

Soy Consumption Alters Endogenous Estrogen Metabolism in Postmenopausal Women¹

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Abstract

Isoflavones are soy phytoestrogens that have been suggested to be anticarcinogenic. Our previous study in premenopausal women suggested that the mechanisms by which isoflavones exert cancer-preventive effects may involve modulation of estrogen metabolism away from production of potentially carcinogenic metabolites [16 α -(OH) estrone, 4-(OH) estrone, and 4-(OH) estradiol] (X. Xu *et al.*, *Cancer Epidemiol. Biomark. Prev.*, 7: 1101–1108, 1998). To further evaluate this hypothesis, a randomized, cross-over soy isoflavone feeding study was performed in 18 healthy postmenopausal women. The study consisted of three diet periods, each separated by a washout of ~3 weeks. Each diet period lasted for 93 days, during which subjects consumed their habitual diets supplemented with soy protein isolate providing 0.1 (control), 1, or 2 mg isoflavones/kg body weight/day (7.1 ± 1.1 , 65 ± 11 , or 132 ± 22 mg/day). A 72-h urine sample was collected 3 days before the study (baseline) and days 91–93 of each diet period. Urine samples were analyzed for 10 phytoestrogens and 15 endogenous estrogens and their metabolites by a capillary gas chromatography-mass spectrometry method. Compared with the soy-free baseline and very low isoflavone control diet, consumption of 65 mg isoflavones increased the urinary 2/16 α -(OH) estrone ratio, and consumption of 65 or 132 mg isoflavones decreased excretion of 4-(OH) estrone. When compared with baseline values, consumption of all three soy diets increased the ratio of 2/4-(OH) estrogens and decreased the ratio of genotoxic: total estrogens. These data suggest that both isoflavones and other soy constituents may exert cancer-preventive effects in postmenopausal women by altering estrogen metabolism away from genotoxic metabolites toward inactive metabolites.

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Introduction

Estrogens play an important role in human breast carcinogenesis. Although the mechanisms are not entirely known, substantial evidence indicates that they likely involve mitogenic properties of the parent estrogens and their metabolites through classical estrogen receptor-mediated processes as well as metabolic activation to genotoxic estrogen metabolites (1–3). 16 α -(OH)E₁,³ the main estrogen metabolite proposed to be a risk factor for breast cancer, shows properties consistent with initiation of mammary cell transformation, including induction of unscheduled DNA synthesis and stimulation of anchorage-independent growth of mammary epithelial cells (4). 16 α -(OH)E₁ exhibits estrogenicity comparable with that of E₂ and low-binding affinity to sex hormone binding globulin (5). It can irreversibly bind to the estrogen receptor and cause long-lasting effects, such as persistent hyperproliferation and up-regulated expression of the *c-myc* oncogene, even after its withdrawal (6). The relative extent of estrogen metabolism via the 16 α -hydroxylation pathway has been shown to be significantly increased in breast cancer patients (7–11) and in healthy women who later develop breast cancer (12), although not in healthy breast cancer survivors (13).

Recent studies suggest that 4-hydroxylated catechol estrogens may be as harmful as 16 α -(OH)E₁ because their electrophilic quinone products react with DNA to form depurinating adducts known to generate mutations that initiate cancer (3). *In vivo* treatment with 4-(OH)E₂, a potent long-acting estrogen (14–16), induces DNA single-strand breaks and other mutagenic products of oxidative damage in liver and kidney of Syrian hamsters (17, 18). *In vitro* studies show that microsomes prepared from human mammary adenocarcinoma and fibroadenoma predominantly catalyze the 4-hydroxylation of E₂, whereas this does not occur in microsomes prepared from normal tissue (19). Aldercreutz *et al.* (20) reported that urinary 4-(OH)E₁ in premenopausal Finnish women at high risk of breast cancer was at least double that in premenopausal Asian women at low risk of breast cancer.

