Urinary Equol Excretion with a Soy Challenge: Influence of Habitual Diet (44241)

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Abstract. **Equol is an isoflavonoid phytoestrogen produced from the soy isoflavone daidzein by gut microflora. Not all humans produce equol from daidzein, presumably due to differences in colonic bacterial populations among individuals. Previously, smaller studies reported that approximately 30% of participants excreted equol when consuming soy. The purpose of our study was to determine the prevalence of equol excreters in a larger sample and to examine what dietary components might influence the tendency to be an equol excreter. Thirty men and thirty women consumed a soy protein beverage containing 22 mg genistein and 8 mg daidzein for 4 days as a supplement to their habitual diets. The mean daily nutrient content of their habitual intakes was determined from 4-day food records. On Day 4, participants provided a 24-hour urine collection. Urinary isoflavonoid (genistein, daidzein, equol, and *O*-desmethylangolensin) excretion was measured by gas chromatography-mass spectrometry. Twenty-one of the 60 participants (35%) excreted equol (> 2000 nmol/day) after 3 days of consuming the soy supplement. Daily equol excretion ranged from 2,134–20,301 nmol/day in the excreters and 21–233 nmol/day in the nonexcreters. There was no difference in equol excreter prevalence between men (43%) and women (27%). Daily excretion of daidzein, genistein, and *O*-desmethylangolensin was similar between equol excreters and nonexcreters and between men and women. Among the women, equol excreters consumed a significantly higher percentage of energy as carbohydrate and greater amounts of plant protein and dietary fiber, both as soluble and insoluble fiber compared to nonexcreters. Such differences were not observed in the men, who overall had significantly higher fiber intakes than the women. These data suggest that, among women, dietary fiber or other components of a high-fiber diet may promote the growth and/or the activity of bacterial populations responsible for equol production in the colon.** [P.S.E.B.M. 1998, Vol 217]

Soybeans are a rich source of isoflavones, biologically active compounds that may play a role in prevention of cancer and other chronic diseases. The predominant isoflavones in soy foods, genistein and daidzein, are present in unfermented soy products primarily as glycosides (1). When consumed by humans, the glycosides are probably hydrolyzed in part by gastric acid (2), and also undergo enzymatic hydrolysis by intestinal microflora (3). Hydrolyzed glycosides can be absorbed from the intestinal lumen or can be metabolized further by bacteria in the colon. This further metabolism yields various intermediate compounds and end products that are also readily absorbed. Equol, *O*-desmethylangolensin (*O*-dma), and dihydrodaidzein have been identified as major metabolites of daidzein (4). In sheep, genistein is metabolized primarily to *p*-ethylphenol (5). *p*-Ethylphenol has not been reported in human urine or plasma; however, low urinary recoveries of genistein suggest that genistein also undergoes significant degradation in humans (2).

A number of soy feeding studies in humans have reported high interindividual variability in excretion of genistein and daidzein and daidzein metabolites (4, 6, 7). Although all individuals in these studies had the capacity to metabolize dietary isoflavones, not all produced equol when fed a source of daidzein. These studies and others, with small numbers of participants and of varying duration, have reported equol excretion prevalence rates of 25%–67% (4, 6–9).

The factors that contribute to the use of a particular

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isoflavone metabolizing pathway (e.g., the capacity to produce equol) are unknown; however, several explanations have been proposed. Setchell *et al.* (9) hypothesized that the composition of the intestinal microflora, intestinal transit time, and variability in the redox potential of the colon might contribute to variation in equol production in humans. Adlercreutz *et al.* (10) reported that equol excretion was positively associated with intake of fat and meat in a Japanese population and suggested that individuals consuming more fat and meat have an intestinal microfloral population more capable of producing equol from daidzein. More recently, Kelly *et al.* (4), observing that patterns of isoflavone excretion remain consistent over several years, postulated that some inherent factor probably plays a more significant role than diet in determining isoflavone metabolism. The relative stability of the composition of human intestinal microfloral populations may account for the consistent patterns of isoflavone excretion. However, external and internal conditions influence microbial populations and their activities and contribute to interindividual differences in bacterial populations. Dietary intake is one of these factors and can mediate its influence directly through a change in substrate availability or indirectly through its effect on host metabolic functions (11).

