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Comparison of regulative functions between dietary soy isoflavones aglycone and glucoside on lipid metabolism in rats fed cholesterol

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Abstract

The effects of dietary soy isoflavones aglycone and glucoside on lipid metabolism were compared in male Sprague–Dawley rats (4 weeks old) given purified diets containing 0.3% cholesterol. The rats were fed a diet supplemented with either isoflavone aglycone-rich powder (IF-A group) or isoflavone glucoside-rich powder (IF-G group) or isoflavone-free diet (control group) for 40 days. The additional level of isoflavone aglycone moiety in the diet was prepared to the same level (approximately 0.096 g/100 g: approximately 0.1% in diet). The activity of hepatic cholesterol 7α-hydroxylase tended to be slightly higher in the rats fed isoflavones than in those fed the isoflavone-free diet. On the other hand, the activity of hepatic Δ6 desaturase in the IF-A group was lower than that of the control group. Reflecting this effect, the Δ6 desaturation indices [(20:3n-6+20:4n-6)/18:2n-6] in liver phospholipids of the IF-A group were lower than those in the control group. Liver and serum total cholesterol levels and liver TG level were also reduced by consumption of isoflavone aglycone. Moreover, serum TG level was lowered by consumption of both isoflavones aglycone and glucoside. The level of serum total isoflavones in the IF-A group was significantly higher than that in the IF-G group. Therefore, we speculate that the absorption speed of isoflavone aglycones might be faster than that of isoflavone glucosides in rats. This study suggests that dietary soy isoflavones, particularly their aglycone form, may exert a beneficial effect on lipid metabolism in rats fed cholesterol.

Keywords: Soybean; Isoflavone; Aglycone; Glucoside; Lipid metabolism; Rats

1. Introduction

Some epidemiological evidence suggest that high consumption of soybean and soy products may confer a reduced risk of cancer [1–3] and incidence of cardiovascular diseases [3,4]. Many studies have elucidated that soy isoflavones, among various soy components, have many bioactivities and pharmacokinetics attributing to healthy maintenance and promotion including anticarcinogenic action [5–7], antiosteoporosis [8–10] and atherosclerosis prevention [10–14].

Soybean and soy products have about 0.1–0.3 mg isoflavones/100 mg protein, and many isoflavones are malonyl- and β-glycoside derivatives [15]. The primary soy isoflavones are genistein, daidzein and much lower amounts of glycitein and their respective β-glucosides, genistin, daidzin and glycitin [15]. Bioavailability of soy isoflavones is different between isoflavones aglycone and glucoside [16]. However, the study concerning bioavailability of soy isoflavones has remained controversial. Richelle et al. [17] demonstrated that similar plasma and urine pharmacokinetics were observed for the aglycone and glycoside drinks in humans. On the other hand, there is also a report that the absorption speed of isoflavone aglycone is faster than that of isoflavone β-glucoside and that the amount of absorbed isoflavones is higher in aglycone form than in β-glucoside form in humans [18]. There are some reports stating that isoflavone aglycones are superior to isoflavone glucosides in various bioactivities [8,13,19,20]. Therefore, we have speculated that dietary isoflavone aglycones may be effective for a variety of bioactivities compared with isoflavone glucosides.

Abbreviations: FA, fatty acid; GLC, gas liquid chromatography; PG, prostaglandin; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2X, prostaglandin I_2X; TLC, thin-layer chromatography.

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At present, the study on soy isoflavones has focused on the exertion of weak estrogenic activities by virtue of structural similarity to a female hormone, estrogens [21,22]; it may thus be possible that dietary soy isoflavones affect a regulation of hormonal homeostasis in women. It has been reported that soy isoflavones are an effective dietary factor in preventing bone loss [9,10]. Moreover, the consumption of intact soy protein resulted in a significant reduction in total cholesterol and LDL cholesterol plus VLDL cholesterol compared with a diet containing soy protein without isoflavones in female cynomolgus monkeys [23]. It has been inferred that the estrogenic activities of soy isoflavones might be responsible for the lipid-lowering effects of soy protein. Generally, dietary soybean protein decreases both the levels of serum cholesterol and TG and therefore reduces the incidence of cardiovascular disease compared with animal protein [23–25]. These reports raise the possibility that coexisting phytochemicals such as isoflavones may contribute to various beneficial effects on lipid metabolism by dietary soybean protein.