Few studies have evaluated the effects of diet on excretion of urinary estrogen metabolites. Consumption of indole-3-carbinol, a phytochemical abundant in cruciferous vegetables, significantly decreases urinary excretion of E₂, E₁, E₃, and 16 α -(OH)E₁ and significantly increases urinary excretion of 2-(OH)E₂ and 2-(OH)E₁, both of which have been proposed to be benign and weak estrogens in men and women (21). We reported recently that urinary 16 α -(OH)E₁, 4-(OH)E₁, and 4-(OH)E₂ were significantly reduced by soy isoflavone consumption in premenopausal women (22) and suggested that this effect may provide a mechanism for the observed inverse

³ The abbreviations used are: E₁, estrone; E₂, estradiol; E₃, estriol; low-iso, low-isoflavone; high-iso, high isoflavone; ODMA, *O*-desmethylangolensin; CYP, cytochrome P-450.

associations between breast cancer and soybean consumption (23–25) as well as urinary excretion of specific soy isoflavonoids and lignans (26, 27).

For this study, we postulated that soy isoflavones, when consumed by postmenopausal women in a soy protein isolate, will modulate estrogen metabolism away from formation of potentially carcinogenic metabolites. To evaluate this hypothesis, a randomized, cross-over soy isoflavone feeding study was performed in 18 healthy postmenopausal women.

Materials and Methods

Subjects. Detailed subject information has been described previously (28). All subjects were recruited from the Minneapolis-St. Paul metropolitan area. Exclusionary criteria included athleticism; regular consumption of a strict vegetarian, high fiber, high soy or low fat diet; cigarette smoking; regular consumption of vitamin and mineral supplementation greater than the Recommended Dietary Allowances; regular use of medication including aspirin; use of hormones or antibiotics within 6 months of the start of the study; menstrual bleeding within 12 months; hysterectomy and oophorectomy; follicle-stimulating hormone concentration <25 IU/l; history of chronic disorders including endocrine or gynecological diseases; benign breast disease; $<90\%$ or $>120\%$ ideal body weight; weight change >10 pounds within the previous year; and inability to abstain from alcoholic beverages during the study. Health history, physical exam, and routine blood and urine screening verified health status of the subjects. Twenty-three women were admitted into the study. Eighteen subjects completed the study. Their prestudy averages for age, years since menopause, body weight, body mass index, and percentage of body fat were 56.9 ± 5.8 years, 7.6 ± 4.7 years, 65.6 ± 10.2 kg, 25.2 ± 3.6 kg/m², and $40.3 \pm 5.6\%$, respectively.

Study Design and Diet. Prior to the study, the protocol was approved by the University of Minnesota Institutional Review Board: Human Subjects Committee. The study was performed using a randomized, cross-over design. The study consisted of three diet periods, each separated by a 26-day washout period. Each diet period lasted for 93 days, during which subjects consumed their habitual diets supplemented with one of three soy protein powders (Protein Technologies International, St. Louis, MO) that were similar in macronutrient content but differed in concentration of isoflavones. The three powders provided 0.11 ± 0.01 (control), 1.00 ± 0.01 (low-iso), or 2.00 ± 0.02 (high-iso) mg total isoflavones (expressed as aglycone units) per kg body weight/day (7.1 ± 1.1 , 65 ± 11 , and 132 ± 22 mg total isoflavones/day for control, low-iso, and high-iso diets, respectively). The concentrations of all 12 isoflavone isomers (the aglycone, glucoside, acetylglucoside, and malonylglucoside forms of daidzein, genistein, and glycitein) were analyzed in the laboratory of Professor Pat Murphy at Iowa State University by a reversed phase high-performance method as described previously (29). On average, the proportions of daidzein, genistein, and glycitein were 33, 58, and 9%, respectively, of the total isoflavones in the soy protein powders. 90% of daidzein, 91% of genistein, and 82% of glycitein were present as their glucoside conjugates, and the remainder were present as the aglycones. The daily nutrient contribution of the soy powder averaged 348 kilocalories, 63 g protein, 21 g carbohydrate, and 1.9 g fat (28).