The objectives of our study were to measure the prevalence of equol excretion with a fixed soy dose in a larger sample size than previously described, to compare the prevalence of equol excreters between men and women, and to compare the energy and nutrient intakes of equol excreters and nonexcreters.

Materials and Methods

Men and women, 20–40 years old were recruited from the University of Minnesota community through posted flyers and an advertisement in the campus newspaper. Potential participants were screened by telephone. Exclusion criteria included: oral antibiotic use within 6 months prior to participation in the study; regular use of prescription medication; pregnancy or lactation: usual soy intake greater than one serving per week (one serving = 28 g soy or tempeh, or 240 ml soy milk); mean alcohol intake of greater than two drinks per day (one drink = 720 ml beer, 240 ml wine, or 90 ml hard liquor); and use of oral contraceptives or other sex steroid treatments within 6 months prior to the study. Of approximately 103 respondents screened, 63 met the participation criteria and agreed to participate in the study by giving informed written consent. Of these 63 participants, 60 (30 women and 30 men) successfully completed the study. Mean (± SD) height and weight for the women and men were 165 ± 7 cm and 60.9 ± 9.5 kg and 177 ± 7.0 cm and 71.5 ± 8.6 kg, respectively.

Participants consumed their usual diets for 4 days, supplemented daily with 34 g powdered soy protein beverage (Altima HP-20 (now called TakeCare), Protein Technologies International, St. Louis, MO). The soy protein powder provided approximately 22 mg genistein and 8 mg daidzein daily, and all participants received soy from the same lot. We chose the 4-day study period to provide a source of daidzein for a long enough time to be able to detect whether a participant could produce equol, but for a short enough time to avoid potential alteration in gut environment. Participants recorded their food intake during the 4 days. Diets were analyzed using the Nutrient Data System (NDS), Version 2.3 (Nutrition Coordinating Center, Minneapolis, MN). The diet analysis did not include the energy and nutrient contribution of the soy protein powder (130 kcal, 20 g protein, 8 g carbohydrate, and 1.5 g fat).

On Day 4, starting after the first morning void, participants collected one 24-hr urine sample, through the first morning void on Day 5. Urine was collected into 1-liter bottles containing 1 g ascorbic acid and stored at 4°C until the collection was complete. Total urine volume was measured, and aliquots were stored with addition of 10% sodium azide (final weight per volume = 0.1% sodium azide) at −20°C until analysis. The isoflavonoids equol, *O*-desmethylangolensin, daidzein, and genistein were extracted from urine by ion-exchange chromatography and were measured by selected ion monitoring (SIM) gas chromatography mass spectrometry as previously described (7, 12). Urine samples were analyzed in batches of 20 samples per batch with quality control urine samples run in duplicate in each batch.

Comparisons of isoflavonoid excretion and nutrient intakes between equol excreters and nonexcreters and between men and women were made using unpaired *t*-tests. Isoflavonoid data were log transformed prior to statistical analyses.