The development of atherosclerosis is influenced by the excessive level of cholesterol and the inflammatory reaction accompanying the imbalance of eicosanoid level. The liver is the primary tissue in cholesterol metabolism, and cholesterol synthesis is regulated by the activity of HMG-CoA reductase. Moreover, hepatic cholesterol 7α-hydroxylase is the key enzyme of bile acid conversion from cholesterol. Therefore, this enzyme controls the major pathway for elimination of cholesterol from the body. On the other hand, Δ6 desaturase is the rate-limiting enzyme related to the conversion of linoleate into arachidonate. Arachidonic acid exists in cell membrane phospholipids at a relatively high level and is a substrate for the synthesis of a variety of eicosanoids, which are a family of chemical mediators and have inflammatory effects. Therefore, the regulation of lipid metabolism is an important factor in the prevention of the development of atherosclerosis. However, the regulative function of soy isoflavones on lipid metabolism and the difference of biological action between soy isoflavones aglycone and glucoside are still unknown. Therefore, we compared the differences of regulative function on lipid metabolism between soy isoflavones aglycone and glucoside in rats fed cholesterol.

2. Methods and materials

2.1. Chemical

[1-^{14}C]Linoleic acid (55 mCi/mmol) and [4-^{14}C]cholesterol (52 mCi/mmol) were the products of New England Nuclear (Boston, MA, USA). Radioactive compounds were purified by thin-layer chromatography (TLC) before use. Isoflavone-rich powders were purchased from Kikkoman [Tokyo, Japan, "Soyact" (isoflavone aglycone-rich powder)] and Fujicco [Kobe, Japan, "Fujiflavone P40" (isoflavone glucoside-rich powder)]. Other reagents were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

2.2. Animal and diets

Male Sprague–Dawley rats (3 weeks old, CLEA Japan, Tokyo, Japan) housed individually in a room with controlled temperature (20–23°C) and light (8–20 h). After rats were acclimatized for 1 week, they were divided into three groups of seven or eight each; the first group was fed an isoflavone-free diet (control group, $n=7$), the second group was fed a 0.365% isoflavone aglycone-rich powder-added diet (IF-A group, $n=8$) and the third group was fed a 0.3% isoflavone glucoside-rich powder-added diet (IF-G group, $n=7$). Since the concentrations as aglycone moiety of Soyact and Fujiflavone P40 were 0.263 and 0.320 g/g, respectively, the additional level of isoflavones into the diet was prepared to the same level as aglycone moiety [The amount of isoflavone aglycone moiety in the IF-A diet: 0.263 g×0.365/100 (0.365% in diet)=0.096 g/100 g; the amount of isoflavone aglycone moiety in the IF-G diet: 0.320 g×0.3/100 (0.3% in diet)=0.096 g/100 g.] Experimental diets were prepared according to the recommendations of the American Institute of Nutrition [26], and their ingredients are shown in Table 1. Diets were prepared weekly and were packed in pouches and stored at 4°C to prevent autoxidation of lipids. After 40 days, rats were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia at night (1:00 am) for analysis of hepatic cholesterol metabolism. The liver was excised immediately and microsome was prepared at 4°C. The serum was prepared by centrifugation after allowing blood to clot at room temper-

Table 1
Diet composition

Ingredient	Group		
	Control	IF-A (g/kg diet)	IF-G
Cornstarch	364.486	360.836	361.486
Casein	200.000	200.000	200.000
α-Cornstarch	132.000	132.000	132.000
Sucrose	100.000	100.000	100.000
Safflower oil	100.000	100.000	100.000
Cellulose	50.000	50.000	50.000
Mineral mix (AIN-93G)	35.000	35.000	35.000
Vitamin mix (AIN-93)	10.000	10.000	10.000
L-Cystine	3.000	3.000	3.000
Choline bitartrate	2.500	2.500	2.500
Tert-butylhydroquinone	0.014	0.014	0.014
Cholesterol	3.000	3.000	3.000
Isoflavone-rich powder	–	3.650	3.000

Abbreviations: Control, rats fed isoflavone-free diet; IF-A, rats fed isoflavone aglycone-rich diet; IF-G, rats fed isoflavone glucoside-rich diet. We used isoflavone-rich powders, which are Soyact (IF-A) and Fujiflavone P40 (IF-G). The levels of aglycone moiety of Soyact and Fujiflavone P40 were 0.263 and 0.32 g/g, respectively. The additional level of isoflavones into the diet was prepared to the same level as aglycone moiety (the amount of isoflavone aglycone moiety in IF-A diet, 0.263 g×0.365/100=0.096 g/100 g; the amount of isoflavone aglycone moiety in IF-G diet, 0.32 g×0.3/100=0.096 g/100 g).

ature. These samples were kept at −30°C until analysis to avoid oxidation. Feces were collected from each rat before the 20 days the rats were killed and then lyophilized. The Hirosaki University Animal Policy approved this animal study, and rats were maintained according to the guidelines for the care and use of laboratory animals of the Hirosaki University.

