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Plasma pharmacokinetics and urinary excretion of isoflavones after ingestion of soy products with different aglycone/glucoside ratios in South Korean women

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Abstract

Asian populations are thought to receive significant health benefits from traditional diets rich in soybeans due to high isoflavone contents. However, available epidemiologic data only weakly support this hypothesis. The present study was carried out to assess the pharmacokinetics of isoflavones in South Korean women after ingestion of soy-based foods. Twenty-six healthy female volunteers (20-30 y old) consumed three different soy products (i.e., isogen, soymilk, and fermented soybeans) with different aglycone/glucoside ratios. Plasma and urine isoflavone concentrations were measured by high-performance liquid chromatography (HPLC) after ingestion of one of the soy products. Pharmacokinetic parameters were determined using the WinNonlin program. The area under the curve (AUC) for plasma daidzein levels of the soymilk group (2,101 ± 352 ng · h/mL) was significantly smaller than those of the isogen (2,628 ± 573 ng · h/mL) and fermented soybean (2,593 ± 465 ng · h/mL) groups. The maximum plasma concentration (C_{max}) of daidzein for the soymilk group (231 ± 44 ng/mL) was significantly higher than those of the isogen (160 ± 32 ng/mL) and fermented soybean (195 ± 35 ng/mL) groups. The half-lives of daidzein and genistein in the soymilk group (5.9 and 5.6 h, respectively) were significantly shorter than those in the individuals given isogen (9.6 and 8.5 h, respectively) or fermented soybean (9.5 and 8.2 h, respectively). The urinary recovery rates of daidzein and genistein were 42% and 17% for the isogen group, 46% and 23% for the fermented soybean group, and 33% and 22% for the soymilk group. In conclusion, our data indicated that soy products containing high levels of isoflavone aglycone are more effective for maintaining plasma isoflavone concentrations. Additional dose-response, durational, and interventional studies are required to evaluate the ability of soy-based foods to increase the bioavailability of isoflavones that positively affect human health.

Key Words: Soy-based foods, isoflavone, pharmacokinetics, plasma, urine

Introduction

Soybeans have traditionally been an important part of the diets of many cultures worldwide. Recently, soybeans have received much attention because of their nutritional profile and beneficial biological effects [1]. According to a previous epidemiological study [2], Asian populations derive significant health benefits from their traditional diets. The lower incidences of certain cancers, cardiovascular disease, osteoporosis, and unpleasant menopausal symptoms seem to be due to the high consumption of soybeans that contain large amounts of isoflavones [3,4].

Isoflavones are naturally occurring plant chemicals (i.e., phytochemicals) that have various physiological activities. Soybean isoflavones have chemical structures that are strikingly similar to those of mammalian estrogens [5] and exert biological effects similar to those of estrogen. Soybean isoflavones can be divided into three chemical groups [daidzeins (4',7-dihydroxyisoflavone), genisteins (4',5,7-trihydroxyisoflavone), and glyciteins (4',7,-dihydroxy-6-methoxyisoflavone)], and their respective β-glycosides [daidzin, genistin, and glycitin (7'-*O*-glucosides, attached at the seventh position)] [6]. In addition, the respective glycosides can be further divided into 7'-*O*-acetylglucosides and 7'-*O*-malonylglucosides. These isoflavones confer various health benefits. In particular, these compounds are currently believed to represent potential alternative therapies for a range of hormone-dependent conditions including cancer, menopause, cardiovascular disease, and osteoporosis [1,7].

The physiological activity of isoflavones depends on the amount of the compounds that can be reasonably consumed as well as their metabolism and absorption in the intestine. After ingestion, soybean isoflavones are hydrolyzed by intestinal glucosidases, leading to the release of aglycones, daidzein, genistein, and glycitein. These compounds may be absorbed or further metabolized into many specific metabolites including equol and *p*-ethylphenol [8]. Once absorbed, isoflavones conjugate with glucuronic or sulfuric acid either in the bowels or liver [8,9].

