Premenopausal Equol Excretors Show Plasma Hormone Profiles Associated with Lowered Risk of Breast Cancer[1]

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Abstract

Increased urinary excretion of equol, a metabolite of the isoflavone daidzein, has been associated with a reduced risk of breast cancer. This risk reduction has generally been presumed to be a consequence of increased isoflavone consumption. However, only 30–40% of the population excretes more than trace amounts of equol, regardless of isoflavone intake. Accordingly, we hypothesized that the observed apparent protective effect of equol is at least in part attributable to hormonal differences between equol excretors and non-excretors, and that these differences are largely independent of isoflavone intake.

We measured plasma hormone and sex hormone binding globulin (SHBG) concentrations in 14 normally cycling premenopausal women during each of three diet periods in which they consumed differing isoflavone doses (0.15, 1.0, and 2.0 mg/kg of body weight/day) as a component of soy protein isolate. The plasma hormone and SHBG concentrations of equol excretors ($n = 5$) were then compared with those of the non-excretors ($n = 9$). Results showed that even at the lowest dose, urinary equol excretion values for excretors far exceeded those for non-excretors consuming the highest dose. At all doses, equol excretors generally had lower concentrations of estrone, estrone-sulfate, testosterone, androstenedione, dehydroepiandrosterone (DHEA), DHEA-sulfate, and cortisol and higher concentrations of SHBG and midluteal progesterone, a hormonal pattern overall consistent with lowered breast cancer risk. In conclusion, the association of equol excretion and lowered breast cancer risk may largely reflect the tendency of equol excretors to have more favorable hormonal profiles, as opposed to merely reflecting increased isoflavone intake. Equol may be a marker for the presence of colonic bacterial enzymatic activity that increases fecal steroid excretion. Alternatively, equol itself, even with very modest isoflavone intake, may exert beneficial effects on the regulation of endogenous hormones.

Introduction

Epidemiological studies have shown that breast cancer rates are low in populations that consume soy (1, 2), as well as in subgroups who consume high quantities of soy products within Asian populations (3–6). Because soy is a rich source of the phytoestrogenic isoflavones and isoflavones have been shown to be anticarcinogenic in animal (7) and cell culture studies (8), most researchers assume that the epidemiological findings are attributable to the presence of isoflavones in soy products. Total urinary isoflavones, often used as a surrogate for intake, have been shown to be lower in breast cancer patients than controls (9, 10), and the isoflavone metabolite equol was specifically shown to be inversely associated with breast cancer risk in a recent case control study performed in a population consuming low amounts of isoflavones (11).

Although not present in plant foods, equol is produced in the gastrointestinal tract by bacterial degradation of daidzein, a predominant soy isoflavone (12). Urinary excretion of equol varies up to 800-fold among individuals (13–17), because 60–70% of the population excretes only trace amounts of equol, regardless of isoflavone intake (13, 15, 16, 18). This high variability in urinary equol excretion is presumably attributable to interindividual differences in colonic bacteria. Although most researchers conclude that the association of urinary isoflavone excretion with low breast cancer risk is a result of differences in isoflavone consumption between cases and controls, it is possible that breast cancer cases excrete significantly less equol than controls (11), largely as a result of differences in phytoestrogen metabolism, rather than intake.

Along these lines, we hypothesized that the ability to excrete equol in more than trace amounts *per se* may be associated with a lower breast cancer risk. Given the association between breast cancer risk and plasma hormone concentrations (19, 20), we specifically hypothesized that equol excretors would have hormone profiles more favorable from a breast cancer standpoint when compared with non-excretors consuming identical doses of isoflavones. Using data from a crossover study designed to investigate the hormonal effects of soy isoflavones in normally cycling premenopausal women (21), we tested this hypothesis by comparing plasma hormone concentrations of equol excretors to those of non-excretors who were provided three isoflavone doses.