Subjects were free living during the entire study. They were instructed to minimize phytoestrogens in their background diet by avoiding soy foods, flaxseed, sprouts, and limiting beans and legumes to one serving/week. Food intake was mon-

itored by 3-day diet records kept prior to the start of the study and on days 35–37, 63–65, and 91–93 of each diet period. Energy, macronutrient, and dietary fiber intakes were analyzed by a computerized nutrient analysis program (Nutritionist IV, version 4.0; The Hearst Corp., San Bruno, CA). Body density was calculated from the sum of the four skinfold thicknesses, and a predictive equation was used to determine the percentage of body fat (30).

Sample Collection and Analysis. Three continuous 24-h urine samples were collected during 3 days before the start of the study (baseline) and days 91–93 of each diet period. Twenty-four-h urines were collected in 3-liter containers containing 3 g of ascorbic acid. Urinary creatinine was measured to evaluate the completeness of urine collection using an enzymatic assay kit (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY). After recording the 24-h urine volume, sodium azide was added to achieve a 0.1% (w/v) concentration. Urine samples were stored at -20°C until analysis. Immediately before analysis, the three 24-h urine aliquots were thawed and proportionally combined to create a 72-h pooled sample.

Fifteen-ml aliquots from each 72-h pooled urine sample were extracted and analyzed for 10 phytoestrogens (equol, ODMA, dihydrodaidzein, daidzein, genistein, glycitein, enterodiol, enterolactone, matairesinol, and coumestrol) and 15 endogenous estrogens and their metabolites [E_1 , E_2 , E_3 , $16\alpha\text{-(OH)}E_1$, $2\text{-(OH)}E_1$, $2\text{-(OH)}E_2$, $4\text{-(OH)}E_1$, $4\text{-(OH)}E_2$, 2-methoxyestrone (2-MeOE₁), 2-methoxyestradiol (2-MeOE₂), 4-methoxyestrone (4-MeOE₁), 4-methoxyestradiol (4-MeOE₂), 16-ketoestradiol (16-ketoE₂), 16-epiestriol (16-epiE₃), and 17-epiestriol (17-epiE₃)] as described previously (22) by an ion-exchange chromatography and capillary gas chromatography-mass spectrometry method originally developed by Adlercreutz and colleagues (31–33).

All samples from each subject were analyzed in duplicate in the same batch. Duplicate pooled postmenopausal quality control urine samples were also analyzed with each batch. For phytoestrogen analysis, intra-assay coefficients of variation ranged from 0.9 to 6.3%, and inter-assay coefficients of variation ranged from 2.5 to 14.2%. For estrogen metabolite analysis, intra-assay coefficients of variation ranged from 1.5 to 7.8%, and inter-assay coefficients of variation ranged from 3.9 to 17.6%, except for 2-MeOE₂ and 17-epiE₃, which had coefficients of variation of 24.9 and 27.4%, respectively.

Statistics. Statistical analysis was performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC) version 6.12. The effects of diet on urinary excretion of phytoestrogens and estrogen metabolites were determined by repeated measures ANOVA, controlling for subject, diet period, and diet. Comparisons between baseline and each diet were evaluated using paired *t* tests. Data were examined for normality and homogeneity of variance before ANOVA. If necessary, log transformation of data was performed before analysis. Significance was considered at $P < 0.05$.

Results

Diet and Body Weight. Body weight, body mass index, percentage of body fat, and mean consumption of energy, macronutrients, and dietary fiber for the 18 subjects in this study have been reported previously (28). There were no significant differences in body weight, body mass index, or percentage of body fat during the study. There were also no significant differences in mean daily consumption of energy, macronutrients, or dietary fiber among the three diet periods, although baseline carbohydrate, fat, and dietary fiber consumption was