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The factors influencing isoflavone bioavailability vary depending on absorption and metabolism of the isoflavones, which in turn depend on the specific chemical forms of the isoflavones and the intestinal environment. Soybeans are good sources of isoflavones, and soy-based foods provide a significant dietary source of isoflavones [10,11]. However, the content and composition of isoflavones vary significantly with respect to processing and the type of soy-based food including tofu, soymilk, soybean paste, and soybean sauce [4]. In addition to the large variations in isoflavone content and composition of soy-based foods, intestinal degradation and absorption differ significantly. This may influence the bioavailability and metabolism of isoflavones, thereby affecting their physiological actions. In non-fermented soy-based foods, isoflavones appear mostly as conjugates. In contrast, aglycones are primarily found in fermented soy products such as miso and tempeh [12]. The conjugated forms of isoflavones must be hydrolyzed by intestinal enzymes and absorbed to perform their biological activities [8,13].

Intestinal microflora plays a key role in the metabolism and bioavailability of isoflavones [8,14]. Isoflavones are not absorbed in the absence of intestinal microflora [4]. Indeed, more than 85% of dietary isoflavones may be degraded in the bowels [15]. Variations in the absorption and bioavailability of isoflavones may depend on the bacterial populations in the intestine [4]. This is particularly important since some isoflavones are consumed in forms with relatively low potency and must be chemically modified in the gut into more active compounds. Intestinal microflora can convert daidzein into several different products including equol (7-hydroxyisoflavan), dihydrodaidzein, and *O*-desmethylangolensin [16,17]. This may be clinically relevant to the efficacy of soybean isoflavones because the estrogenic potency of equol is much greater than that of its precursor, daidzein [18]. However, equol production varies widely between individuals and is strongly affected by differences in intestinal microflora [14]. High carbohydrate concentrations, which increase intestinal fermentation, result in more extensive biotransformation of isoflavones with greatly increased formation of equol, a daidzein metabolite [19].

Watanabe *et al.* [20] studied the pharmacokinetics of isoflavones after consumption of baked soybean powder. Additionally, Izumi *et al.* [13] evaluated differences between the absorption of aglycones and glucosides using refined isoflavones. Although several studies have measured the plasma concentrations and urinary excretion of daidzein and genistein [14,15,21], few have been conducted in Asian populations to investigate the pharmacokinetics of isoflavones from soy foods with different aglycone/glucoside ratios. In the present study, the plasma pharmacokinetics and urinary excretion of the isoflavones daidzein and genistein were evaluated in South Korean women after ingestion of three soy products with different aglycone/glucoside ratios. This investigation was performed to determine whether the ingestion of different soy-based foods can increase the bioavailability of isoflavones that positively influence human health.

Subjects and Methods

Subjects

Twenty-six healthy female volunteers living in Seoul who were nonsmokers and not taking any medications, hormones, or dietary supplements participated in this study after signing a written informed consent form. The ages of the subjects ranged from 20 to 30 y (mean ± SD; 25.4 ± 2.8 y) with body weights ranging from 45 to 68 kg (53.0 ± 6.9 kg). The average ages of the isogen consumption group (n = 9), fermented soybean consumption group (n = 9), and soymilk consumption group (n = 8) were 24.1 ± 2.4, 26.1 ± 2.6 and 26.3 ± 2.7 y old, respectively. The average body weights were 53.6 ± 7.4, 52.6 ± 7.3, and 52.6 ± 6.4 kg, respectively. The subjects ate their usual diet except soy food. Their typical daily activities were not restricted during the experimental period. The study protocol conformed to the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Kyung Hee University, Seoul, Korea [KHSIRB-13-030(EA)].

Soy product administration

The subjects received one of three soy products: isogen (refined soy isoflavone), fermented soybeans (soybeans fermented for 3 mo), or commercial soymilk (Dr. Chung's Food Co., Ltd., Cheongju, Korea). Isoflavone contents of the three soy products were analyzed by high-performance liquid chromatography (HPLC) before administration, and the total concentration of daidzein and genistein per unit of soy product was calculated. The doses of each soy product were adjusted so that the levels of total isoflavones were equal. A total of 64.8 mg of isoflavones was administered as 81.8 mg isogen, 43.8 g fermented soybeans, or 600 mL soymilk. Isogen was supplied in capsules, the fermented soybeans were mixed with water before consumption, and the soymilk was ingested as a commercial product. The subjects were instructed to consume the dose within 30 min. Each soy product was supplied to the subjects at 9:00 am after breakfast.

