

## Pharmacokinetics of Soybean Isoflavones in Plasma, Urine and Feces of Men after Ingestion of 60 g Baked Soybean Powder (Kinako)<sup>1,2</sup>

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**ABSTRACT** To take advantage of the various pharmacologic activities of soy bean isoflavones, more detailed studies of the absorption and excretion rates of these compounds in humans and subsequent evaluation of their bioavailabilities are required. We conducted a pharmacokinetic study of soybean isoflavones in seven healthy male volunteers. After ingestion of 60 g of kinako (baked soybean powder, containing 103  $\mu\text{mol}$  daidzein and 112  $\mu\text{mol}$  genistein), changes of the isoflavone and metabolite concentrations in plasma, urine and feces were measured by gas chromatography-mass spectrometry. The plasma concentration of genistein increased after 2 h and reached its highest value of  $2.44 \pm 0.65 \mu\text{mol/L}$  6 h later. The plasma concentration of daidzein peaked at  $1.56 \pm 0.34 \mu\text{mol/L}$  at the same time, but it was always lower than that of genistein. Peak plasma concentration of O-desmethylangolensin (O-DMA) and equol appeared after the daidzein peak in four and two subjects, respectively. In contrast with plasma, daidzein was the main component in urine. Urinary daidzein excretion started to increase shortly after the rise in its plasma concentration and reached  $2.4 \mu\text{mol/h}$  8 h after ingestion of kinako. Genistein excretion in urine paralleled that of daidzein, but the value at 6 h was about half ( $1.1 \mu\text{mol/h}$ ). The majority of ingested isoflavones after ingestion of kinako were recovered on d 2 or 3 in the feces. Total recovery of daidzein, O-DMA and equol from urine and feces was 54.7%, calculated from daidzein intake; 20.1% of administered genistein was recovered as genistein. The half-lives of plasma genistein and daidzein were 8.36 and 5.79 h, respectively. The individual plasma and urinary concentrations of equol and O-DMA were quite variable; subjects were classified as high and low metabolizers. The high plasma concentration of isoflavones for at least several hours after a single ingestion of soy protein suggests that these compounds may interact with macromolecules and have biological effects. *J. Nutr.* 128: 1710–1715, 1998.

**KEY WORDS:** • phytoestrogen • genistein • daidzein • humans • kinetics

There is interest in the various pharmacological effects of soy isoflavones such as genistein and daidzein because of their potential role in the prevention of estrogen-related cancers and some diseases caused by estrogen deficiency (Adlercreutz et al. 1982, 1986, 1991a and 1995a, Braezinski et al. 1996, Fotsis et al. 1993, Messina et al. 1994, Setchell et al. 1984, Watanabe and Koessel 1993, Wei et al. 1995). The Japanese consume  $\sim 70$  g soybean per capita per day as assessed by the National Dietary Survey in 1995. Such high levels of soybean products in Japanese diets result in high plasma and urine concentrations of daidzein and genistein and their metabolites (Adlercreutz et al. 1991a and 1993). We hypothesized that these high levels may be responsible for the low mortality

from breast, endometrial, ovarian and prostatic cancers in the Japanese population (Adlercreutz 1990, Watanabe and Koessel 1993).

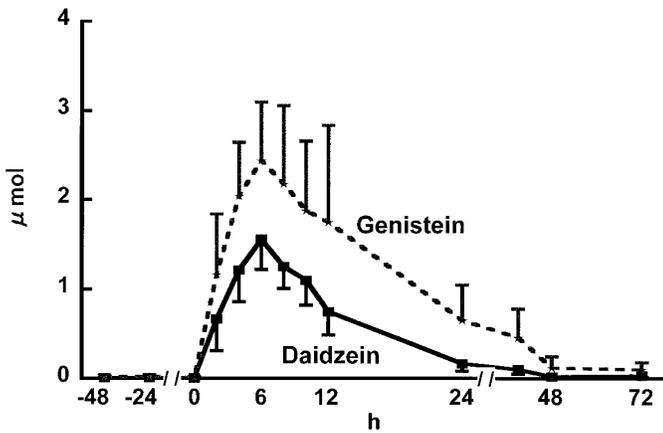
Only one pharmacokinetic study has been conducted in which isoflavones were measured in human subjects simultaneously in plasma, urine and feces (Xu et al. 1994). In that study, genistein and daidzein, but no metabolites, were determined by high performance liquid chromatography (HPLC)<sup>4</sup>. Hutchins et al (1995) determined the metabolites O-desmethylangolensin (O-DMA) and equol in addition to genistein and daidzein in urine after intake of fermented and unfermented soy foods, but no analyses were done in blood or feces. King et al. (1996) studied the pharmacokinetics of pure genistein or genistein after administration of a soy extract to rats and followed genistein concentrations in plasma, urine and feces for 48 h.