Table 1 Urinary phytoestrogens (nmol/24 h)^a

	Baseline (n = 18)	Control (n = 18)	Low-Iso (n = 17) ^b	High-Iso (n = 18)
Isoflavonoids				
Genistein	840 (679–1039) ^c	1564 (1341–1824) ^d	6608 (5629–7757) ^e	13148 (11271–15337) ^f
Daidzein	1002 (790–1272) ^c	1569 (1195–2059) ^d	6688 (5041–8873) ^e	14059 (10709–18457) ^f
Dihydrodaidzein	716 (590–869) ^c	1071 (865–1325) ^d	4163 (3329–5206) ^e	7383 (5966–9137) ^f
ODMA	677 (552–830) ^c	960 (799–1153) ^d	4900 (4047–5932) ^e	10405 (8661–12498) ^f
Equol	222 (186–266) ^c	354 (253–497) ^d	735 (524–1030) ^e	1097 (782–1537) ^e
Glycitein	293 (260–331) ^c	425 (385–469) ^d	1739 (1568–1928) ^e	3674 (3327–4057) ^f
Coumestrol	33.1 (30.8–35.5) ^{c,d}	31.9 (29.6–34.4) ^c	33.7 (31.2–36.3) ^{c,d}	35.4 (32.9–38.1) ^d
Lignans				
Enterodiol	345 (298–399) ^c	291 (221–383) ^c	511 (383–681) ^d	553 (420–730) ^d
Enterolactone	1306 (1221–1398) ^c	1382 (1163–1641) ^c	2404 (2010–2877) ^d	3820 (3220–4532) ^e
Matairesinol	23.6 (22.3–25.0)	25.6 (23.8–27.7)	23.5 (21.7–25.5)	25.6 (23.7–27.6)
Total	6100 (5466–6807) ^c	9072 (8199–10039) ^d	35137 (31619–39046) ^e	67508 (61006–74703) ^f

^a Because of unequal variance, all data were log-transformed before ANOVA. Geometric means (95% confidence intervals) are presented in the table. Within a row, means not sharing a superscript (c, d, e, or f) are significantly different ($P < 0.05$). When data were analyzed relative to urinary creatinine (nmol phytoestrogens/mmol creatinine), the same results were obtained.

^b One subject did not collect urine samples at the end of the low-iso diet period.

Table 2 Urinary estrogens and estrogen metabolites (nmol/24 h)^a

	Baseline (n = 18)	Control (n = 18)	Low-Iso (n = 17) ^b	High-Iso (n = 18)
E ₂ ^c	2.94 ± 0.19	3.92 ± 0.25	4.14 ± 0.26	3.97 ± 0.25
	2.71 (2.44–3.02) ^d	3.55 (3.08–4.09) ^e	3.43 (2.96–3.97) ^e	3.35 (2.91–3.86) ^e
E ₁ ^c	5.28 ± 0.34	6.26 ± 0.40	6.52 ± 0.42	5.69 ± 0.40
	4.32 (3.79–4.93) ^d	5.17 (4.59–5.81) ^{d,e}	5.18 (4.58–5.86) ^e	4.46 (3.97–5.02) ^{d,e}
E ₃	5.17 ± 0.40	5.07 ± 0.30	5.03 ± 0.31	4.52 ± 0.30
16α-(OH)E ₁	2.27 ± 0.29	2.30 ± 0.23	1.92 ± 0.24	2.14 ± 0.23
4-(OH)E ₂	0.78 ± 0.02	0.77 ± 0.04	0.78 ± 0.04	0.73 ± 0.04
4-(OH)E ₁ ^c	2.21 ± 0.14	1.93 ± 0.10	1.63 ± 0.10	1.51 ± 0.10
	1.64 (1.48–1.81) ^d	1.52 (1.36–1.70) ^d	1.27 (1.13–1.42) ^e	1.25 (1.12–1.39) ^e
2-(OH)E ₂ ^c	2.37 ± 0.14	2.92 ± 0.29	2.70 ± 0.31	2.86 ± 0.29
	1.93 (1.70–2.20) ^d	2.48 (2.16–2.85) ^e	2.21 (1.92–2.56) ^{d,e}	2.16 (1.88–2.47) ^{d,e}
2-(OH)E ₁ ^c	7.81 ± 0.37	9.34 ± 0.71	9.92 ± 0.75	9.93 ± 0.71
	5.88 (5.40–6.41) ^d	7.13 (6.41–7.92) ^e	7.17 (6.43–8.00) ^e	6.83 (6.15–7.60) ^e
4-MeOE ₁	0.21 ± 0.08	0.20 ± 0.08	0.19 ± 0.08	0.19 ± 0.07
2-MeOE ₂	1.42 ± 0.09	1.53 ± 0.11	1.32 ± 0.11	1.59 ± 0.11
2-MeOE ₁ ^c	2.09 ± 0.11	2.45 ± 0.11	2.52 ± 0.11	2.50 ± 0.11
	1.77 (1.61–1.94) ^d	2.06 (1.86–2.29) ^e	1.99 (1.79–2.22) ^{d,e}	1.97 (1.78–2.19) ^{d,e}
16-ketoE ₂	1.87 ± 0.12	1.79 ± 0.12	1.71 ± 0.12	1.82 ± 0.12
17-epiE ₃	0.85 ± 0.03	0.83 ± 0.06	0.96 ± 0.06	0.87 ± 0.06
16-epiE ₃	1.67 ± 0.11	1.71 ± 0.09	1.68 ± 0.09	1.84 ± 0.09
Total estrogens ^c	33.2 ± 1.3	37.0 ± 1.7	37.5 ± 1.8	35.9 ± 1.7
	29.6 (27.5–32.0)	32.7 (30.1–35.5)	32.3 (29.6–35.2)	30.4 (28.0–33.0)