Results

Urinary Isoflavonoid Excretion. An equol excreter was defined as an individual with urinary equol excretion of greater than 2000 nmol/day with the soy challenge (Fig. 1). Nonexcreters excreted less than 250 nmol equol/day. None of the participants had daily equol excretion values between 250–2000 nmol. Prevalence of equol excreters in the 60 participants was 35% (21 of 60), and there was no significant difference in prevalence of equol

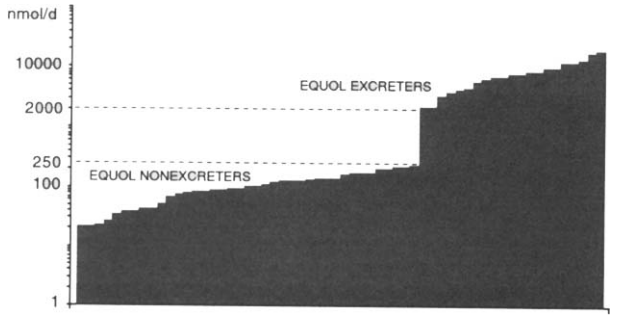

Figure 1. Urinary equol excretion of each of the 60 study participants as nmol/d on a log scale. Equol excreters were defined as those individuals excreting > 2000 nmol/d and nonexcreters as excreting < 250 nmol/d.

excretion in men (13 of 30; 43%) compared to women (8 of 30; 27%). Daily *O*-dma, daidzein, and genistein excretion were not different between the equol excreters and nonexcreters or between men and women (Table 1).

Nutrient Intakes. Among the women, equol excreters compared to nonexcreters consumed significantly more carbohydrate, dietary fiber (both as soluble and insoluble fiber), plant protein and percentage of energy as carbohydrate (Table II). In addition, the mean fat-to-fiber ratio was lower in the equol excreting women. These differences in women remained significant when adjusting individually for body weight and energy intake. Among men, there were no dietary differences between equol excreters and nonexcreters (Table II), and adjustment for body weight did not influence the results. Overall, dietary β-carotene and vitamin C intakes were not different between the equol excreters and nonexcreters. There was no correlation between fiber intake and equol excretion in the equol excreters or nonexcreters.

Comparison of nutrient intakes between the 30 men and 30 women revealed few sex differences. Distribution of energy intake as carbohydrate, protein, and fat (mean ± SD) was similar for men and women, 53 ± 8%, 15 ± 2% and 31 ± 8%, and 56 ± 10%, 15 ± 2% and 29 ± 8%, respectively. When adjusted for body weight, the only nutrient intakes that differed between men and women were total dietary fiber ($p = 0.04$) and soluble fiber ($p = 0.02$); women consumed less of both.

Discussion

Differences in isoflavone metabolizing pathways used may contribute to interindividual variation in isoflavone exposure and consequently to variation in physiologic effects of a given soy dose. This study provides some new information related to equol excretion and raises additional questions that warrant further exploration.

Thirty-five of the 60 participants excreted equol at a level greater than 2000 nmol/day. This is similar to the prevalence rates reported in a number of other smaller studies delivering set daily doses of soy: 4 of 20 men and women (20%) (8); 5 of 17 men (29%) (7); 4 of 12 men and women (33%) (4); and 2 of 6 women (33%) (6). Only one study has found a significantly higher prevalence—4 of 6 men and women (67%) (9). Although a quantitative definition of equol excreter depends, in part, on the amount of daidzein consumed, it appears that among Western populations not usually consuming soy, approximately one-third are equol excreters. The prevalence of equol excreters among populations that regularly eat soyfoods or the effects of long-term soy consumption on equol excretion have not been determined.

This study is the first to compare habitual diets of individuals who excrete equol and do not excrete significant amounts of equol when presented with a defined soy challenge. In an observational study of 19 men and women consuming a traditional Japanese diet, Adlercreutz *et al.* (10) reported that equol excretion correlated positively with the intake of total fat ($P < 0.01$), fat-to-fiber ratio ($P < 0.05$), and meat ($P < 0.05$). They hypothesized that individuals consuming more meat and fat may have the intestinal flora capable of producing equol from daidzein. Our study of individuals consuming a "Western" diet does not support this hypothesis. Rather, the female equol excreters in our study had a significantly higher intake of dietary fiber and a lower fat-to-fiber ratio than the nonexcreters. Major differences in diet, as well as age and potentially colonic microfloral populations, between the Japanese men and women and the participants in our study may contribute to these discrepant findings. The Japanese were consuming approximately 60% of their daily energy intake from carbohydrate and less than 20% from fat and had a fat-to-fiber ratio of 2.5. The men and women in our study were consuming approximately 54% of energy from carbohydrate and 30% from fat, and had a fat-to-fiber ratio of 4. Soyfood intake