Materials and Methods

Subjects and Experimental Design. The current study used data from a study reported previously of the hormonal effects of isoflavones in premenopausal women (21). The University of

Received 10/8/99; revised 3/8/00; accepted 3/20/00.
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[1] Supported by NIH Grant CA-66016 and General Clinical Research Center Grant MO1-RR00400 from the National Center for Research Resources.
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Minnesota Institutional Review Board: Human Subjects Committee approved the study protocol. Potential subjects were selected after a phone questionnaire, interview, and health screen. Exclusionary criteria included vegetarian, high fiber, high soy, or low fat diets; regular consumption of vitamin and mineral supplementation greater than the Recommended Dietary Allowances; athleticism; cigarette smoking; antibiotic or hormone use within 6 months; history of chronic disorders including endocrine or gynecological diseases; benign breast disease; regular use of medication including aspirin; current pregnancy or lactation; irregular menstrual cycles; <90% or >120% ideal body weight; weight change >10 pounds within the previous year or weight change >5 pounds within the previous 2 months; consumption of more than two alcoholic beverages per day; and a history of food allergies. Fourteen subjects completed the entire study. The randomized crossover design included three diet periods of three menstrual cycles and 9 days each, separated by an approximately 3-week washout.

Experimental Soy Supplement. Subjects consumed their habitual diets but were provided with detailed dietary instructions to minimize phytoestrogen consumption (21). Their diets were supplemented with three soy protein isolate powders, each similar in macronutrient composition but different in isoflavone concentration (Supro Brand Isolated Soy Protein; Protein Technologies International, St. Louis, MO). The three soy powders provided 0.15 ± 0.01 (control), 1.01 ± 0.04 (low-iso), and 2.01 ± 0.03 (high-iso) mg total isoflavones/kg body weight/day (10 ± 1.1, 64 ± 9.2 and 128 ± 16 mg isoflavones/day, respectively), expressed as unconjugated phytoestrogen units. The proportions of genistein, daidzein, and glycitein in the soy powders averaged 55, 37, and 8%, respectively. The daily nutrient contribution of the soy protein isolates has been described previously (21).

Study Procedures. Fasting body weight was obtained in a hospital gown approximately every 7–10 days. Skinfold thicknesses were obtained once at the start of the study and once at the end of each diet period to determine the percentage of body fat, as described previously (21).

For determination of day of ovulation, day of LH[3] surge was determined from home urinary LH testing (OvuQUICK Self-Test; Quidel, San Diego, CA) beginning on day 9 of each menstrual cycle until detection of the surge. Basal body temperature was also taken during diet period one and was continued throughout the study for subjects whose OvuQUICK test results were ambiguous.

Fasting blood was drawn in heparinized tubes every other day beginning 7 days after the LH surge in menstrual cycle 2 and continuing until the end of each diet period. Plasma was separated, ascorbic acid and sodium azide were each added to a final concentration of 0.1%, and aliquots were frozen at −20°C until analysis.

Twenty-four-h urine collections were performed during the MF phase (days 7–9) of menstrual cycle 4 of each diet period. Urine was collected in plastic containers containing 1 g of ascorbic acid per liter and separated into aliquots after the addition of sodium azide to a final concentration of 0.1%. Aliquots were frozen at −20°C until analysis.

Food records were obtained for three consecutive days during the follicular phase (days 7–9) and luteal phase (7–9 days after the LH surge) of every menstrual cycle.

Analytical Methods. All plasma samples were analyzed for E_2, E_1, E_1-S, progesterone, LH and FSH. Plasma samples from days 2 to 5 of menstrual cycles 3 and 4 were also analyzed for testosterone, androstenedione, DHEA, DHEA-S, cortisol, insulin, and SHBG. All samples from each subject were analyzed in duplicate in the same batch, along with a plasma pool control, to reduce interassay variability. All hormones were analyzed by RIA techniques, as described elsewhere (21).

Before analysis, three-day urines were thawed and proportionally combined to create a pooled sample. Urine was analyzed for equol concentration using ion-exchange chromatography followed by gas chromatography-mass spectrometry, as described previously (15).

Three-day food records were analyzed using Nutritionist IV, Version 4.0 (The Hearst Corporation, San Bruno, CA), and averages were calculated for energy, macronutrients, and dietary fiber.