2.3. Measurement of hepatic key enzymes of lipid metabolism

The activities of hepatic cholesterol 7α-hydroxylase and Δ6 desaturase were measured by the methods of Van Cantfort et al. [27] and Svensson [28], respectively. The level of microsomal protein was determined by the method of Bradford [29].

2.4. Lipid analyses

Liver and serum lipids were extracted by the method of Folch et al. [30], and the concentrations of liver and serum phospholipid, liver and serum total cholesterol and liver TG were measured as described previously [31]. The serum TG and HDL cholesterol were measured using triglyceride-E and HDL-cholesterol-E tests (Wako Pure Chemical Industries), respectively. Lipid classes of liver were separated using preparative TLC, and their fatty acid (FA) composition was analyzed by gas liquid chromatography (GLC) with a flame ionization detector after transmethylation [32]. The levels of fecal neutral and acidic steroids were analyzed by GLC using 5α-cholestane and 23-nordeoxycholic acid (Steraloids, Wilton, NH, USA) as internal standards, respectively [33,34].

2.5. Determination of tissue eicosanoid concentrations

The aortic 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), which is a prostaglandin I_2 (PGI_2)-stable hydrolytic product, was extracted according to the methods of Bruckner et al. [35]. The level of 6-keto-$PGF_{1\alpha}$ was determined with HPLC-UV. The HPLC-UV system was composed of a Shimadzu LC-10AD liquid chromatograph with an SPD-M10A diode array detector (195 nm) using Asahipak HIKARISIL-C18 (4.6×250 mm, 5 μm, Asahi Chemical Industry). The mobile phase consisted of 0.1% phosphoric acid in water/acetonitrile (2:1, v/v) and the flow rate was 1.0 ml/min.

The kidney was homogenized in the presence of indomethacin. The homogenate was centrifuged at 10,000 rpm for 20 min (4°C), and the supernatant of homogenate was acidified to pH 3.0 with HCl and extracted with ethyl acetate to recover PGs. The ethyl acetate extracts were dried under nitrogen gas and purified with Sep-Pak C18 cartridge (Waters USA) after dissolving in ion-exchanged water. PGs were eluted with ethyl acetate and converted into the corresponding fluorescent derivatives by reaction with 3-bromomethyl-6, 7-dimethoxy-1-methyl-1, 2-dihydroquinoxaline-2-one and determined by HPLC with a spectrofluorometric detector according to the methods of Yamaguchi etal. [36].

2.6. Analysis of isoflavone level in serum and feces

The serum (50 μl) was incubated with Sulfatase H5 (25.3 U) for 45 min at 37°C to hydrolyze conjugates and then extracted with diethyl ether [37]. Extracts were diluted in methanol for HPLC analysis. Isoflavones in feces were extracted by ethanol after lyophilized feces were crushed to fine powder. Extracts were dried under nitrogen gas and applied to a Sep-Pak C18 cartridge dissolving in ion-exchanged water. Isoflavones were eluted with 80% methanol [16]. Isoflavones were determined using an HPLC system with esa coulArray (MC Medical). The HPLC system was composed of a Shimadzu LC-10ADvp liquid chromatograph with an esa coulArray operating at potentials of 340, 420, 500 and 580 mV. The column used was MCM column C18 (4.6×150 mm, 5 μm, MC Medical). The column and the detector were thermostated to 35°C. Elution was carried out at a flow rate of 1.0 ml/min with the following solvent gradient system: (A) 50 mM sodium acetate buffer (pH4.8)/CH_3CN (75:25) and (B) 50 mM sodium acetate buffer (pH4.8)/CH_3CN (50:50) — consisting of a linear increase from 0% to 100% of solvent B for 10 min, B 100% for 10 min and B 0% for 1 min followed by holding at B 0% for 10 min, which equilibrates the system for subsequent injections.

2.7. Statistical analysis

Data were analyzed by Duncan's new multiple range test to determine the exact nature of the difference ($P<.05$) among the groups [38] The statistical analysis of isoflavone level was done by Student's t test to determine significant differences at $P<.05$.