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<sup>4</sup> Abbreviations used: HPLC, high performance liquid chromatography; O-DMA, O-des methylangolensin.



**FIGURE 1** Plasma genistein and daidzein concentrations in men after ingestion of 60 g kinako (baked soybean powder). Each point represents the mean  $\pm$  SD,  $n = 7$ .

In soy products, isoflavones occur to a variable extent as glycosides (Brown 1988, Eldridge and Kwolek 1983). This may affect absorption because the glycosidic bond is first split in the upper colon (Hutchins 1995). In rats, however, the overall recovery of genistein seems to be independent of glycosidation (King et al. 1996).

In this study, a more detailed pharmacokinetic evaluation was conducted in seven healthy Japanese men after consumption of a single dose of baked soybean powder (kinako). The isoflavone content of the kinako was carefully measured and the metabolism of the isoflavones followed for 3 d by measuring genistein, daidzein, O-DMA and equol, as well as enterolactone and enterodiol, in plasma, urine and feces by isotope dilution gas chromatography-mass spectrometry in the selected ion monitoring mode.

## MATERIALS AND METHODS

**Subjects.** Seven healthy men participated in this study after giving their written informed consent. Their ages ranged from 23 to 54 y (mean: 33.3 y, median: 30 y). Body height ranged from 165 to 178 cm (median: 171 cm), and body weights from 60 to 81 kg (median: 70 kg). The study design was approved by the ethics committee of the National Institute of Health and Nutrition of Japan. The participants remained in the dormitory of the institute from d 4 to 6, and ate specially prepared meals. Their usual daily movements and light sports were not restricted during this experiment.

**Food and kinako supply.** The participants were requested to consume a soy product-free diet 6 d before and throughout the 10-d study period. They were requested to strictly avoid tofu, natto (fermented soybean), miso (fermented soybean paste) and shoyu (soy sauce). We also requested that lignan-rich foods, such as rye bread, whole grain bread, oatmeal, beer, bourbon whiskey and soba noodles, be avoided. All foods and beverages were measured and reported in dietary records, and the dietary records were checked by trained dietitians. On the morning of d 4, 60 g of kinako (baked soybean powder) (Nisshin Seifun, Tokyo, Japan) dissolved in 200 g of 30 g/L agar water was added to the subjects' breakfast. From d 4 to 6, meals adjusted to the energy requirement of each subject were provided by the dietitian. Energy requirements were calculated according to the height, weight and physical activity of the individuals based on the Recommended Dietary Allowance for Japanese (1995). Breakfast time was 0700 h, lunch time 1200 h and dinner time 1800 h.

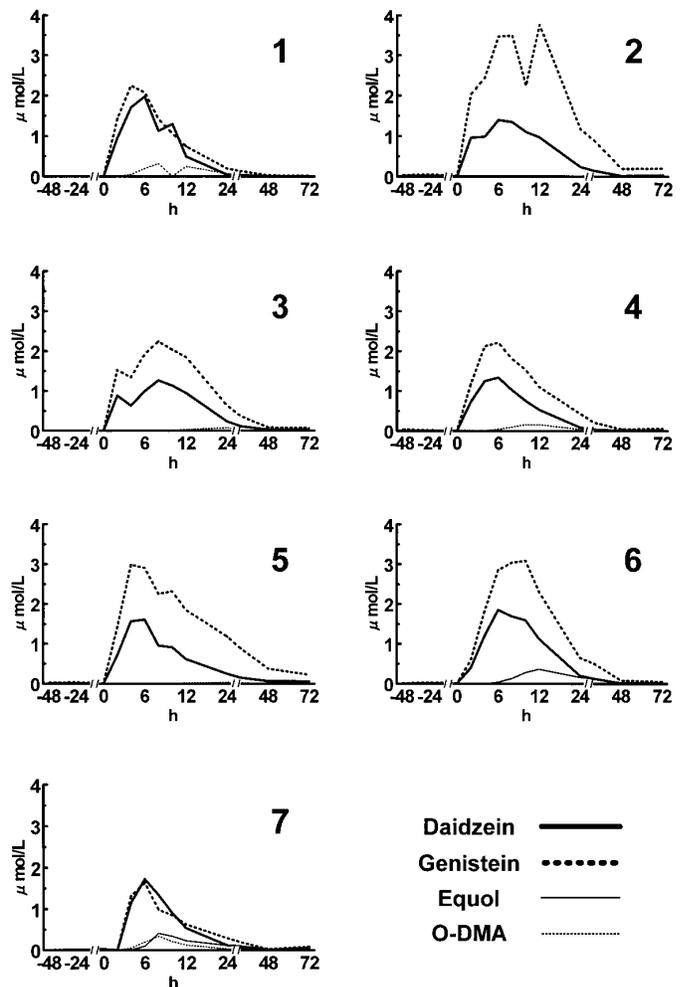
**Blood samples.** Blood (8 mL) was collected in vacuum syringes containing heparin powder every day at 1100 h. On d 4, blood was collected 7 times, i.e., every 2 h after kinako ingestion; additional blood was collected from fasting subjects in the morning on d 5–7.