^a Least squares mean ± SE. Within a row, means not sharing a superscript (d or e) are significantly different ($P < 0.05$). When data were analyzed as nmol estrogen metabolite/mmol urinary creatinine, the same results were obtained. Only trace amounts of 4-MeOE₂ were found in the samples of these postmenopausal women.

^b One subject did not collect urine samples at the end of the low-iso diet period.

^c Because of unequal variance, data were log-transformed before ANOVA. Geometric means (95% confidence intervals) are presented below the least squares means.

significantly higher, and baseline protein consumption was significantly lower than during the study (28). On average, the daily consumption at baseline was 1772 kcal, 69 g of protein, 262 g of carbohydrate, 54 g of fat, and 18 g of dietary fiber, whereas the daily consumption during the three soy diet periods was 1779 kcal, 113 g of protein, 236 g of carbohydrate, 46 g of fat, and 13 g of dietary fiber (28).

Urinary Phytoestrogens. As a result of unequal variance, all phytoestrogen data were log-transformed before data analysis. Table 1 shows the geometric means and 95% confidence intervals for the urinary phytoestrogen data. Although on average the control diet only provided 7 mg of total isoflavones/day, urinary excretion of isoflavonoids (genistein, daidzein, dihydrodaidzein, ODMA, equol, and glycitein) in the control diet were significantly greater than in baseline samples. As ex-

pected, urinary excretion of isoflavonoids and lignans (enterodiol and enterolactone) were significantly increased by the low-iso and high-iso diets in a dose-dependent manner, when compared with the control diet. When data were analyzed relative to urinary creatinine (nmol phytoestrogen per mmol creatinine), the same results were obtained.

Urinary Estrogens and Estrogen Metabolites. Urinary excretion of 4-(OH)E₁ was significantly decreased by both the low-iso and high-iso diets, when compared with the control diet and baseline samples (Table 2). Urinary excretion of E₂, E₁, 2-(OH)E₂, 2-(OH)E₁, and 2-MeOE₁ was not significantly different among the three soy diets but tended to be increased by all three soy diets when compared with baseline samples. Urinary excretion of E₃, 16α-(OH)E₁, 4-(OH)E₂, 4-MeOE₁, 2-MeOE₂, 16-ketoE₂, 17-epiE₃, 16-epiE₃, and total estrogens