3. Results

3.1. Growth and liver weight

On average, a rat weighing 113 g was fed 19.4 g of purified diet per day and gained 280 g of body weight for 40 days. No statistically significant differences were found in these indices among the groups. The average of liver weight (g per 100 g body weight; mean±S.E.) was also comparable among the groups (4.6±0.1, 4.8±0.2 and 4.9±0.1 g per 100 g body weight for the control, IF-A and IF-G groups, respectively).

3.2. Key enzyme activities of liver microsomes

Among the various lipid metabolism enzymes, we analyzed the activities of cholesterol 7α-hydroxylase and Δ6 desaturase (Fig. 1). The activity of hepatic cholesterol 7α-hydroxylase tended to be slightly higher in the rats fed isoflavones than in those fed the isoflavone-free diet. Hepatic Δ6 desaturase activity in the rats fed the isoflavone aglycones, but not isoflavone glucosides, was

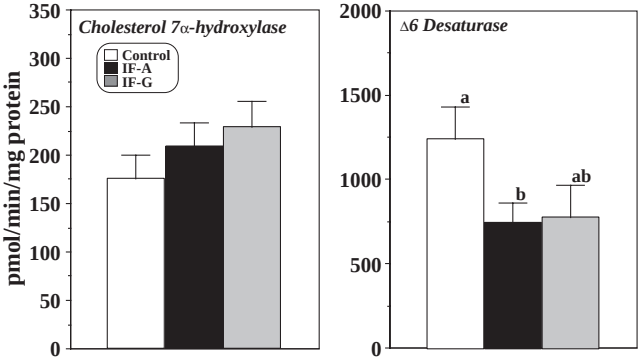

Fig. 1. Effects of dietary soy isoflavones on hepatic microsomal key enzyme activities of cholesterol catabolism and linoleic acid desaturation in the rats fed cholesterol. Data are presented as mean±S.E. for seven or eight rats in each group. Values without a common superscript letter denotation are significantly different at $P<.05$. Abbreviations: Control, rats fed isoflavone-free diet (open bars); IF-A, rats fed isoflavone aglycone-added diet (solid bars); IF-G, rats fed isoflavone glucoside-added diet (hatched bars).

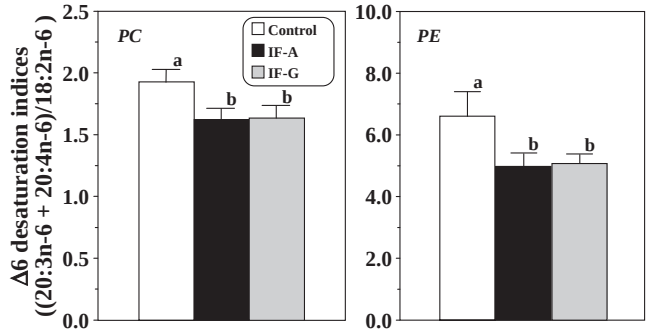

Fig. 2. Effects of dietary soy isoflavones on linoleic acid desaturation indices of liver phospholipids in the rats fed cholesterol. Data are presented as mean±S.E. for seven or eight rats in each group. Values without a common superscript letter denotation are significantly different at $P<.05$. Abbreviations are the same as those in Fig. 1.

significantly lower than that in those fed the isoflavone-free diet.

3.3. Levels of liver and serum lipids

The effects of soy isoflavones aglycone and glucoside on the lipid profile were analyzed in the rats fed cholesterol. Levels of liver cholesterol and TG were significantly lower in the IF-A group than in the control group (Table 2). Level of serum cholesterol in the IF-A group was also significantly lowered compared with that in the control group. By contrast, HDL-cholesterol level in the IF-A group was significantly higher than that in the control group. The levels of serum TG and phospholipid were decreased in the rats fed soy isoflavone-added diets when compared with those fed the isoflavone-free diet.

3.4. FA desaturation indices and tissue eicosanoid level

As shown in Fig. 2, Δ6 desaturation indices [(20:3+20:4)/18:2] of liver PC and PE in the rats fed soy isoflavones were significantly lowered compared with those in the rats fed the isoflavone-free diet. The eicosanoid levels of the aorta tended to be lower in the IF-A group than in the control group. Dietary soy isoflavone aglycone tended to decrease prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels of the kidney compared with the isoflavone-free diet (Table 3).