Data Analysis. Day of ovulation was assumed to be the day after the LH surge, as determined by a positive OvuQUICK result. However, if the OvuQUICK results were ambiguous, plasma hormones, urinary LH, and basal body temperature measurements were used as adjuncts, allowing for precise identification of the day of ovulation by a reproductive endocrinologist (W. R. P.). Follicular phase length was defined as day 1 of the menstrual cycle through the day of ovulation. Luteal phase length was defined as the remainder of the cycle, through the day before the next menses.

For reproductive hormone analyses, the menstrual cycle was divided into four phases, each reflecting a distinct hormone milieu: EF (days 2 and 4), MF (days 7 and 9), PO (ovulation −3, ovulation −1, and ovulation +1) and ML (ovulation +5, ovulation +7, and ovulation +9). For subjects whose blood was not drawn on the days used in the phase definitions, interpolation using values obtained on the surrounding days was used to determine the values for the data analyses.

Statistical Analysis. Although there are no established levels of equol excretion that separate equol excretors from non-excretors, subjects fell clearly into the two categories (Table 1), particularly at the higher levels of isoflavone consumption.

Comparisons between equol excretors and non-excretors were made within each of the three diets, using unpaired, two-tailed Student's *t* tests (SAS Institute, Inc., Cary, NC; Ref. 22). Because of the unequal variance between menstrual cycle phases, plasma hormone comparisons were made within each phase separately, for each of the three diets. Effects of isoflavone consumption on urinary equol, genistein, and daidzein were evaluated using repeated measures ANOVA, controlling for subject and diet. The interactions between equol excretion status and diet were evaluated to determine whether the effects of diet significantly differed between equol excretors and non-excretors. Because of unequal variance and nonnormality, urinary genistein and daidzein data were log-transformed and are presented as the geometric mean and 95% confidence interval. $P < 0.05$ is considered significant.

Results

Urinary isoflavone results are presented in Table 1. Urinary daidzein and genistein significantly increased as isoflavone dose increased, and there were no significant differences between equol excretors and non-excretors for any of the diet periods. As expected, equol excretors excreted substantially greater amounts of equol during all three diet periods, when

[3] The abbreviations used are: LH, luteinizing hormone; FSH, follicle stimulating hormone; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; E_2, estradiol; E_1, estrone; E_1-S, estrone sulfate; SHBG, sex hormone binding globulin; EF, early follicular; MF, midfollicular; PO periovulatory; ML, midluteal.

Table 1 Urinary isoflavone excretion (nmol/24 h)[a]

	Control (n = 13[b])	Low-iso (n = 13[c])	High-iso (n = 14)
Daidzein[d]			
Equol excretors	810* (5.5%) (2.83,2319)	4402† (4.7%) (2293,8452)	8250‡ (4.4%) (6176,11021)
Equol non-excretors	1214* (8.3%) (914,1613)	6053† (6.5%) (4403,8321)	10485‡ (5.6%) (7552,14557)
Genistein[d]			
Equol excretors	1097* (5.4%) (523,2301)	7647† (5.9%) (6120,9554)	15438‡ (5.9%) (10380,22961)
Equol non-excretors	965* (4.7%) (755,1234)	6317† (4.8%) (4133,9656)	13969‡ (5.4%) (10045,19426)
Equol[e]			
Equol excretors	996 ± 364* (454–2402)	11125 ± 440† (10099–12243)	17743 ± 3509‡ (9749–30811)
Equol non-excretors	56.2 ± 9.7 (24–60)	58.3 ± 10.2 (34–131)	76.1 ± 8.7 (24–109)

[a] Values within rows with different symbols(*,†,‡) are significantly different ($P < 0.05$).
[b] One subject did not collect urine at the end of the control diet period.
[c] One subject used antibiotics at the end of the low-iso diet period; therefore, her data were excluded.
[d] Data were log-transformed because of unequal variance and nonnormality. Values are presented as geometric mean (% dose), with 95% confidence intervals in parentheses.
[e] Mean ± SE, with the range in parentheses.

compared with non-excretors. As isoflavone dose increased, urinary equol increased in a dose-dependent fashion for the equol excretors ($P = 0.003$) but remained relatively constant for the non-excretors ($P = 0.22$).