After centrifugation of tubes at  $2000 \times g$  for 10 min at 4°C, 4 mL of plasma was separated and stored at  $-80^{\circ}\text{C}$  for later measurement of isoflavones.

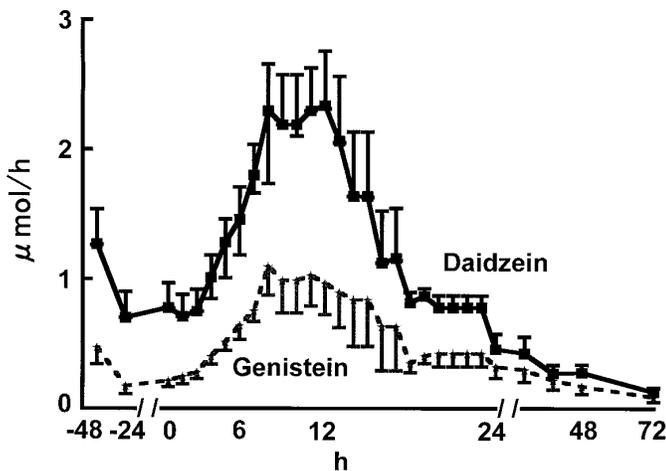
**Urine samples.** A 24-h urine sample (from the second urination of the day to the first urination on the next day) was collected using the U-mate (24-h urine measuring device with proportional sampling method, Sumitomo Bakelite, Akita, Japan). Vitamin C (final concentration  $\sim 10$  g/L) and  $\text{NaN}_3$  (final concentration 0.003 mol/L) (Sigma, Tokyo, Japan) were added to the storage bottle of U-mate to prevent isoflavone oxidation and bacterial contamination. On d 4, each urine sample was separately stored and labeled for time and volume. Each person urinated 6–8 times on that day. About 10 mL of urine were stored in a bottle at  $-80^{\circ}\text{C}$  until assayed.

**Feces.** Feces were collected for 3 d, from the morning of d 5 to d 7. One gram of indigo carmine (Sigma) was consumed when the subject ingested the kinako so that defecation of meals containing kinako could be confirmed by the red color of the stools. All subjects had regular daily bowel movements. After homogenizing the stool, samples ( $\sim 60$  g) were stored in two bottles at  $-80^{\circ}\text{C}$ .

**Determination of isoflavones and lignans.** All samples were analyzed in the Department of Clinical Chemistry, University of Helsinki. The samples were sent frozen in a dry-ice container. Plasma daidzein, genistein, O-DMA, equol, enterolactone and enterodiol were measured by isotope dilution capillary gas chromatography-mass spectrometry using selected ion monitoring with a slight modification of a method described in detail (Adlercreutz et al. 1994). The samples



**FIGURE 2** Changes in plasma genistein, daidzein, O-desmethy-langlansin (O-DMA) and equol concentrations in seven men after ingestion of 60 g kinako (baked soybean powder).



