Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans

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ABSTRACT Soybean consumption may be protective against hormone-dependent cancers, possibly in part because of the isoflavones daidzein and genistein, which are weakly estrogenic. This paper reviews our studies of the metabolism and disposition of these phytoestrogens in humans. During 1 mo of daily soy ingestion in a metabolic unit [1.065 L (36 oz) soymilk, providing 80–210 mg of each isoflavone daily], women initially excreted more isoflavone conjugates in urine than did men. Recoveries of conjugates of genistein, daidzein, and equol were 24%, 66%, and 28% of the amounts ingested in women, respectively, and 15%, 47%, and 15%, respectively, of those in men. A progressive decrease in urinary excretion of genistein and daidzein was observed in women but not in men during the study. At least 10% of ingested daidzin was excreted in urine as daidzein was observed in women but not in men during the progressive decrease in urinary excretion of genistein and daidzein was observed in women but not in men during the study. At least 10% of ingested daidzin was excreted in urine as genistein was observed in women and equol conjugate in one man and one woman after the first soy ingestion. Three more women but no more men developed the ability to produce and excrete large amounts of equol. Absorption rate constants (k) of the isoflavones were estimated to be 0.24–0.50 h⁻¹. The elimination rates (kₛ) for genistein, daidzein, and equol were 0.1, 0.16, and 0.08 h⁻¹, respectively, in women and 0.19, 0.25, and 0.13 h⁻¹, respectively, in men. Thus, the excretion half-life values of genistein were longer in women (7, 4, and 9 h, respectively) than in men (4, 3, and 5 h, respectively) after the first soy ingestion. The excretion half-life shortened progressively in women but lengthened progressively in men over the study period. Thus, isoflavone metabolism and disposition were affected by the duration of soy ingestion and by sex.

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INTRODUCTION

Epidemiologic studies suggest that consumption of legumes protects against the development of colon, breast, and prostate cancers (1–4). Soybeans (soy) are among the most widely consumed legumes. Soy-derived chemopreventive compounds include isoflavones (daidzein and genistein) (4), a protease inhibitor (Bowman-Birk protease inhibitor) (5), phytosterols (6), saponins (7), and inositols (8). Of these components, metabolites of isoflavones (9–12) and of the Bowman-Birk protease inhibitor (S Wan, L-JW Lu, KE Anderson, and AR Kennedy, unpublished observations, 1998) have been detected in animals and humans after soy ingestion. Soy consumption is inversely associated with breast cancer risk in premenopausal Chinese women in Singapore (3) and with prostate cancer risk in men of Japanese ancestry in Hawaii (2). Legumes are also a major source of protein for vegetarians, a group with low risk for many cancers (1). The prevalence of prostate carcinoma is lower among Japanese in Japan than among US whites and blacks (13), which may be attributed to a higher intake of soy phytoestrogens by the Japanese (9).

Soy isoflavones (daidzein and genistein) exhibit estrogenic activities and may act as estrogen agonists or antagonists (14). Daidzein and genistein inhibit cell proliferation (15) and induce cell differentiation (16). Genistein inhibits angiogenesis (17), is a specific inhibitor of tyrosine kinase (18), and inhibits topoisomerase II (15, 19). Although these isoflavones may play important roles in the reduction of cancer risk, adverse reproductive effects have been observed in sheep. These effects were found to be associated with the ability of sheep to metabolize daidzein (and its precursor, formononetin) to equol (20).

Because metabolism and adverse reproductive effects of isoflavones vary greatly among species, it is extremely difficult to extrapolate results from animals to humans. Therefore, it is important to investigate the metabolism and disposition of soy isoflavones in humans during soy ingestion (12, 21, 22).

Two different studies were conducted, one in free-living subjects [6 adolescents (12)] and one in subjects in a metabolic unit [12 adults (21, 22)]. The metabolism and disposition of isoflavones were studied during repeated soymilk ingestion. The study designs were described in detail elsewhere (12, 21, 22). These studies aimed to determine the persistence of diet-induced effects on isoflavone metabolism and disposition. The daily dose of isoflavones ranged from 80 to 200 mg of each isoflavone.