Sample collection

Before the study, the study participants consumed their usual soy product-free diet for 2 weeks. Blood was collected in heparin vacuum tubes from the mid-arm veins 0, 1, 2, 3, 5, 8, and 24 h after soy product ingestion. The collected blood was centrifuged at 1290 × g for 30 min at 4℃ to recover plasma that was stored at -70℃ until further use.

On the first day of the study, 24-h urine samples were collected from the second urination of the first day to the first urination of the next day. Vitamin C was added to the urine storage bottles to prevent isoflavone oxidation. Total volume of the collected urine was measured, and approximately 50 mL of urine was stored at -70℃ until use.

Isoflavone analysis by HPLC

The isoflavone concentrations in the three soy products as well as the plasma and urine samples were analyzed by HPLC (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) as previously described [22-24]. A Spherex5 C18 column (250 × 4.6 mm I.D.; 5 μm; Phenomenex Co., Torrance, CA, USA) was used, and the column temperature was maintained at 35℃. The mobile phase conditions were slightly modified from the methods of other researchers [22], 5 mM NaH_2PO_4 (pH 4.6) and methanol mixing solution (45:55, v:v). Chromatographic separation was monitored at a flow rate of 1 mL/min. Daidzein and genistein concentrations were measured at 260 nm, and equol levels were measured at 210 nm at the maximum absorbance.

Total daidzein and genistein in the three soy products and plasma samples were hydrolyzed with 1 M HCl at 100℃ for 2 h. Aglycone was not hydrolyzed and prepared with the same procedure used for total daidzein and genistein. Total daidzein and genistein in the urine were analyzed using enzymatic hydrolysis methods as previously described [22,23]. To separate the sulfates and glucuronides from urinary isoflavones, 2 or 3 mL of urine were mixed with an enzyme solution (β-glucuronidase and sulfatase in 0.2 M acetate buffer, pH 5) and incubated at 37℃ for 24 h to allow sufficient reaction time. The prepared samples were extracted with methanol, and dried under nitrogen at room temperature. Isoflavones from the samples were dissolved with methanol containing an internal standard (10 μg/mL apigenin) for 2 h at room temperature. After centrifugation at 14,400 × g for 20 min at 4℃, a 20 uL aliquot of the clear supernatant was injected into the HPLC system.

One subject's plasma and urine samples were used for the recovery study. Different amounts of daidzein (0.1-100 μg/mL), genistein (0.1-100 μg/mL), and equol (0.1-50 μg/mL) were added to the plasma samples. Hydrolysis, extraction, and other procedures were the same as those used for plasma sample analysis. The recovery rates for each isoflavone from the plasma samples were estimated based on the known amounts of daidzein, genistein, and equol that had been added to the plasma. The recovery rates were estimated in quadruplicate. The same method was used to evaluate the urine samples and soy products. The recovery rates of daidzein and genistein in the plasma were 63.0% and 62.4%, respectively. The recovery rates of daidzein, genistein, and equol in urine were 58.8%, 54.1%, and 34.8%, respectively. Isoflavone concentrations in the plasma and urine samples obtained from the subjects were adjusted relative to the recovery rates.

Pharmacokinetic analysis

Plasma isoflavone concentrations were calculated at each times. The following plasma pharmacokinetic parameters were calculated using the WinNonlin program (Pharsight Corp., CA, USA): the area under the plasma concentration-time curve (AUC), maximum concentration (C_{max}), time of maximum concentration (T_{max}), half-life ($t_{1/2}$), total body clearance (Cl/F), and volume of distribution (V_d/F).