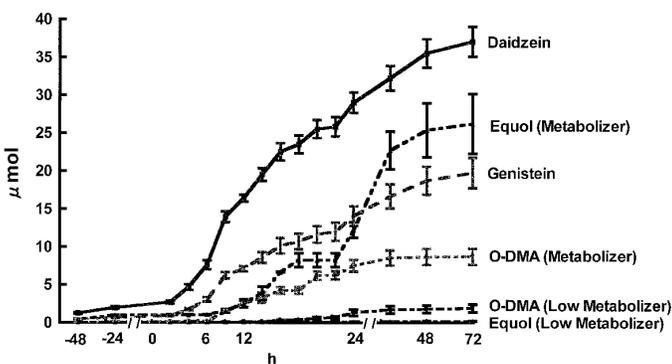
**FIGURE 3** Urinary daidzein and genistein excretion after ingestion in men of 60 g kinako. Each point the the mean  $\pm$  SD,  $n = 7$ .

were purified by ion exchange chromatography. Instead of separating the sulfate and free fraction from the glucuronide fraction, the sulfates and glucuronides were hydrolyzed in consecutive steps and all free phytoestrogens were extracted with diethyl ether. The interassay coefficient of variation varied from 8.7 to 13.6% for all compounds occurring at concentrations  $>0.01 \mu\text{mol/L}$ . The methods for assaying total urinary and unconjugated fecal isoflavonoids and lignans as well as the assay for phytoestrogens in food have been described in detail, including complete documentation of reliability (Adlercreutz et al. 1991b and 1995b, Mazur et al. 1996). Four different portions of kinako agar gel were measured in duplicate, and the average concentrations of the isoflavones were used for calculation of ingested doses (103  $\mu\text{mol}$  daidzein and 112  $\mu\text{mol}$  genistein).

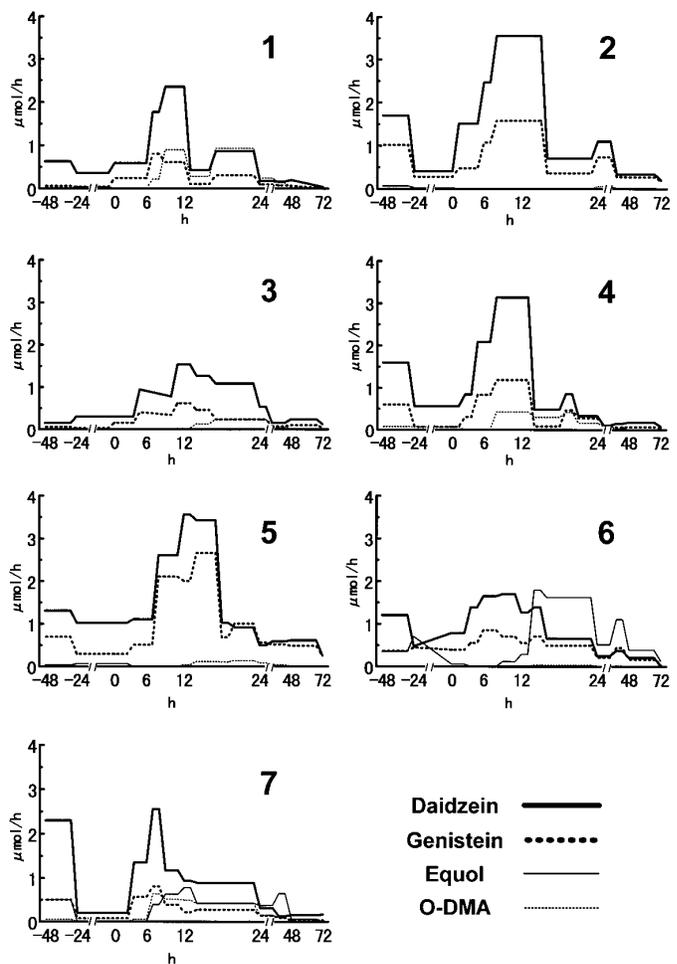
Because the lignan levels in plasma and urine were very low throughout this experiment, these values were not included. The half-lives of plasma isoflavones were calculated by the equation,  $dS(+)/dt = -\lambda dt$ , by using data from the highest average concentration (6 h after kinako intake) to the value 48 h later.  $T_{(\text{half-life})}$  was given by  $T = \log 2/\lambda$ . Values in the text are means  $\pm$  SD,  $n = 7$ .

## RESULTS

**Plasma isoflavones and equol.** Genistein levels were  $\sim 0$  after subjects avoided soybeans and soy products for 6 d. The highest mean level occurred at 6 h after ingestion of kinako



**FIGURE 4** Cumulative excretions of daidzein, genistein, *O*-desmethylangolensin (*O*-DMA) and equol in the urine of men after ingestion of 60 g kinako (baked soybean powder). "Metabolizers" excreted metabolites of daidzein in the urine at levels  $>1\%$  of ingested daidzein. Values are mean  $\pm$  SD,  $n = 7$ .