3.5. Fecal steroid excretion

The excreted steroid levels in feces are summarized in Table 4. Dietary isoflavone aglycones, compared with isoflavone glucosides, significantly increased the excreted level of total neutral steroids into feces. The excreted level of coprostanol into feces was higher in the rats fed isoflavone aglycone than in those fed the isoflavone-free diet (Table 4). On the other hand, the excreted level of total acidic steroids into feces tended to be higher in both the IF-A and IF-G groups than in the control group. Moreover, the level of fecal total steroid excretion in the IF-A group was highest among the three groups.

3.6. The levels of isoflavone in serum and feces

To examine the absorption of dietary isoflavone, the levels of serum isoflavones and excreted isoflavones into feces in the rats fed isoflavone-added diets were analyzed. The level of serum total isoflavones in the IF-A group was significantly higher than that in the IF-G group (Fig. 3). The serum genistein and daidzein levels were significantly higher in the IF-A group than in the IF-G group. On the other hand, the excreted level of total isoflavones into feces in the rats fed the isoflavone aglycone was significantly lower than that in those fed the isoflavone glucoside. The level of excreted daidzein in

Table 2
Effects of type of dietary soy isoflavone on levels of liver and serum lipids in rats

Group	Liver			Serum			
	Total cholesterol	Triglyceride	Phospholipid	Total cholesterol	HDL-cholesterol	Triglyceride	Phospholipid
	(μmol/g tissue)			(μmol/ml)			
Control	259.7±6.7[a]	53.2±2.0[a]	32.6±0.3	1.68±0.08[a]	0.56±0.04[a]	1.04±0.08[a]	0.68±0.01[a]
IF-A	234.1±9.3[b]	41.0±3.3[b]	33.9±0.6	1.43±0.07[b]	0.68±0.03[b]	0.71±0.03[b]	0.57±0.03[b]
IF-G	278.8±7.8[a]	49.4±4.6[a]	33.9±0.3	1.53±0.08[ab]	0.60±0.03[ab]	0.70±0.02[b]	0.61±0.06[ab]

Data are presented as mean±S.E. for 7 or 8 rats in each group.
Values without a common superscript letter are significantly different at $P<.05$.
Abbreviations are the same as those in Table 1.

Table 3
Effect of type of dietary soy isoflavone on production levels of tissue eicosanoids in rats

Group	Aorta	Kidney	
	6-keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$
	(nmol/g tissue)		
Control	18.6±4.9	1.6±0.2	0.2±0.0
IF-A	10.0±1.4	1.2±0.1	0.1±0.0
IF-G	15.3±2.0	1.4±0.1	0.2±0.0

Data are represented as mean±S.E. for 7 or 8 rats in each group. Abbreviations are the same as those in Table 1.

the IF-G group was considerably higher than that in the IF-A group.

4. Discussion

Soybean protein is thought to affect many biological measures of coronary heart disease risk accompanying modulations of various lipid metabolism in a favorable manner. Many researches suggest that coexisting isoflavones must associate with various biological actions exerted by dietary soybean protein. However, purified isoflavones and powder containing high levels of isoflavone were not used in many experimental cases. Moreover, it has been controversial that isoflavone glucoside or isoflavone aglycone favorably exerts a potent regulative function on lipid metabolism. Therefore, we compared the regulative function of isoflavones aglycone and glucoside on lipid metabolism in rats fed cholesterol.

We found that dietary isoflavones modulated cholesterol and essential FA metabolism. Dietary isoflavones, irrespective of their form, tended to increase the activity of hepatic cholesterol 7α-hydroxylase, although not significantly. The fecal excretion of acidic steroids tended to be higher in the isoflavone-fed groups than in the control group relevant to this modulation of enzyme activity. The same observation was shown in our previous study using rats fed cholesterol-added diet [39]. Therefore, dietary isoflavones may promote hepatic cholesterol catabolism irrespective of their form. Reflecting these observations, the level of liver total cholesterol must be significantly lowered in the IF-A group compared with the control group. The metabolites of dietary isoflavones may modulate a hepatic cholesterol 7α-hydroxylase mRNA level because mRNA levels for cytochrome *p*450-2A and phosphoribosylpyophosphate synthase-associated protein were up-regulated in the gerbil liver after consumption of isoflavone-containing protein [40]. Ni et al. [41] also demonstrated that dietary ethanol-extracted soy protein isolate, compared with the intact soy protein isolate, had a significantly less hepatic mRNA level of cholesterol 7α-hydroxylase in exogenously hypercholesterolemic rats.