Statistical analysis

Data were analyzed with the Statistical Analysis System version 8.1 (SAS Institute Inc., Cary, NC, USA). All the values are expressed as the mean ± standard deviation (SD). Daidzein and genistein concentrations were analyzed separately. Comparisons between the groups of subjects were made using a general lineal model (GLM) and Duncan's multiple range test was used to analyze the data. *P*-values < 0.05 were considered statistically significant.

Results

Isoflavone composition of the three soy products

Isoflavone composition of the three soy products is shown at Table 1. The total daidzein and genistein concentrations in isogen, a refined isoflavone product, were 42.4 and 36.8 mg/100 mg, respectively. The fermented soybean product contained 69.8 mg/100 g of total daidzein and 78.2 mg/100 g of total genistein. Soymilk was found to contain 5.2 and 5.6 mg/100 mL of total daidzein and genistein, respectively.

The soy products had different proportions of aglycones. All the isoflavones (100%) in isogen were aglycones. In contrast, 60.1% and 52% of daidzein and genistein in the fermented soybean, respectively, were aglycones whereas only 1.77% and 1.81% of daidzein and genistein in the soymilk, respectively, were aglycones. No equol was found in any soy product.

Plasma concentration of isoflavones

Plasma pharmacokinetic parameters of the isoflavones after the three soy products were ingested are shown in Table 2. Plasma concentrations of daidzein and genistein of the isogen group peaked at 3.78 and 4.67 h, respectively, after ingestion. The concentration of daidzein was higher than that of genistein at

Table 1. Isoflavone compositions of the three soy products

	Isogen (mg/100 mg)	Fermented soybeans (mg/100 g)	Soymilk (mg/100 mL)
Daidzein			
Total	42.4 ± 0.56	69.8 ± 0.61	5.20 ± 0.09
Aglycone (% aglycone)	42.4 ± 0.56 (100%)	42.0 ± 0.21 (60.1%)	0.09 ± 0.01 (1.77%)
Genistein			
Total	36.8 ± 0.47	78.2 ± 0.84	5.60 ± 0.29
Aglycone (% aglycone)	36.8 ± 0.47 (100%)	40.7 ± 0.14 (52.0%)	0.10 ± 0.01 (1.81%)

Values are presented as the mean ± standard deviation (SD) or percentage. Isogen, refined soy isoflavone; fermented soybeans, 100% soybeans fermented for 3 mo; soymilk, commercial soymilk purchased from Dr. Chung's Food Co., Ltd.

Table 2. Pharmacokinetic parameters of the isoflavones in plasma after administration of the three soy products

Products (% aglycone)	AUC (ng · h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	Cl/F (L/h)	V_d/F (L)
Isogen						
Daidzein (100%)	2,629 ± 573.1^{A}	230.4 ± 44.2^{NS}	3.78 ± 1.2^{NS}	9.75 ± 3.8^{A}	12.2 ± 5.4^{B}	211.4 ± 66.0^{B}
Genistein (100%)	2,356 ± 672.8^{NS}	160.1 ± 32.4^{Y}	4.67 ± 2.5^{NS}	8.53 ± 2.2^{X}	15.1 ± 5.8^{NS}	226.5 ± 45.1^{Y}
Fermented soybean						
Daidzein (60.1%)	2,594 ± 465.2^{A}	214.0 ± 52.9	2.88 ± 1.5	9.54 ± 1.9^{A}	12.9 ± 3.7^{B}	295.4 ± 76.1^{A}
Genistein (52.0%)	2,279 ± 724.6	195.7 ± 35.4^{XY}	3.50 ± 0.8	8.22 ± 2.1^{X}	17.4 ± 6.1	347.0 ± 93.4^{X}
Soymilk						
Daidzein (1.77%)	2,101 ± 352.4^{B}	211.2 ± 58.3	3.71 ± 2.1	5.92 ± 1.7^{B}	19.0 ± 5.4^{A}	131.4 ± 25.8^{C}
Genistein (1.81%)	2,326 ± 332.4	231.1 ± 44.3^{X}	4.86 ± 1.9	5.64 ± 0.7^{Y}	13.5 ± 2.4	104.5 ± 27.6^{Z}