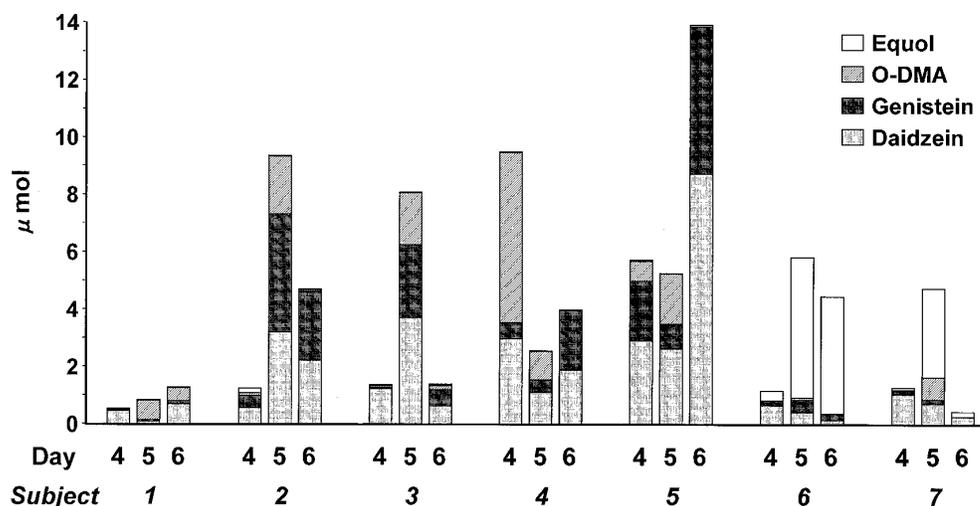


**FIGURE 5** Different patterns of urinary daidzein, genistein, *O*-desmethylangolensin (*O*-DMA) and equol excretion in seven men after ingestion of 60 g kinako (baked soybean powder).

(Fig. 1). Genistein reached  $3.76 \mu\text{mol/L}$  in subject 2, but the mean concentration was  $2.44 \pm 0.65 \mu\text{mol/L}$ . A few subjects showed a second peak or shoulder 12 h after ingestion. The genistein concentration had decreased to the basal level after 48 h, but a few subjects showed a slight elevation of the values above basal levels even 48 or 72 h later (Fig. 2).

The mean peak plasma daidzein concentration was lower than that of genistein ( $1.56 \pm 0.34 \mu\text{mol/L}$ ). In subjects 1 and 7, however, daidzein concentration was as high as that of genistein. The plasma *O*-DMA concentration increased after the increase of daidzein concentration in subjects 1, 3, 4 and 7, and equol level increased only in subjects 6 and 7 (Fig. 2).

**Urinary excretion of isoflavones and equol.** Daidzein and genistein excretions reached a plateau from 8 to 12 h after kinako intake (Fig. 3). Urinary genistein excretion paralleled daidzein excretion, but the value was about half that of daidzein. Excretion of daidzein began to rise shortly after the increase in its plasma level and reached a mean value of  $2.4 \mu\text{mol/h}$  7 h after kinako intake. Urinary excretion of genistein was highest at 8 h ( $1.2 \mu\text{mol/h}$ ). The cumulative excreted dose of daidzein was  $37.0 \pm 13.9 \mu\text{mol}$ ; that of genistein was  $19.7 \pm 14.0 \mu\text{mol}$  (Fig. 4). Excretion of *O*-DMA increased after the peak daidzein excretion in subjects 1, 3, 4 and 7 (Fig. 5). Equol excretion was detected only in subjects 6 and 7, and



**FIGURE 6** Excretion in the feces of daidzein, genistein, O-desmethylangolensin (O-DMA) and equol isoflavones and equol in seven men after ingestion of 60 g kinako (baked soybean powder). Day 4 is the first day after ingestion of kinako.

it occurred several hours after the peak daidzein excretion. These metabolites were quite low in subjects 1–5; thus they were categorized as “low metabolizers.” The excretion of these metabolites was <1 % of ingested daidzein. Characteristically, almost all subjects showed two or three, more or less clear peaks of daidzein and genistein excretion over the 2 d (Fig. 5). In Subjects 1, 2, 3, 4 and 6, daidzein and genistein urinary excretions showed several peaks. Urinary O-DMA appeared a few hours later in urine. In subject 6, daidzein and genistein produced a second lower peak 13–14 h after kinako intake, and equol rapidly increased after the first peak of daidzein excretion.