Table 3 Urinary estrogen metabolite ratios^a

	Baseline (n = 18)	Control (n = 18)	Low-Iso (n = 17) ^b	High-Iso (n = 18)
Genotoxic:Total	0.16 ± 0.01 ^c	0.14 ± 0.01 ^d	0.12 ± 0.01 ^d	0.13 ± 0.01 ^d
2-(OH)E ₁ :16α-(OH)E ₁ ^e	3.82 ± 0.33	4.54 ± 0.37	5.09 ± 0.38	5.13 ± 0.37
	3.13 (2.51–3.90) ^c	3.95 (3.38–4.62) ^{c,d}	4.39 (3.73–5.16) ^d	4.03 (3.45–4.72) ^{c,d}
2E ₁ -total:16α-total	1.33 ± 0.08	1.51 ± 0.07	1.56 ± 0.07	1.53 ± 0.07
2E ₁ -total:4E ₁ -total	4.13 ± 0.12 ^c	5.16 ± 0.31 ^d	6.25 ± 0.33 ^f	5.90 ± 0.31 ^{d,f}
2E ₂ -total:4E ₂ -total	3.29 ± 0.19 ^c	3.91 ± 0.30 ^d	3.72 ± 0.32 ^{c,d}	3.84 ± 0.30 ^{c,d}
2-total:4-total	3.80 ± 0.11 ^c	4.67 ± 0.24 ^d	5.14 ± 0.25 ^d	5.05 ± 0.24 ^d

^a Least squares mean ± SE. Within a row, means not sharing a superscript (c, d, or f) are significantly different ($P < 0.05$). Genotoxic:Total, [16α-(OH)E₁ + 4-(OH)E₂ + 4-(OH)E₁]/total estrogens; 2E₁-total:16α-total, [2-(OH)E₁ + 2-MeOE₁]:[16α-(OH)E₁ + E₃ + 17-epiE₃]; 2E₁-total:4E₁-total, [2-(OH)E₁ + 2-MeOE₁]:[4-(OH)E₁ + 4-MeOE₁]; 2E₂-total:4E₂-total, [2-(OH)E₂ + 2-MeOE₂]:[4-(OH)E₂]; 2-total:4-total, [2-(OH)E₁ + 2-(OH)E₂ + 2-MeOE₁ + 2-MeOE₂]:[4-(OH)E₁ + 4-(OH)E₂ + 4-MeOE₁]. Only trace amounts of 4-MeOE₂ were found, and data were not included in the calculation of ratios.

^b One subject did not collect urine samples at the end of low-iso diet period.

^c Because of unequal variance, data were log-transformed before ANOVA. Geometric means (95% confidence intervals) are presented below the least squares means.

was not significantly different among the three soy diets or between baseline samples and any soy diet.

The ratio of 2E₁-total to 4E₁-total (which reflects the ratio of 2-hydroxylation:4-hydroxylation for E₁) was significantly increased by the low-iso diet when compared with the control diet (Table 3). There were no other significant differences in ratios among the three soy diets, although there appeared to be trends toward relatively increased 2-hydroxylation with the low-iso diet. There were, however, a number of differences between the three diets and samples collected at baseline. The ratio of genotoxic estrogen metabolites [16α-(OH)E₁ + 4-(OH)E₂ + 4-(OH)E₁] to total estrogens was significantly decreased by all three soy diets compared with baseline samples, and the ratios of 2E₁-total to 4E₁-total (which reflects the ratio of 2-hydroxylation:4-hydroxylation for E₁) and 2-total:4-total (which reflects the ratio of 2-hydroxylation:4-hydroxylation for both E₁ and E₂) were significantly increased by all three soy diets compared with baseline samples. The ratio of 2-(OH)E₁:16α-(OH)E₁ was significantly increased by low-iso diet when compared with baseline samples.

Discussion

As expected, increased urinary excretion of isoflavonoids was observed in our postmenopausal subjects after consumption of a soy protein isolate providing 7, 65, or 132 mg of soy isoflavones for 93 days in a dose-dependent manner. Consumption of the soy protein isolate providing 7 mg of isoflavones/day also significantly increased urinary isoflavonoid excretion, when compared with the subjects' baseline samples. Daidzein and genistein were the predominant urinary isoflavonoids in this study, although we previously reported ODMA to be the major urinary isoflavonoid in premenopausal subjects (22). This difference in urinary phytoestrogen profile suggests that age or menopausal status may influence the activities of the colonic microfloral enzymes responsible for isoflavone metabolism.

