panel of urinary estrogen metabolites was detected at the end of 2 well controlled dietary interventions among premenopausal women, but the analytic challenges of measuring these metabolites may have prevented the detection of an effect. Since the potential of isoflavones to modulate estrogen metabolizing enzymes is primarily based on limited evidence from experimental studies⁸ and on urinary excretion profiles in humans, this research area needs further elucidation.

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Abbreviations

E₁	Estrone
2-OHE₁	2-hydroxy estrone
4-OHE₁	4-hydroxy estrone
16α-OHE₁	16 α -hydroxy estrone
2-MeOE₁	2-methoxy estrone
E₂	Estradiol
2-OHE₂	2-hydroxy estradiol
16-keto E₂	16-keto estradiol
E₃	Estriol
GCMS	Gas chromatography mass spectrometry
LCMS	Liquid chromatography mass spectrometry

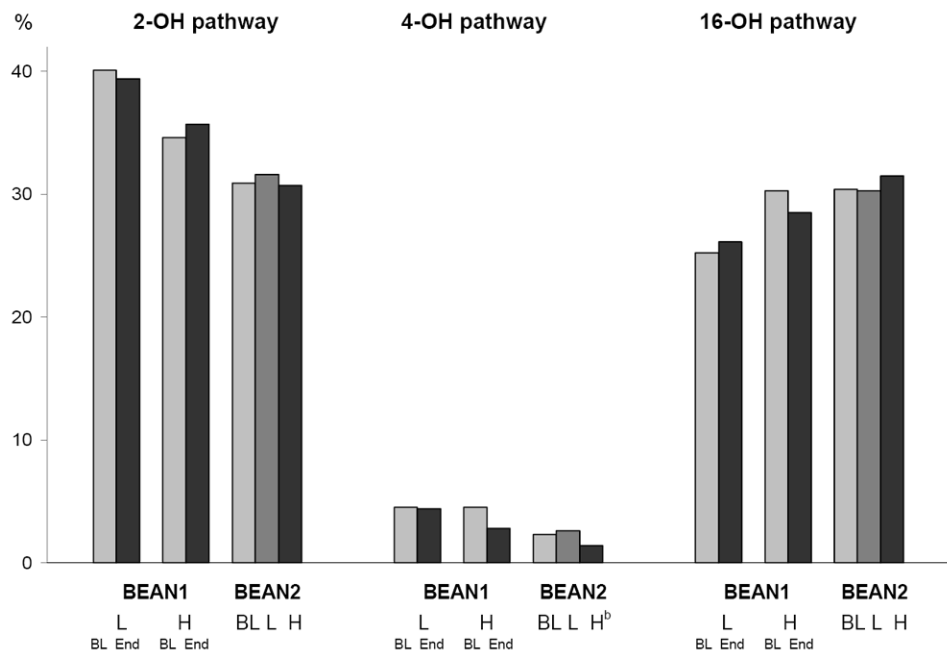


Figure 1.

Urinary estrogen metabolite excretion by pathway in BEAN1 and BEAN2^a

^aMedian percentages for each metabolite pathway were calculated after conversion to molar concentrations:

$$2\text{-OH pathway} = (2\text{-OHE}_1 + 2\text{-OHE}_2 + 2\text{-MeOE}_1) / \text{total estrogen metabolites}$$

$$4\text{-OH pathway} = (4\text{-OHE}_1) / \text{total estrogen metabolites}$$

$$16\text{-OH pathway} = (E_3 + 16\alpha\text{-OHE}_1 + 16\text{-ketoE}_2) / \text{total estrogen metabolites}$$

BL = baseline; End = 2 years;

L=control group or low-soy diet (month 6 or 13); H=intervention group or high-soy diet (month 6 or 13)