**Feces.** All seven subjects excreted stools every morning after breakfast, and marker color was detected in the feces mainly on d 4, 24 h after kinako ingestion. O-DMA was the main compound in d-4 stool after kinako ingestion in subject 4, but daidzein and genistein were dominant in d-7 stools in subject 2,4 and 5. Equol was predominant in the stools of subjects 6 and 7 (Fig. 6). The ratio of the total amount of daidzein excreted in urine to that in feces ranged from 5 to

30, that of genistein ranged from 4 to 50, that of O-DMA ranged from 0.5 to 11.8 and that of equol ranged from 0.7 to 6.4 in the individual subjects (Table 1).

**Absorbed and excreted amount of isoflavones.** The 60-g kinako sample contained 103 μmol daidzein and 112 μmol genistein after hydrolysis of the glycosides. The amounts of the other isoflavones were negligible in the kinako. The mean recovery rate of daidzein in urine was 35.8%, and the metabolic conversions of daidzein to O-DMA and equol were 4.0 and 7.0%, respectively (Table 1). The recovery in feces was 4.4% of ingested daidzein for daidzein, 1.9% for O-DMA and 1.6% for equol. The recovery rate of genistein in urine and feces was 17.6 and 1.6%, respectively.

The half-lives of plasma genistein and daidzein were calculated from the equation  $dS/dt = -\lambda t$ . Isoflavone concentrations from the highest plasma concentration to that 48 h later were used for calculations. Regression equations for genistein and daidzein, calculated from highest peak to lowest point, were  $y_{(\mu\text{mol/L})} = -0.036x_{(h)} + 3.593$  ( $R^2 = 1.000$ ) and  $y = -0.052x + 3.481$  ( $R^2 = 0.994$ ), respectively. The half-lives

**TABLE 1**

*Total recovery of isoflavones and isoflavane equol in urine and feces of men for 3 d after ingestion of 60 g kinako (baked soybean powder)*

Subject	Daidzein		O-DMA		Equol		Total	%	Genistein	
	Urine	Feces	Urine	Feces	Urine	Feces			Urine	Feces
	μmol							μmol		
1	27.28	1.08	11.70	0.99	0.16	0.08	51.60	24.0	10.12	0.20
2	47.01	5.28	0.97	1.90	0.22	0.21	81.53	37.9	27.24	6.15
3	28.06	4.91	3.55	1.78	0.07	0.10	49.94	23.2	11.02	2.78
4	29.67	5.28	6.16	6.12	0.13	0.05	58.27	27.1	11.92	2.69
5	64.56	12.59	2.38	2.14	0.30	0.11	128.67	59.8	47.91	7.14
6	32.07	1.07	0.59	0.08	33.51	8.21	92.03	42.8	20.10	0.73
7	30.16	1.77	8.22	0.74	18.80	2.93	69.67	32.4	9.68	0.24
Mean	36.97	4.57	4.80	1.97	7.60	1.67	75.96		19.71	2.85
SD	13.90	4.04	4.10	1.97	13.37	3.07	27.96		14.03	2.82
Recovery, %	35.8	4.4	4.0 <sup>1</sup>	1.9 <sup>1</sup>	7.0 <sup>1</sup>	1.6 <sup>1</sup>	35.3		17.6	2.5

<sup>1</sup> These values are the percentage of recovery of daidzein ingested.

<sup>2</sup> Percentage of recovery of daidzein, including its metabolites in urine and feces together. O-DMA; O-desmethylangolensin.

of plasma genistein and daidzein from these equations were 8.36 and 5.79 h, respectively.