Isoflavones in soymilk or urine samples were analyzed by a gas chromatography–flame ionization detection method as described (12) and confirmed by mass spectrum analysis. Loga-
rithmic values of urinary isoflavone excretion (expressed as mg/h) were used to calculate elimination rate constants \( (k_e) \) and elimination half-lives \( (t_{1/2}) \) (21, 22). The method of residuals (23) was used to estimate the isoflavone concentrations resulting from absorption. If the logarithmic values of these concentrations were linear, the data were then used to calculate the absorption rate constant \( (k_a) \), which was used to calculate absorption \( t_{1/2} \).

**PREFERENTIAL URINARY EXCRETION OF DAIDZEIN OVER GENISTEIN METABOLITES**

The soymilk used for our studies was a homogenized, pasteurized soybean preparation that contained no preservatives. The isoflavone content varied significantly by lot \( (\bar{x} \pm SD: 34 \pm 15 \) and \( 38 \pm 15 \) mg equivalent of daidzein and genistein, respectively) (12, 22). About 80% of both daidzein and genistein in soymilk exists as glucosides. The carbohydrate, protein, and fat contents of different lots of soymilk used were analyzed and varied <1% (analysis by Protein Technologies Inc, St Louis). Isoflavone concentrations in soybeans are known to be affected by season and storage (24), which may explain the variation in isoflavone concentrations in our soymilk.

All subjects (>40 subjects studied to date in our metabolic unit) excreted isoflavones mostly as conjugates in urine, as reported by others (9), and 95% of the amount excreted in urine was excreted within 24 h (Figure 1). There was considerable interindividual variability in excretion of daidzein and genistein conjugates in urine. Even though all subjects ingested slightly more genistein than daidzein (mostly as genistin and daidzin, respectively), all subjects excreted more conjugates of daidzein (30–100% of the intake recovered) than of genistein (0.5–50% of the intake recovered) (12, 21, 22). This differential excretion was observed after acute (12) and chronic (21, 22) soy ingestion, in different ethnic groups, in vegetarians, and in omnivores. Preliminary results from the analysis of blood samples from some of these subjects showed that concentrations of daidzein in plasma within the first 10 h after soy milk ingestion were also higher than those of genistein (25). This preferential urinary excretion of daidzein over genistein metabolites has been observed by others (26–28). These data suggest that daidzein may be more bioavailable than genistein. The biological significance of this differential bioavailability remains to be investigated.

**EFFECTS OF CHRONIC SOY INGESTION ON URINARY EXCRETION KINETICS OF ISOFLAVONES**

Because individuals vary substantially in their ability to excrete isoflavone metabolites, factors modifying the bioavailability of isoflavones before and during controlled soy feedings were studied in subjects in a metabolic unit. This study design used each subject as his or her own control and thereby controlled for interindividual variation.

A typical time course of urinary excretion of conjugates of genistein, daidzein, and equol after the first soymilk ingestion is shown in Figure 1. Urinary excretion of daidzein and genistein in all subjects increased promptly after each dose of soymilk, reached a peak 8–10 h later, and decreased thereafter. The excretion rates before the peak represent a balance of absorption and excretion, whereas the excretion rates after the peak reflect primarily excretion. The excretion rates before and after the peaks were used to calculate \( k_e \), \( k_a \), and \( t_{1/2} \) of absorption and excretion, respectively (21, 22).