^bP value = 0.01 for the high-soy diet

Table 1

Baseline characteristics of BEAN1 and BEAN2 participants^a

	BEAN1			BEAN2		
	Control	Intervention	p value	Group A (high to low-soy)	Group B (low to high-soy)	p value
Number of participants	95	93		40	39	
Ethnicity	Non-Asian	57 (61)	0.86	29 (72)	29 (74)	0.85
	Asian	36 (39)		11 (28)	10 (26)	
Equol producer status ^b	No	66 (71)	0.29	17 (43)	21 (54)	0.31
	Yes	7 (7)		23 (57)	18 (46)	
Age (years)	42.8±2.9	43.2±2.8	0.40	41.3±5.6	37.6±5.8	0.01
Body mass index (kg/m ²)	26.0±6.1	26.2±5.6	0.82	25.8±5.2	26.0±6.1	0.83
Dietary isoflavone intake (mg/d)	5.0±7.5	4.2±4.8	0.38	16.3±38.8	21.7±37.3	0.53
Urinary isoflavonoids (nmol/mg creatinine)	5.5±15.1	8.4±18.6	0.24	7.3±11.3	3.0±6.3	0.04
Total estrogen metabolites (ng/mg creatinine)	53.9 (35.2, 80.8)	54.8 (35.3, 73.5)	0.53	43.4 (28.8, 61.7)	48.7 (33.8, 76.1)	0.21
Estrore (E ₁)	10.0 (6.8, 12.9)	10.3 (6.9, 14.5)	0.81	7.4 (5.4, 12.6)	9.2 (6.6, 15.7)	0.11
2-OHE ₁	13.8 (6.1, 23.3)	10.2 (4.0, 20.2)	0.14	9.6 (4.3, 21.6)	11.6 (5.3, 21.5)	0.78
4-OHE ₁	2.5 (1.5, 3.8)	2.1 (1.2, 3.7)	0.36	0.8 (0.5, 2.0)	1.3 (0.8, 2.2)	0.03
16α-OHE ₁	2.2 (1.0, 3.4)	1.9 (1.0, 3.2)	0.70	2.5 (1.3, 4.2)	2.2 (1.2, 3.4)	0.92
2-MeOE ₁	4.6 (2.3, 9.2)	4.6 (1.8, 7.7)	0.45	2.1 (0.9, 4.0)	2.6 (1.4, 4.5)	0.39
Estradiol (E ₂)	3.2 (2.5, 4.7)	3.9 (2.5, 5.2)	0.57	2.7 (2.0, 4.4)	3.3 (2.5, 5.1)	0.24
2-OHE ₂	0.9 (0.5, 2.4)	1.2 (0.3, 2.1)	0.99	1.2 (0.3, 2.9)	1.3 (0.4, 3.8)	0.73
16-keto E ₂	2.1 (1.1, 2.9)	2.1 (1.2, 2.7)	0.92	1.6 (1.1, 2.7)	2.0 (1.0, 2.6)	0.83
Estriol (E ₃)	9.7 (5.7, 15.9)	10.9 (7.1, 16.0)	0.64	9.9 (5.7, 15.1)	11.0 (6.6, 17.9)	0.53
2-OH pathway (%) ^c	40.1 (26.1, 50.9)	34.6 (25.2, 46.5)	0.09	31.2 (21.9, 45.8)	30.0 (19.8, 45.7)	0.50
4-OH pathway (%) ^d	4.5 (3.2, 6.2)	4.5 (2.9, 6.3)	0.77	2.2 (1.6, 3.0)	3.1 (1.7, 4.4)	0.05
16-OH pathway (%) ^e	25.2 (19.0, 36.3)	30.3 (19.9, 41.5)	0.32	32.3 (22.1, 46.9)	28.2 (19.9, 42.7)	0.80
2/16α-OHE ₁ ratio	6.8 (3.6, 12.4)	6.2 (2.1, 12.3)	0.60	3.8 (2.2, 8.5)	5.3 (1.8, 8.3)	0.74

^aData are shown as N (%) or mean ±std for all but the estrogen metabolites, which are shown as medians (Q1, Q3); P-values were calculated from χ^2 and Student's *t* tests; for the estrogen metabolites log transformed estrogen values were used. N=94 for total estrogen metabolites for the BEAN1 control group.

^bMissing if urinary daidzein excretion < 2 nmol/mg; yes if daidzein > 2 nmol/mg and equol/daidzein < 0.018; no if daidzein > 2 nmol/mg and equol/daidzein > 0.018.

$$\frac{(2\text{-OHE}_2 + 2\text{-OHE}_1 + 2\text{-MeOE}_1)/\text{total estrogen metabolites after conversion to molar concentrations}}{(4\text{-OHE}_1)/\text{total estrogen metabolites after conversion to molar concentrations}}$$
$$\frac{(E_3 + 16\alpha\text{-OHE}_1 + 16\text{-ketoE}_2)/\text{total estrogen metabolites after conversion to molar concentrations}}{p}$$

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Table 2
Effect of the high-soy diet on urinary estrogen metabolite excretion in BEAN1 and BEAN2^a