Values are presented as the mean ± SD or percentage. Data for daidzein and genistein were analyzed separately. Values in a column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test. NS, not significant at $P < 0.05$ according to Duncan's multiple range test (daidzein and genistein were not significantly affected by the type of soy product).
Isogen, refined soy isoflavone; fermented soybean, 100% soybean fermented for 3 mo; soymilk, commercial soymilk purchased from Dr. Chung's Food Co., Ltd.
AUC, area under the plasma concentration-time curve; C_{max}, maximum concentration; T_{max}, time of maximum concentration; $t_{1/2}$, half-life; Cl/F, total body clearance; V_d/F, volume of distribution.

all time points, and the AUC of daidzein was greater than that of genistein. The mean plasma half-lives of daidzein and genistein were 9.8 and 8.5 h, respectively. After consumption of the fermented soybeans, the plasma concentrations of daidzein and genistein peaked at 2.88 and 3.50 h, respectively. The concentration of daidzein was higher than that of genistein at all time points although a greater quantity of genistein was ingested. The AUC of daidzein was greater than that of genistein. The mean plasma half-lives of daidzein and genistein were 9.5 and 8.2 h, respectively. In the participants who consumed soymilk, the plasma concentrations of daidzein and genistein peaked at 3.71 and 4.86 h, respectively, after ingestion. The AUC of genistein was greater than that of daidzein. The mean plasma half-lives of daidzein and genistein were 5.9 and 5.6 h, respectively.

Urinary excretion of isoflavones

Each study group had different urinary excretion patterns and recovery rates after consuming the three different soy products (Table 3). Consumption of the three soy products influenced urinary isoflavone excretion. After isogen ingestion, the urinary excretion rates of daidzein, genistein, and equol were 42.1%, 16.8%, and 12.8%, respectively. The urinary excretion rates of daidzein, genistein, and equol for the fermented soybean consumption group were 45.8%, 23.4%, and 13.9%, respectively. After the administration of soymilk, the urinary excretion rates of daidzein, genistein, and equol were 33.8%, 22.1%, and 9.6%, respectively. The urinary excretion rate of daidzein was lower than that of the isogen and fermented soybean groups. The urinary excretion rate of genistein was the lowest after isogen ingestion. The urinary excretion of genistein was significantly less than that of daidzein after administration of the three soy products. The soymilk group exhibited the lowest equol excretion rate among the three groups. It was also noted that equol excretion varied greatly from subject to subject.

Table 3. Urinary isoflavone excretion and recovery after administration of the three soy products expressed as percentages of the dose ingested for 24 h

	Isogen	Fermented soybeans	Soymilk
Intake (total 64.8 mg)			
Daidzein (% aglycone)	34.7 (100%)	30.6 (60.1%)	31.2 (1.77%)
Genistein (% aglycone)	30.1 (100%)	34.2 (52.0%)	33.6 (1.81%)
Urinary excretion (total, mg)			
Daidzein	14.69 ± 5.62^{A}	14.01 ± 3.68^{A}	10.54 ± 2.49^{B}
Genistein	5.09 ± 1.61^{Y}	8.00 ± 2.68^{X}	7.41 ± 2.29^{X}
Equol	4.44 ± 2.06^{NS}	4.27 ± 4.82	2.99 ± 2.37
Urinary recovery (%)			
Daidzein	42.3 ± 16.2^{A}	45.8 ± 12.0^{A}	33.8 ± 8.0^{B}
Genistein	16.9 ± 5.4^{Y}	23.4 ± 7.8^{X}	22.1 ± 6.8^{X}
Equol	12.8 ± 6.0^{NS}	13.9 ± 15.8	9.6 ± 7.6

Values are presented as the mean ± SD or percentage. Data for daidzein, genistein, and equol were analyzed separately. Values within the same row with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.
NS, not significant at $P < 0.05$ according to Duncan's multiple range test (daidzein, genistein, and equol were not significantly affected by the type of soy product consumed).
Isogen, refined soy isoflavone; fermented soybeans, 100% soybeans fermented for 3 mo; soymilk, commercial soymilk purchased from Dr. Chung's Food Co., Ltd.