Table I. Daily Urinary Isoflavonoid Excretion (nmol/day) among Equol Excreters and Nonexcreters After 3 Days of Soy Protein Supplementation

Isoflavonoid	Women equol excreters $n = 8$	Women equol nonexcreters $n = 22$	Men equol excreters $n = 13$	Men equol nonexcreters $n = 17$
Equol	9,159 ± 6,988[a] (2,241–20,301)	108 ± 65 (22–233)	8,084 ± 3,371 (2,134–13,997)	106 ± 56 (21–223)
Daidzein	10,336 ± 4,444 (4,058–17,732)	11,752 ± 4,887 (541–22,575)	11,913 ± 4,115 (3,938–18,948)	14,017 ± 4,993 (389–22,057)
O-dma[b]	3,336 ± 2,572 (583–8,080)	3,640 ± 3,986 (0–13,289)	3,907 ± 4,365 (0–13,323)	3,921 ± 3,407 (0–11,180)
Genistein	8,233 ± 11,805 (1,126–37,220)	6,333 ± 4,784 (967–19,812)	5,810 ± 3,956 (770–14,347)	5,405 ± 3,979 (60–13,375)
Sum of isoflavonoids	31,063 ± 16,085 (11,091–64,669)	21,832 ± 8,352 (8,360–42,666)	29,744 ± 9,339 (16,633–52,110)	23,448 ± 8,686 (839–38,575)

[a] nmol/day, mean ± SD (range)
[b] *O*-desmethylangolensin

Table II. Nutrient Intakes of Female and Male Equol Excreters and Nonexcreters

Nutrient	Women equol excreters $n = 8$	Women equol nonexcreters $n = 22$	Men equol excreters $n = 13$	Men equol nonexcreters $n = 17$
Energy, kcal	1931 ± 499[a]	1806 ± 493	2417 ± 607	2293 ± 677
Protein, g	66 ± 17	68 ± 21	88 ± 29	84 ± 28
Animal protein, g	38 ± 18	48 ± 17	56 ± 24	54 ± 21
Plant protein, g	26 ± 9[b]	20 ± 6	32 ± 10	29 ± 12
Energy from protein, %	14 ± 2	15 ± 2	14 ± 3	15 ± 2
Fat, g	54 ± 29	63 ± 27	85 ± 37	77 ± 25
Energy from fat, %	24 ± 9	30 ± 8	30 ± 8	31 ± 7
Carbohydrate, g	299 ± 74[b]	238 ± 59	293 ± 89	297 ± 102
Energy from carbohydrate, %	63 ± 10[b]	54 ± 9	55 ± 9	52 ± 7
Dietary fiber, g	17.4 ± 4.5[c]	12.3 ± 4.1	21.2 ± 5.9	18.6 ± 7.3
Soluble fiber, g	5.5 ± 1.2[c]	3.9 ± 1.4	6.6 ± 2.2	6.2 ± 2.5
Insoluble fiber, g	11.8 ± 3.4[b]	8.1 ± 3.0	13.8 ± 4.2	12.2 ± 4.9
Dietary fiber/1000 kcal	9.5 ± 3.4[b]	7.1 ± 2.3	9.2 ± 2.9	8.2 ± 2.0
Fat:fiber ratio	3.3 ± 1.9[c]	5.2 ± 2.0	4.3 ± 2.2	4.6 ± 1.8
β-carotene, μg	4109 ± 5120	2450 ± 306	3331 ± 3057	2592 ± 1894
Vitamin E, mg α-TE	6.9 ± 3.0	6.7 ± 4.0	11.1 ± 8.6	8.8 ± 5.2
Vitamin C, mg	86 ± 55	104 ± 76	148 ±97	137 ± 99

[a] Mean ± SD
[b] Differs significantly from women equol nonexcreters, $P < 0.05$
[c] Differs significantly from women equol nonexcreters, $P < 0.01$

was also higher among the Japanese. These differences suggest that components of diet that determine the capacity of colonic microflora to produce equol may differ among populations, depending on the populations' usual diets.