Subject characteristics and dietary intake data for the low-iso diet period are shown in Table 2. There were no significant differences between equol excretors and non-excretors for body weight, body mass index, percentage of body fat, or consumption of energy, macronutrients, or dietary fiber. Menstrual cycle and phase lengths were not significantly different between equol excretors and non-excretors, although equol excretors did tend to have longer lengths. The same general results were observed during the control and high-iso diet periods (data not shown).

Table 3 shows reproductive hormone concentrations during the low-iso diet period, when isoflavone intake was likely closest to typical intakes in Asia (23, 24). Within specific cycle phases, equol excretors had significantly lower concentrations of E_1 and E_1-S and significantly higher concentrations of progesterone and FSH, as well as higher progesterone:E_2 ratios, when compared with non-excretors. Similar differences were observed during the control and high-iso diet periods, indicating that these differences exist regardless of isoflavone intake. For example, within the EF phase of the control diet, plasma E_1 was 57.6 ± 5.1 and 85.2 ± 6.4 pmol/l ($P = 0.007$), and E_1-S was 1.52 ± 0.3 and 2.78 ± 0.5 nmol/l ($P = 0.03$), for equol excretors and non-excretors, respectively. Within the EF phase of the high-iso diet, plasma E_1 was 60.5 ± 5.4 and 83.5 ± 5.7 pmol/l ($P = 0.01$), and E_1-S was 1.14 ± 0.2 and 3.06 ± 0.5 nmol/L ($P = 0.003$), for equol excretors and non-excretors, respectively. Within the ML phase of the control diet, plasma E_1 was 144.5 ± 13.1 and 181.6 ± 18.7 pmol/l ($P = 0.24$), E_1-S was 5.87 ± 1.1 and 8.72 ± 1.8 nmol/l ($P = 0.18$), and progesterone was 64.4 ± 8.8 and 39.7 ± 3.4 nmol/l ($P = 0.005$), for equol excretors and non-excretors, respectively. Within the ML phase of the high-iso diet, plasma E_1 was 140.9 ± 12.3 and 177.1 ± 15.3 pmol/l ($P = 0.14$), E_1-S was 4.81 ± 1.2 and 9.84 ± 2.2 nmol/l ($P = 0.06$), and progesterone was 48.5 ± 8.1 and 39.2 ± 1.8 nmol/l ($P = 0.30$), for equol excretors and non-excretors, respectively.

Within the EF phase, equol excretors had significantly lower concentrations of testosterone, androstenedione, DHEA, DHEA-S, and cortisol and higher concentrations of SHBG (Table 4). There were also nonsignificant trends toward decreased prolactin and insulin. The similarity of these comparisons among the three diet periods suggests that the hormonal differences between equol excretors and non-excretors exist regardless of isoflavone intake.

Table 2 Subject characteristics and dietary intake[a]

	Equol excretors (n = 5)	Equol non-excretors (n = 9)	P
Age (yr)	28.6 ± 1.7	25.3 ± 1.6	0.22
Body weight (kg)	62.2 ± 3.5	64.7 ± 2.7	0.59
BMI (kg/m^2)	22.1 ± 1.1	23.2 ± 0.6	0.40
% body fat[b]	26.7 ± 2.5	28.9 ± 1.6	0.44
MC length (days)[c]	31.2 ± 2.0	28.7 ± 1.3	0.28
FP length (days)[d]	17.4 ± 2.6	16.6 ± 1.3	0.75
LP length (days)[e]	13.9 ± 0.5	12.1 ± 0.7	0.12
Energy (kcal)[f]	2314 ± 100	2266 ± 59	0.66
Protein (g)[f]	116.4 ± 4.7	113.4 ± 2.9	0.57
Carbohydrate (g)[f]	292.9 ± 14.1	301.6 ± 10.1	0.61
Fat (g)[f]	77.5 ± 3.8	71.8 ± 3.1	0.26
Dietary fiber (g)[f]	9.70 ± 0.8	8.45 ± 0.4	0.12

[a] Mean ± SE within the low-iso diet period.
[b] Determined from skinfold thicknesses.
[c] MC, menstrual cycle.
[d] FP, follicular phase.
[e] LP, luteal phase.
[f] Based on seven 3-day food records per subject; includes contributions from the soy powder.