Discussion

Isoflavone aglycones are absorbed more efficiently than isoflavone glucosides. Izumi *et al.* [13] studied the pharmacokinetics of isoflavone aglycones and glucosides, and found that plasma concentrations of aglycones are significantly higher than those of glucosides after intake of isoflavones. We obtained similar results in the current study showing that soymilk contained less than 2% aglycones, and the AUC of daidzein was smaller than those of isogen and fermented soybeans. Isogen contained 100% of the isoflavone aglycone and fermented soybeans contained > 50% of the isoflavone aglycones. Plasma concentrations of daidzein in the groups with high aglycone contents were higher than those with low aglycone contents. However, the plasma

concentrations of genistein were similar among all groups.

Isoflavone glucosides are very poorly absorbed from the gut compared to isoflavone aglycones due to their greater hydrophilicity and molecular weight [25]. It is assumed that glucosides must be converted into aglycones to be absorbed in the human body. Friend and Chang [26] reported that glucosidases of intestinal microflora in the bowels can separate aglycones from glucosides, thereby promoting their absorption. Our results demonstrating that the three soy products contained different amounts of aglycone partially support these previous findings. Nevertheless, the plasma concentration of genistein for the fermented soybean (high aglycone content) group was not greater than that of the soymilk (low aglycone levels) group. Genistein contains a hydroxyl group in the fifth position of the A-ring. Structural differences between genistein and daidzein may result in genistein being broken down by the gut microflora [27]. In addition, aglycones are more easily degraded by the microflora than glucosides.

Many studies [13,20,28] have shown that the plasma concentration of genistein is higher than that of daidzein. In contrast, our results show that the plasma concentration of daidzein was higher than that of genistein after the subjects were given isogen, which contains a high level of isoflavone aglycone. However, glucosides were predominant in the subjects given soymilk and the plasma concentration of genistein was higher than that of daidzein. These patterns were similar to ones observed in soymilk administration studies by Xu *et al.* [15] and Zhang *et al.* [29].

In our investigation, the plasma half-life of daidzein was longer than that of genistein after the consumption of all the three soy products. In contrast, Watanabe *et al.* [20] reported that the half-life of genistein in plasma is longer than that of daidzein after consumption of baked soybean powder containing isoflavone glucosides. Izumi *et al.* [13] also found that the half-life of genistein in plasma is higher and elevated for a longer period of time than that of daidzein after isoflavone aglycone intake.

When isogen and fermented soybeans were ingested, the AUC of daidzein was greater than that observed with soymilk due to the absorption phase time gap. This gap is usually attributed to the aglycone ratio and the type of soy product. The results of our study could be explained by the relatively low percentage of aglycones in soymilk. The relative bioavailability of soymilk is lower than that of fermented soybeans because the aglycone ratio in soymilk is lower than that in fermented soybeans. Bioavailability increases as the percentage of aglycones increases.

The half-lives of isoflavones were significantly shorter after soymilk intake compared to isogen and fermented soybean intake. This is because total daidzein and genistein contents were measured after the hydrolysis of isoflavones that exist in plasma in the bound forms. Once isoflavones are absorbed in the intestine, they exist in plasma as isoflavone glucuronides, isoflavone sulfates, and isoflavone glucosides through the processes of glucuronidation, sulfatation, and glycosidation, respectively. Since the ratio, formation, and elimination of each type of isoflavone are different, the half-lives could differ as well. Furthermore, aglycone ratios in the soy products and intersubject variation are other possible reasons for differences observed for the half-lives. Daidzein clearance was significantly more rapid in the soymilk group than in the isogen and fermented soybean groups; this can be attributed to the short half-life of isoflavones. It is necessary to provide the same amounts of isoflavones according to their half-life to accurately measure the plasma isoflavone concentration in a steady state. However, it was assumed in our study that the difference between minimum and maximum concentrations was quite remarkable because isoflavones were consumed every 24 h.