Sex-related differences in urinary recoveries of isoflavone metabolites and in elimination rate constants during chronic soy ingestion are shown in Figure 2 (21, 22). A sex-related difference in percentage urinary recoveries of daidzein but not genistein was observed after short-term isoflavone ingestion either in a metabolic unit [Figure 2, A and B, 0 d of soymilk pretreatment (21, 22)] or in a free-living environment (12). Women excreted more of their daidzin intake (as daidzein glucuronide and sulfate, \( \bar{x} \pm SD: 66 \pm 24\% \)) than did men (47 ± 15%; \( P = 0.06 \)). This was also observed in 15–17- y-olds (12). The 15–17- y-old females excreted 69 ± 14% of their daidzin intake as daidzein glucuronide and sulfate and age-matched males excreted 40 ± 7% in urine (\( P = 0.02 \)). Thus, females (15–19 y old) excreted more daidzein than did males. No significant differences between males and females in urinary recoveries of genistein glucuronide and sulfate were observed after acute soy ingestion. However, a difference in urinary recoveries of isoflavones after chronic soy ingestion was observed. In men, chronic soy ingestion did not affect the percentage recovery of either isoflavone (Figure 2A). In women, however, chronic soy ingestion caused a progressive decrease in the percentage urinary recoveries (Figure 2B), peak concentrations, and total amounts (results not shown) of both isoflavones. These data suggest that chronic soy ingestion may decrease the bioavailability of isoflavones in women because of an increase in the reductive metabolism of daidzein to equol and possibly to \( O \)-desmethylandolensin (not measured in our studies). The bioavailability of isoflavones may also decrease in women because of ring opening of genistein to \( p \)-ethylphenol (not measured), as was observed in sheep during long-term grazing on high-isoflavone forages (20, 29–31). Repeated soy isoflavone exposure might increase the activities of the isoflavone-metabolizing enzymes in women. Because the female subjects entered the study within 3–6 d after the onset of menses,
FIGURE 2. Urinary recoveries (A and B), elimination rate constants (C and D), and absorption rate constants (E and F) in 6 men and 6 women during 4 wk of soymilk ingestion. Study subjects were admitted to a metabolic unit for a total of 33 d. During study days 1–4, 16–18, and 30–32, subjects ingested an identical basal diet low in soy. On study days 3 (0 d pretreatment on x axis), 16 (12 d pretreatment), 17 (13 d pretreatment), 30 (25 d pretreatment), and 31 (26 d pretreatment), subjects fasted overnight and then ingested 1.065 L soymilk without other food. Subjects collected urine at 3-h intervals on days 2–4, 16–18, and 30–32. Subjects ingested a 0.355-L portion of soymilk during each meal on days 5–15 and 19–29 and did not ingest soymilk on days 4, 18, and 32. Urinary recoveries were calculated from the amount excreted divided by the amount of glycone plus aglycone ingested. The total amounts excreted are the sums of glucuronide conjugates (>99% of total excreted) and aglycones (<1% of total excreted) estimated after β-glucuronidase digestion. Recoveries for equol (E), daidzein (D), and equol plus daidzein were based on intake of daidzin plus daidzein, whereas those of genistein (G) were based on intake of genistin plus genistein. Values are means ± SDs. Repeated-measures ANOVA using the general linear models procedure and paired t tests were used to compare within-subject differences. P values refer to comparisons with baseline. Reproduced from references 21 and 22 with permission.
cyclic changes in ovarian hormones may also explain the altered isoflavone metabolism during 1 mo of soy feeding.

As shown in Figure 2, C and D, the urinary \( k_t \) of daidzein and genistein conjugates after the first dose of soymilk (ie, without prior soy ingestion) was smaller in women (\( \bar{x} = 0.16 \pm 0.02 \) h\(^{-1} \) for daidzein and 0.10 \( \pm 0.01 \) h\(^{-1} \) for genistein) than in men (0.25 \( \pm 0.05 \) h\(^{-1} \) for daidzein and 0.19 \( \pm 0.03 \) h\(^{-1} \) for genistein). Thus, the elimination \( t_{1/2} \) of both isoflavones was longer in women (4.4 \( \pm 0.7 \) h for daidzein and 6.7 \( \pm 0.8 \) h for genistein) than in men (2.9 \( \pm 0.5 \) h for daidzein and 3.8 \( \pm 0.7 \) h for genistein; \( P < 0.001 \)). In men, the \( k_t \) for both isoflavones decreased progressively during 1 mo of soy ingestion, whereas in women, the \( k_t \) for both isoflavones increased progressively. Thus, chronic soy ingestion increased the \( t_{1/2} \) of isoflavones in both men but decreased the \( t_{1/2} \) of both isoflavones in women. The elimination \( t_{1/2} \) of daidzein metabolites in urine correlated well with that of genistein metabolites during 1 mo of soy ingestion in both men and women. These data suggest that conjugation (eg, glucuronol- or sulfua-transferase) enzyme activities or \( \beta \)-glucosidase in intestinal bacteria may be affected by chronic soy ingestion.