## DISCUSSION

Soybeans contain high amounts of genistein and daidzein (Eldridge and Kwolek 1983, Walter 1941, Walz 1931, Wang and Murphy 1994). In this study, the 60 g of baked soybean powder contained slightly less genistein (103  $\mu\text{mol}$  = 27.8 mg) than daidzein (112  $\mu\text{mol}$  = 28.5 mg). In the study by Xu et al. (1994), the soy milk given to women contained much larger amounts of isoflavonoids, and the relative amount of daidzein was higher, which may explain the similar peak levels of plasma daidzein and genistein in that study. In our experience, when similar amounts of genistein and daidzein are given, the genistein concentration in plasma is almost always higher than the daidzein concentration (Fig. 2). In this study, as in the study by Xu et al. (1994), the urinary daidzein excretion was much higher than that of genistein. In their first study, Xu et al. (1994) found that only 1–2% of the isoflavones ingested were excreted in the feces. In this study, 2.9% (0.2–7.14  $\mu\text{mol}$ ) of the genistein ingested and 4.6% (1.07–12.6  $\mu\text{mol}$ ) of the daidzein were found in the feces. Average urinary recovery of daidzein and genistein was 21 and 9% in the study by Xu et al. (1994), respectively, as opposed to 35.8 and 17.6%, respectively, in this study. When all metabolites of daidzein and the total amounts recovered in urine and feces were calculated in this study, the total recovery of daidzein was 54.7% and that of genistein was 20.1%. In a later study, Xu et al. (1995) found that the individual variation in fecal excretion was large, and that in some subjects it was 10–20 times higher than in others. The recovery of isoflavones in urine and the plasma levels in those cases were also much higher, similar to our own values in this study. They concluded that the breakdown of isoflavones by the microflora in the gut determines the recovery of the compounds. Equol and O-DMA excretion to the urine may depend on the different composition of intestinal microflora. We tentatively categorized them as metabolizer and nonmetabolizer. Karr et al. (1997) found that urinary isoflavone excretion was dose dependent in both equol-excretors and nonexcretors.

In this study, a soy-free diet resulted in a reduction in blood isoflavone levels to very low values after 6 d (Fig. 1), but some excretion of isoflavones in urine was observed (Fig. 3). In plasma, genistein reached basal levels after 72 h, but the decrease in daidzein level was more rapid. The half-life of plasma genistein was longer (8.4 h) than that of daidzein (5.8 h). The rapid appearance of daidzein and genistein in plasma is most likely due to absorption in the upper gastrointestinal tract of free aglycones formed during processing of kinako. Two peaks could be observed in many of the subjects, particularly in the urine, and could be explained by enterohepatic circulation of isoflavones, which has in fact already been described (Sfakianos 1977, Yueh and Chu 1977). Fecal excretion of isoflavones and the isoflavone equol was often higher on d 5 or 6 than on d 4, when the color marker for kinako intake was first detected. This suggests that much of the fecal isoflavones and equol may represent biliary excretion.

In the study on rats by King et al. (1996), absorption of genistein in soy-containing genistein-glycosides was found to be slower than if unconjugated genistein was given, but total recovery was similar in urine and feces after 48 h. The rat is a fecal excretor of steroids, and the recovery of genistein in feces in this study was higher than in urine, varying in the different experiments between 21 and 22% in the feces and between 17.5 and 19.9% in the urine.

Our results demonstrated that O-DMA is a metabolite that appears slightly earlier than equol in the body. O-DMA is a major metabolite of daidzein (Bannwart et al. 1984, Kelly et al. 1993, Yueh and Chu 1977), and it has been suggested that equol is also a metabolite of genistein (Axelson et al. 1984). Because many other metabolites occur that were not measured in this study (Joannou et al. 1995, Kelly et al. 1993 and 1995), recovery, particularly of the daidzein administered, would be at least 60–70% if all metabolites could be measured.

Most of the isoflavonoids in plasma occur in their glucuronide form (Adlercreutz et al. 1993), but small amounts also occur as sulfates. In addition, sulfoglucuronides and disulfates occur in urine (Adlercreutz et al. 1995c) but at much lower levels than the monoconjugates. It has been shown that in humans, estrogen sulfates are taken up by the liver and excreted mainly as glucuronides in the bile (Adlercreutz 1962); the metabolism of isoflavones is likely similar.

We conclude that genistein, the most interesting isoflavone from an anticancer point of view (Adlercreutz et al. 1987 and 1991a, Adlercreutz and Mazur 1997, Fotsis et al. 1993), is the most important isoflavone in plasma of the subjects that consumed the soy products, because it reached the highest concentration and had the longest half-life. This is advantageous from the point of view of preventive medicine. Because the plasma levels of genistein decrease relatively rapidly, the traditional custom in Japanese populations of consuming soy products both morning and evening, a total of about 40 mg of isoflavonoids a day (Watanabe et al. 1997), may be important in the prevention of cancer.

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