The between-subject variation in urinary phytoestrogen excretion was great. Within a diet, excretion of genistein, daidzein, ODMA, and equol varied by 2-, 5-, 10-, and 120-fold, respectively. This high variability in phytoestrogen excretion after soy consumption is consistent with previous reports (22, 34–36) and is likely attributable to the dependence of phytoestrogen formation and absorption on the composition of gut microflora (37), which varies substantially among individuals (38). This variability was particularly high for equol, because only 6 of the 18 subjects excreted significant quantities, consistent with reports that only 30–40% of human subjects produce large quantities of equol after soy consumption (22, 39).

In addition to providing isoflavones, soy contains small amounts of plant lignans, which are converted to the mammalian lignans enterodiol and enterolactone by human colonic microflora (40). Although the quantities are low, lignan excretion was increased significantly after consumption of soy protein isolate containing 65 or 132 mg of isoflavones. Increased urinary lignan excretion is likely attributable to the presence of plant lignans in soy, although it is also possible that soy isoflavone consumption alters the profile of intestinal microflora or their enzyme activities.

In general, the effects of soy isoflavones on endogenous estrogen metabolism in postmenopausal women were less pronounced than we reported previously in premenopausal women (22). Urinary excretion of 4-(OH)E₁, a proposed genotoxic estrogen metabolite (2, 3), was reduced by consumption of the low-iso and high-iso diets when compared with baseline and control diet samples, suggesting a beneficial effect of soy isoflavones on endogenous estrogen metabolism. At the same time, the proportion of estrogen metabolized by 2-hydroxylation (a pathway producing benign and weak estrogen metabolites; Ref. 41) was increased by all three soy diets, with the low-iso diet appearing to have the greatest effect. It is possible that this increased 2-hydroxylation was attributable to increased protein and decreased carbohydrate consumption during the three soy diet periods, although the dietary changes we observed were much smaller than those shown to increase urinary 2-hydroxylation in previous studies (42, 43). The significant effect of the control diet, which provided a slightly higher level of isoflavones than consumed in a typical Western diet, suggests the involvement of other soy phytochemicals or protein. It is possible, but less likely, that the small amount of isoflavones provided in the control diet exerted these effects.

Urinary excretion of E₁ and E₂ tended to be increased during consumption of all three soy diets when compared with baseline. This increased urinary E₁ and E₂ may have been attributable to elevated excretion of E₁ and E₂ sulfate conjugates because soy isoflavones have been shown to inhibit steroid sulfatase and subsequent tissue uptake of estrogens (44). Similar results have been observed in postmenopausal women taking tamoxifen, likely through the same mechanism (45).

Although few data are available, a likely mechanism by which soy consumption modulates estrogen metabolism is via effects of isoflavones on the activities of specific CYP isoenzymes responsible for estrogen hydroxylations. Genistein has been shown to inhibit rat liver CYP1A1 (46), an enzyme that catalyzes the conversion of catechol estrogens to their electrophilic quinones that may be responsible for the genotoxicity of

4-hydroxylated estrogens (47). α -Naphthoflavone, a synthetic flavone and inhibitor of CYP1A1 and CYP1B1, completely suppresses E₂-induced tumorigenesis through inhibition of the formation of 4-(OH)E₂ and its electrophilic quinone (47). Consumption of the synthetic isoflavone, ipriflavone by rats, results in a strong suppression of liver CYP3A4 (48), an enzyme that catalyzes the 4-hydroxylation of estrogens in humans (49). Isoflavones also inhibit enzymes involved in estrogen synthesis (50–52) and steroid sulfohydrolysis (44) that may be particularly important for postmenopausal women because biologically active estrogens in their mammary tissue are derived almost exclusively from inactive sulfoconjugates (53, 54).

This is the first study to report that soy consumption in postmenopausal women leads estrogen metabolism in a favorable direction by preferentially decreasing production of genotoxic 4-hydroxylated estrogen metabolites and increasing relative 2-hydroxylation. This mechanism may contribute to the observed inverse associations between soy consumption and breast cancer risk.

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