The urinary excretion \( t_{1/2} \) observed in our study subjects after the oral ingestion of genistin and daidzin (glucosides of genistein and daidzein, respectively) was similar to the 4.7 h estimated in mice after oral administration of genistein (the aglycone) by Supko and Malspeis (32). However, the plasma \( t_{1/2} \) of genistein to mice after intravenous injection of genistein was estimated to be 3–7 min during the initial disposition phase and \( \approx 40 \) min during a terminal decay phase (32). Thus, the longer excretion \( t_{1/2} \) of isoflavones after oral administration may relate to the slow absorption of the isoflavones throughout the gastrointestinal tract, as suggested by Supko and Malspeis (32). Furthermore, hydrolysis of soy isoflavone glucosides to isoflavone aglycones was found to be necessary before systemic absorption; in humans, this occurs primarily in the distal parts of the gastrointestinal tract (10). We estimated the absorption \( t_{1/2} \) of daidzein and genistein to be 0.7–2 h and 1.2–3 h, respectively, after the first dose of soy. The absorption rate constants of daidzein were similar in both sexes and were not affected by chronic soy ingestion (Figure 2, E and F). The absorption \( t_{1/2} \) of genistein was slightly longer in women (>2 h) than in men (<2 h; \( P = 0.04–0.07 \)) with and without chronic soy ingestion.

**METABOLISM OF DAIDZEIN TO EQUOL**

Formation of the equol metabolite enhances the reproductive effects of isoflavones in sheep (20). We investigated the ability to metabolize daidzein to equol in our study populations (12, 21, 22; L-JW Lu and KE Anderson, unpublished observations, 1998). We observed that \( \approx 30\% \) of both men (\( n = 11 \)) and women (\( n = 11 \)) could excrete substantial amounts of ingested daidzin as equol conjugates in urine (>10% of the daidzin intake) after an initial dose of soymilk. This was observed in various ethnic groups, in vegetarians, and in omnivores. This interindividual variability in metabolizing daidzein to equol is similar to that reported by others (26, 28, 33, 34). However, we further showed that some women (but no men) were initially unable to metabolize daidzein to equol, but developed this ability during chronic soy ingestion (Figure 2; 21,22). One of the 6 men and 1 of the 6 women who participated in the 1-mo soy diet study could initially metabolize daidzein to equol (16–28% of the daidzin intakes), with initial equol formation and excretion \( t_{1/2} \) estimated to be 2–3 and 5–9 h, respectively. These 2 individuals retained the ability to metabolize daidzein to equol and excreted more equol during 1 mo of soy ingestion (Figure 2, A and B). None of the 5 men unable to produce equol after the first soy dose developed the ability to metabolize daidzein to equol during 1 mo of the soy diet (Figure 2A), but 3 of 5 women who were initially unable to metabolize daidzein to equol developed this ability after 2 wk of soymilk ingestion, during the midmenstrual cycle (Figure 2B). Thus, in women, ability to metabolize daidzein to equol can be increased by prolonged soy ingestion. In these studies, we estimated the \( k_t \) of equol to be 0.03–0.18 h\(^{-1} \) (Figure 2, C and D), which is less than that of daidzein. Because equol is a stronger estrogen than daidzein (35), it will be of interest to determine whether the ability of some subjects to metabolize daidzein to equol influences the endocrine effects of isoflavone ingestion.

In summary, we showed that most of the isoflavones excreted in urine were excreted within 24 h of ingestion of soymilk and that conjugates of daidzein were recovered in the urine in preference to conjugates of genistein after both acute and chronic soy ingestion. These data suggest that genistein may be less bioavailable than daidzein when administered orally. We also observed sex-related differences in the urinary recovery of isoflavone intakes and excretion \( t_{1/2} \) and in the ability to metabolize daidzein to equol as a function of the duration of soy diet exposure.

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**REFERENCES**