MF urinary excretion of E_2 and E_1 were lower in the equol excretors, when compared with non-excretors. During the low-iso diet period, urinary E_2 was 5.07 ± 0.73 and 6.71 ± 0.34 nmol/day for equol excretors and non-excretors, respectively (lsmean ± SE; $P = 0.04$) and urinary E_1 was 12.8 ± 2.1 and 17.1 ± 3.0 nmol/day for equol excretors and non-excretors, respectively (lsmean ± SE; $P = 0.05$). Results were similar during the control and high-iso diet periods (data not shown). There were no significant differences in 13 other urinary estrogen metabolites (15) between equol excretors and non-excretors.

There were no significant interactions between equol excretor status and diet for either the plasma hormones or urinary estrogens during any diet period. In other words, the effects of diet observed previously on plasma hormones (21) and urinary estrogens (15) were similar in equol excretors and non-excretors.

Table 3 Reproductive hormone concentrations during the low-iso diet period[a,b]

	Equol excretors (n = 5)	Equol non-excretors (n = 9)	P
E_2 (pmol/l)			
EF	90.7 ± 14.1	103.4 ± 14.7	0.56
MF	176.9 ± 48.5	157.9 ± 20.2	0.68
PO	402.0 ± 63.1	390.6 ± 29.4	0.86
ML	279.4 ± 45.9	294.8 ± 25.0	0.75
E_1 (pmol/l)			
EF	64.0 ± 6.8*	86.9 ± 6.9†	0.04
MF	104.3 ± 21.8	110.6 ± 10.0	0.77
PO	171.3 ± 24.0	222.3 ± 27.0	0.22
ML	122.1 ± 9.6*	179.8 ± 15.2†	0.004
E_1-S (nmol/l)			
EF	1.55 ± 0.4*	3.26 ± 0.5†	0.03
MF	4.14 ± 1.6	4.81 ± 0.7	0.67
PO	7.62 ± 2.3	15.7 ± 3.7	0.14
ML	4.88 ± 1.0*	10.7 ± 2.0†	0.02
Progesterone (nmol/l)			
EF	2.23 ± 0.8	2.58 ± 0.6	0.72
MF	1.49 ± 0.3	2.07 ± 0.4	0.27
PO	4.52 ± 1.1	5.47 ± 0.7	0.44
ML	56.9 ± 6.0*	31.5 ± 4.0†	0.002
Progesterone:E_2			
EF	31.0 ± 11.9	27.4 ± 5.1	0.79
MF	12.7 ± 3.3	15.2 ± 1.8	0.48
PO	25.9 ± 15.8	21.9 ± 2.7	0.82
ML	270.9 ± 65.2*	108.4 ± 11.2†	0.04
LH (IU/l)			
EF	5.74 ± 0.5	6.82 ± 1.5	0.51
MF	8.00 ± 1.4	8.91 ± 2.2	0.73
PO	18.5 ± 4.5	19.3 ± 3.1	0.89
ML	6.21 ± 0.4	6.50 ± 1.2	0.82
FSH (IU/l)			
EF	4.13 ± 0.3	4.30 ± 0.3	0.69
MF	3.86 ± 0.4	4.55 ± 0.3	0.13
PO	4.00 ± 0.6	4.03 ± 0.5	0.96
ML	2.67 ± 0.3*	1.87 ± 0.2†	0.04

[a] Mean ± SE within the low-iso diet period.
[b] Values within rows with different symbols(*,†) are significantly different ($P < 0.05$).