In a population habitually consuming a diet high in fat and low in fiber, higher intake of dietary fiber may promote the growth and/or activity of bacterial populations responsible for equol production in the colon. Comparison of bacterial populations between groups eating diets high in fiber versus low in fiber have suggested differences in composition of intestinal microflora between groups. Healthy adult urban Canadians consuming a low-fiber and high-fat diet showed a greater percentage of the total bacterial population as *Bacillus* and *Clostridium* and a low percentage as *Bifidobacteria* and *Eubacterium* species. In contrast, the dominant bacterial species in rural Japanese consuming high-fiber, low-fat diets consisted of *Bifidobacteria* and *Eubacterium* species (11). Under controlled dietary conditions, increasing intake of whole grains, dietary fiber, and resistant starch increases the total bacterial number in the colon; however, whether supplementing dietary components change species composition of the microflora and/or bacterial activities remains controversial (13). Some studies suggest that acute dietary fiber supplementation may alter the gut microfloral composition; however, in general, the composition of intestinal microflora is thought to be very stable (11). A systematic comparison of the prevalence of equol excretion among populations with significant differences in fiber and soy intake would provide useful data.

The differences in carbohydrate and fiber intake between equol excreters and nonexcreters were observed only in women. Equol excretion prevalence was not statistically different between men and women, but a greater percentage of men than women tended to be equol excreters. Sex differences in colonic function exist, including differences in gastrointestinal transit time, fecal bulk, and bile acid excretion under controlled dietary conditions (14). In addition, men and women respond differently to amount and type of dietary fiber fed (14). We have also observed sex differences in mammalian lignan production: men excreted more enterolactone and less enterodiol than women when consuming the same diets (8). Thus, a sex-dependent interaction between diet and gut physiology related to effects of sex hormones on gut function and colonic microfloral populations may contribute to these sex differences. Another explanation for the observed differences in diet in this study relates to a measurement issue. Although the men and women were similarly trained in how to keep food records, women may be more likely to estimate accurately portion sizes and to provide detailed food records. Study participants also are known to modify their diets to facilitate recording their food intake. If men are more inclined to do this than women, this might decrease the possibility of observing dietary differences between male equol excreters and nonexcreters.

It is not clear what, if any, the health implications are in having the capacity to convert daidzein to equol. Equol is known to be considerably more estrogenic compared to both daidzein and *O*-dma (15). There are also differences in excretion rates of these compounds. Urinary equol and *O*-dma levels are usually still elevated five times above baseline levels by the third day after a soy challenge, whereas daidzein and genistein are generally back to baseline levels 2–3 days post-challenge (16). The combination of equol's greater estrogenicity and longer time in circulation than daidzein or *O*-dma may result in greater physiologic effects

in equol excreters who regularly consume sources of isoflavones. Among individuals who do not habitually consume soy, physiologic effects of equol are less of an issue, but equol excreter status might be a useful marker of a particular colonic microfloral profile and potential disease susceptibility. The relative consistency with which individuals remain equol excreters or nonexcreters [(4) and unpublished observations] suggests that equol excretion may be a useful long-term biomarker; however, this needs to be examined more rigorously.

The interactions between colonic environment and external and internal factors that modulate it are complex. The results of this study confirm that approximately one-third of individuals habitually consuming a traditional Western diet without soy excrete equol, and provide some evidence, at least in women, that dietary factors may play a role in equol excretion.

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