The three soy products contained different levels of isoflavone glucosides and aglycones. Moreover, the rates of isoflavone recovery from urine differed depending upon what soy product was administered. Results from our experiment showed that the urinary excretion of daidzein after the intake of soymilk, in which less than 2% of the total isoflavones were aglycones, was lower than that observed after isogen and fermented soybean ingestion. The urinary excretion rates of daidzein from participants consuming soy-based foods containing low aglycone ratios were lower than those administered soy-based foods containing high aglycone ratios. The urinary excretion rate of genistein was the lowest after ingestion of soy-based foods containing high aglycone ratios. The urinary equol excretion rates, especially considering the variation observed among the subjects, was lower after the consumption of soy-based foods containing low aglycone contents.

Isoflavone glucosides may be poorly hydrolyzed by mammalian intestinal digestive enzymes. Glucosides are more hydrophilic than aglycones, and their greater molecular weight also limits their absorption [25]. Urinary isoflavones in study found that subjects who ingest soy-based foods containing high levels of isoflavone glucosides have lower rates of urinary isoflavone excretion. It is likely that the hydrolysis of isoflavone glucosides into aglycones by β-glucosidases of the gut microflora is required for soy isoflavone absorption [14]. Another study [30] reported a similar conclusion. In that investigation, the urinary excretion of isoflavones from subjects given tempeh or soybean pieces (the aglycone/glucoside ratio of tempeh was 20-fold greater than that of soy beans) was compared. The recovery of daidzein was 70% greater with tempeh than soybean pieces. Genistein was also recovered to a significantly greater extent after tempeh ingestion than after the consumption of soybean pieces. Zhang *et al.* [29] reported that different isoflavone aglycone contents of soy-based foods affect the main type of isoflavone in urine. However, Xu *et al.* [31] found that although four different soy-based foods used in the study contained various amounts of isoflavone glucosides and aglycones, the resulting urinary recovery rates of daidzein and genistein were not significantly different. A study of the renal excretion of various compounds in the urine of 12 volunteers before and after a soy challenge showed significant variations in the levels of excreted daidzein and genistein as well as their metabolites equol, *O*-desmethylangolensin, and 6'-hydroxy -*O*-desmethylangolensin [32]. These findings indicate that there

are large variations in intestinal degradation and absorption rates in addition to large variations in the isoflavone contents of soy-based products.

Izumi *et al.* [13], Watanabe *et al.* [20], and King and Bursill [28] reported that the urinary excretion rate of daidzein is much higher than that of genistein; our results corroborate these findings. Griffiths and Smith [27] showed that isoflavones and flavonoids possessing a hydroxyl group in the fifth position of the A-ring, such as genistein, are much more susceptible to C-ring cleavage by rat gut bacteria. The structural differences between daidzein and genistein may result in reduced microfloral degradation of daidzein. Consequently, more isoflavones broken down by bacteria would result in lower isoflavone concentrations detected in urine. This may explain why less genistein was recovered from urine in the present study. The sum of urinary recovery rates for daidzein and equol ranged from 43.4% to 59.7% in the present investigation. Since urine was only collected for 24 h, total urinary isoflavone excretion could not be measured in the present study considering the half-lives of isoflavones in plasma. To overcome this drawback, urine must be collected for a long time after the intake of isogen and fermented soybeans. Future recovery studies are required.

Soy isoflavones reportedly have estrogenic [18], antioxidative [33,34], antiosteoporotic [35,36], and anticarcinogenic [37] activities. Therefore, these compounds are expected to be effective for ameliorating various conditions such as menopausal symptoms, coronary heart disease, osteoporosis, and cancer. To obtain the desired effects, it will be necessary to maintain constant plasma concentrations of the isoflavones for long periods of time. The dose and intake duration are likely to be the major factors that influence the clinical and biological outcomes of an isoflavone-rich soy-based diet.

In summary, the results of our study suggest that soy-based foods containing high isoflavone aglycone contents are more effective for achieving high isoflavone concentrations in plasma. Therefore, isoflavone aglycone-rich soy-based foods such as fermented soybeans, tempeh, and soy paste may be useful for promoting human health. Further research including dose-response as well as durational and interventional studies is required to evaluate the ability of soy-based food intake to increase the bioavailability of isoflavones that positively influence human health.

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