Table 4 Plasma hormone concentrations[a,b]

	Equol excretors (n = 5)	Equol non-excretors (n = 9)	P
Testosterone (nmol/l)			
Control	0.70 ± 0.08*	1.04 ± 0.08†	0.01
Low-iso	0.73 ± 0.07*	1.04 ± 0.07†	0.004
High-iso	0.73 ± 0.06*	1.05 ± 0.07†	0.008
Androstenedione (nmol/l)			
Control	4.98 ± 0.5*	6.83 ± 0.5†	0.03
Low-iso	5.48 ± 0.4*	7.09 ± 0.5†	0.04
High-iso	5.72 ± 0.4	7.05 ± 0.5	0.10
DHEA (nmol/l)			
Control	18.9 ± 1.9*	33.0 ± 2.3†	0.0003
Low-iso	20.8 ± 1.9*	34.6 ± 2.8†	0.0004
High-iso	21.7 ± 2.2*	32.9 ± 2.8†	0.01
DHEA-S (nmol/l)			
Control	2795 ± 323*	5550 ± 692†	0.002
Low-iso	2961 ± 442*	5705 ± 657†	0.002
High-iso	2694 ± 291*	5360 ± 622†	0.0007
Cortisol (nmol/l)			
Control	366.7 ± 46.9	450.0 ± 21.7	0.08
Low-iso	408.3 ± 31.7*	503.5 ± 29.0†	0.04
High-iso	367.6 ± 26.3*	459.1 ± 24.7†	0.03
Prolactin (μg/l)			
Control	11.3 ± 1.0	14.7 ± 1.5	0.07
Low-iso	12.6 ± 1.3	15.6 ± 1.8	0.24
High-iso	13.4 ± 2.0	16.2 ± 2.2	0.39
SHBG (nmol/l)			
Control	40.8 ± 3.8*	27.7 ± 2.6†	0.007
Low-iso	38.4 ± 3.9	28.5 ± 2.8	0.05
High-iso	39.2 ± 3.3*	28.1 ± 2.9†	0.02
Insulin (pmol/l)			
Control	48.3 ± 11.4	58.9 ± 12.8	0.58
Low-iso	42.3 ± 5.3	64.8 ± 11.3	0.08
High-iso	39.2 ± 4.8	48.8 ± 6.7	0.33

[a] Mean ± SE; samples taken from days 2 to 5 of menstrual cycles 3 and 4.
[b] Values within rows with different symbols(*,†) are significantly different ($P < 0.05$).

Discussion

The objective of this study was to test the hypothesis that premenopausal equol excretors have a plasma hormone profile that is associated with lowered breast cancer risk. The hypothesis was supported by our results in that equol excretors had a more favorable profile when compared with non-excretors at all isoflavone doses studied (0.15, 1.0, and 2.0 mg/kg of body weight/day). This suggests that the inverse association observed previously between urinary equol excretion and breast cancer risk (11) may not have been wholly attributable to differences in isoflavone intake between cases and controls, as suggested by Ingram *et al.* (11), but rather to differences associated with the ability to produce equol.

Of the 14 subjects in this study, 36% were equol excretors (5 of 14 subjects), a percentage consistent with previous studies (13, 15, 16, 18). As isoflavone dose increased, equol excretion increased in the equol excretors but remained relatively constant and low in the equol non-excretors, also in agreement with previous work (25).

The large interindividual variability in equol excretion is thought to be attributable to differences in composition of the intestinal flora, because gut microflora play an important role in isoflavone metabolism (26). Supportive of this are studies showing that germ-free rats fed soy do not excrete equol (27, 28). Although it has been reported that diet may promote growth and/or activity of bacterial populations responsible for equol production (16, 17), there were no differences in dietary intake between equol excretors and non-excretors noted in the current study. Other parameters linked to equol excretion status include intestinal transit time and redox level of the intestine (29).

This is the first study to show that equol excretors have hormone profiles associated with lowered risk of breast cancer (19, 20). In general, equol excretors had lower concentrations of E_1, E_1-S, androgens, and prolactin and higher concentrations of SHBG and progesterone, as well as trends toward longer menstrual cycle and phase lengths, when compared with equol non-excretors. Decreased estrogen concentrations are hypothesized to be protective against breast cancer (30), and numerous epidemiological studies have reported a positive association between estrogens and breast cancer risk (31–34). Although E_2 did not differ between equol excretors and non-excretors, increased SHBG concentrations have been associated with lower concentrations of free estradiol and decreased breast cancer risk (35, 36). Studies on the relationship between androgens and breast cancer risk have yielded inconsistent and at times apparently conflicting results (37, 38). However, much of this has to do with the fact that women with decreased adrenal androgen concentrations appear to have a genetic predisposition toward premenopausal breast cancer (38). In contrast, both higher