	BEAN 1 (randomized 2-year study)				BEAN 2 (randomized 13-month crossover study)			
	Time	Control	Intervention	<i>p</i> value ^b	Baseline	Low-soy (month 6 or 13)	High-soy (month 6 or 13)	<i>p</i> value ^b
Total estrogen metabolites (ng/mg creatinine)	Baseline	53.9 (35.2, 80.8)	54.8 (35.3, 73.5)	0.81	47.1 (31.8, 66.5)	55.8 (38.4, 76.3)	53.2 (33.6, 80.4)	0.99
	After 2 yrs	45.9 (28.9, 70.9)	49.7 (33.2, 74.4)					
Estrone (E ₁)	Baseline	10.0 (6.8, 12.9)	10.3 (6.9, 14.5)	0.53	8.8 (6.3, 13.6)	10.7 (7.4, 15.6)	12.5 (6.8, 17.6)	0.45
	End	8.5 (5.1, 12.0)	8.4 (5.5, 13.6)					
2-OHE ₁	Baseline	13.8 (6.1, 23.3)	10.2 (4.0, 20.2)	0.45	10.0 (4.8, 21.5)	14.6 (5.6, 23.8)	11.5 (5.6, 18.6)	0.76
	End	10.5 (3.9, 20.3)	9.6 (4.1, 22.0)					
4-OHE ₁	Baseline	2.5 (1.5, 3.8)	2.1 (1.2, 3.7)	0.06	1.1 (0.6, 2.1)	1.3 (0.6, 2.3)	0.8 (0.3, 1.9)	<0.01
	End	2.1 (1.1, 3.6)	1.2 (0.5, 2.7)					
16 α -OHE ₁	Baseline	2.2 (1.0, 3.4)	1.9 (1.0, 3.2)	0.69	2.3 (1.3, 4.0)	3.0 (1.6, 5.1)	2.6 (1.4, 6.7)	0.65
	End	1.4 (0.7, 2.8)	1.6 (0.7, 2.9)					
2-MeOE ₁	Baseline	4.6 (2.3, 9.2)	4.6 (1.8, 7.7)	0.66	2.3 (1.1, 4.4)	2.5 (1.1, 4.7)	2.7 (1.2, 4.5)	0.66
	End	3.9 (1.7, 6.8)	4.5 (1.8, 6.8)					
Estradiol (E ₂)	Baseline	3.2 (2.5, 4.7)	3.9 (2.5, 5.2)	0.22	2.9 (2.1, 4.8)	3.7 (2.6, 5.2)	3.5 (2.3, 6.3)	0.49
	End	3.0 (2.0, 4.5)	3.2 (2.1, 4.7)					
2-OHE ₂	Baseline	0.9 (0.5, 2.4)	1.2 (0.3, 2.1)	0.90	1.3 (0.4, 3.2)	1.4 (0.6, 3.2)	1.4 (0.5, 3.0)	0.89
	End	1.1 (0.3, 2.2)	0.9 (0.4, 2.1)					
16-keto E ₂	Baseline	2.1 (1.1, 2.9)	2.1 (1.2, 2.7)	0.57	1.8 (1.0, 2.7)	2.1 (1.3, 3.0)	1.7 (1.0, 4.1)	0.89
	End	1.5 (0.9, 2.6)	1.6 (1.0, 2.8)					
Estriol (E ₃)	Baseline	9.7 (5.7, 15.9)	10.9 (7.1, 16.0)	0.29	9.9 (5.8, 16.2)	12.0 (6.5, 17.2)	10.7 (5.8, 22.1)	0.73
	End	8.9 (4.8, 14.1)	9.3 (5.1, 16.5)					
2-OH pathway (%) ^c	Baseline	40.1 (26.1, 50.9)	34.6 (25.2, 46.5)	0.16	30.9 (21.3, 45.7)	31.6 (22.4, 43.5)	30.7 (22.0, 46.7)	0.81
	End	39.4 (23.8, 49.1)	35.7 (21.3, 47.5)					
4-OH pathway (%) ^d	Baseline	4.5 (3.2, 6.2)	4.5 (2.9, 6.3)	0.12	2.3 (1.7, 3.8)	2.6 (1.4, 3.7)	1.4 (0.7, 2.6)	0.01
	End	4.4 (3.2, 6.7)	2.8 (1.4, 5.0)					
16-OH pathway (%) ^e	Baseline	25.2 (19.0, 36.3)	30.3 (19.9, 41.5)	0.37	30.4 (21.2, 43.3)	30.3 (23.3, 45.6)	31.5 (19.9, 43.2)	0.50
	End	25.2 (19.0, 36.3)	30.3 (19.9, 41.5)					

	BEAN 1 (randomized 2-year study)			BEAN 2 (randomized 13-month crossover study)				
	Time	Control	Intervention	<i>p</i> value ^b	Baseline	Low-soy (month 6 or 13)	High-soy (month 6 or 13)	<i>p</i> value ^b
2/16 α -OHE ₁ ratio	End	26.1 (18.5, 39.4)	28.5 (18.1, 42.9)					
	Baseline	6.8 (3.6, 12.4)	6.2 (2.1, 12.3)	0.63	5.1 (2.1, 8.3)	4.5 (1.9, 8.4)	4.4 (1.9, 8.7)	0.24
	End	7.5 (2.9, 14.5)	5.2 (2.4, 14.2)					

^aNumbers are medians (Q1, Q3)

^b*p*-values for the effect of high-soy diet were calculated from mixed-effects models using log-transformed values of urinary estrogen metabolites as exposure variables.

^c(2-OHE₁ + 2-OHE₂ + 2-MeOE₁)/total estrogen metabolites after conversion to molar concentrations

^d(4-OHE₁)/total estrogen metabolites after conversion to molar concentrations

^e(E₃ + 16 α -OHE₁ + 16-ketoE₂)/total estrogen metabolites after conversion to molar concentrations.