adrenal and ovarian androgen concentrations are associated with an increased risk for postmenopausal breast cancer (38). Specifically, higher concentrations of the adrenal androgens DHEA (39, 40) and DHEA-S (34, 39, 41, 42) have been linked to increased risk for postmenopausal breast cancer. Furthermore, higher concentrations of androgens produced both by the ovary and the adrenal gland, including testosterone (32, 35, 41–43) and androstenedione (35, 42), have been linked to increased risk for both pre- and postmenopausal breast cancer. Increased breast cancer risk has also been associated with decreased concentrations of progesterone (30), presumably in part related to the increased frequency of ovulatory dysfunction in hyperandrogenic women with polycystic ovaries (38). Finally, increased breast cancer risk has also been associated with increased concentrations of prolactin (44) and shorter menstrual cycle length (45).

Notably, differences in both equol excretion and plasma hormone concentrations between equol excretors and non-excretors were detectable in subjects consuming only 10 mg isoflavones/day. This level significantly exceeds the estimated British and American intakes of 1–3 mg/day (46, 47), although it is below the most recent estimated Asian intakes of 25–40 mg/day (23, 24). Very low urinary isoflavone excretion values were reported in the Australian case control study, showing an inverse association between equol excretion and breast cancer (11), suggesting that the study population consumed a typical Western diet, with average daily isoflavone intake less than or near our lowest dose. Thus, differences in plasma hormones between equol excretors and non-excretors may be present even with isoflavone doses consistent with a Western diet.

It should be noted that the Australian case-control study by Ingram *et al.* (11) has a number of limitations, as noted in an editorial by Messina *et al.* (48). These include lack of data on dietary fiber intake (raising a concern about the association between lignan and fiber consumption), lack of markers for completeness of urine collection, high variability in analytical methods, and the inability to measure urinary genistein. Although these limitations may weaken the findings by Ingram *et al.* (11), they do not completely negate their potential significance.

In our study, the hormonal differences noted between equol excretors and non-excretors were similar for all three isoflavone doses. This suggests that these differences were not attributable to a direct effect of equol but rather that the ability to produce equol in more than trace amounts may be a marker for the presence of colonic bacterial enzyme activity that increases fecal steroid excretion. In addition to influencing equol synthesis, intestinal flora play an important role in the metabolism of estrogens and androgens (49, 50). Vegetarian and Oriental women excrete greater quantities of fecal estrogens and have lower plasma estrogen concentrations, when compared with omnivorous women (51–53), presumably because of decreased enterohepatic circulation. Perhaps the intestinal microflora responsible for this effect are found in highest quantities in equol excretors.

An alternative explanation is that the hormonal differences noted between equol excretors and non-excretors were a consequence of the smallest isoflavone dose being enough to alter plasma hormone concentrations in equol excretors. The mechanism(s) by which equol itself might affect the regulation of endogenous hormones in a direction beneficial to breast cancer risk are not clear, although our observations of decreased estrogens and increased SHBG concentrations in the equol excretors are consistent with *in vitro* data showing that equol is a weak inhibitor of aromatase, an enzyme catalyzing estrogen synthesis (54), and a stimulus for SHBG synthesis in HepG2 liver carcinoma cells (55). Along these lines, there are epidemiological data showing a positive association between urinary equol and plasma SHBG concentrations (56).

In summary, this is the first study to report that equol excretors and non-excretors show different plasma hormone profiles. The differences observed suggest that interindividual variation in isoflavone metabolism, specifically the ability to excrete equol, may mediate the observed protective effect of equol against breast cancer (11). It is of great interest to consider that modification of equol production status may influence plasma hormones in a direction associated with lowered breast cancer risk. It is also important to note that differences in plasma hormones between equol excretors and non-excretors may contribute to the inconsistent results seen in studies examining the hormonal effects of soy isoflavones in premenopausal women. Future studies of isoflavone effects in humans should therefore consider equol production status.

Acknowledgments

The soy powders were generously donated by Protein Technologies International (St. Louis, MO).

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Cancer Epidemiol Biomarkers Prev 2000;9:581-586.