Table 4
Effects of dietary soy isoflavone on fecal steroid excretion in rats

	Control	IF-A	IF-G
Dried fecal weight (g/day)	1.12±0.12	1.44±0.12	1.17±0.09
Neutral steroid (mg/day)			
Coprostanol	5.8±1.3[a]	9.3±2.1[b]	7.5±0.7[ab]
Cholesterol	15.5±2.5	18.6±2.5	15.7±2.4
Total neutral steroid	18.1±2.0[a]	25.1±3.3[b]	17.8±2.3[a]
Acidic steroid (mg/day)			
Lithocolic	0.15±0.07	0.11±0.03	0.14±0.05
Deoxycholic	0.35±0.09	0.29±0.03	0.40±0.12
Chenodeoxycholic	0.07±0.02	0.09±0.02	0.13±0.03
Hyodeoxycholic	0.15±0.06	0.66±0.38	0.60±0.46
Ursodeoxycholic	0.10±0.03	0.14±0.04	0.13±0.03
Cholic	0.23±0.07	0.24±0.05	0.32±0.05
α-Muricholic	1.67±0.51	2.99±0.73	2.55±0.63
β-Muricholic	2.28±0.41	1.98±0.47	2.58±0.62
Total acidic steroid	5.03±0.99	6.30±0.51	6.72±0.92
Total steroid (mg/day)	22.6±3.2[a]	31.2±3.5[b]	25.5±2.7[ab]

Data are presented as mean±S.E. for 7 or 8 rats in each group.
Values without a common superscript letter are significantly different at $P<.05$.
Abbreviations are the same as those in Table 1.

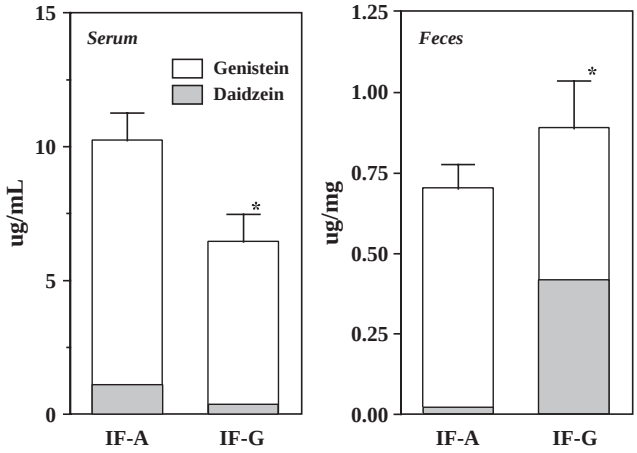

Fig. 3. The level of serum isoflavones and the rate of isoflavones excretion in feces of the rats fed soy isoflavone-added diet. Data are presented as mean±S.E. for seven or eight rats in each group. Asterisk indicates significant difference from the corresponding value in rats fed isoflavone-free diet at $P<.05$ (Student's *t* test). Abbreviations: IF-A, rats fed isoflavone aglycone-added diet; IF-G, rats fed isoflavone glucoside-added diet. Open bars indicate genistein; solid bars, daidzein.

The level of serum cholesterol was also lowered by consumption of soy isoflavones, particularly in the IF-A group. Same observations were found in some studies using other animal species such as rabbits [42], hamsters [43] and monkeys [44] and in human [45] experiments. Moreover, the level of HDL cholesterol increased in the IF-A group compared with the control group in our experiment as reported in other studies [42]. A similar tendency was found in an experiment using male New Zealand White rabbits fed 5 mg/kg body weight of isoflavone aglycone from soybean [42]. On the other hand, the effective cholesterol-lowering function was not significant when isoflavone glucoside was given to rats [46], ovariectomized cynomolgus monkeys [44] or humans [47]. Thus, dietary isoflavone aglycones, compared with isoflavone glucosides, may highly be attributed to a reduction in plasma cholesterol and increase of HDL-cholesterol levels. Daidzein, among isoflavone aglycones, may be highly associated with the cholesterol-lowering

function because defatted soy ontjom, which is fermented soy food, having a high level of daidzein reduced plasma cholesterol in rats [48] or ethanol-extracted isolated soy protein with daidzein had significantly less plasma cholesterol in golden Syrian hamsters [43].

Furthermore, the regulative function of dietary soy isoflavone on tissue TG level was observed in this study. The rats fed dietary isoflavones, irrespective of their form, significantly decreased serum TG level. On the other hand, the liver TG level was significantly lowered in only the IF-A group compared with the control group. Yousef et al. [42] observed the same tendency in male New Zealand White rabbits given isoflavone aglycone. Peluso et al. [11] demonstrated that dietary isoflavones lowered the liver TG level by 33% relative to the isoflavone-free diet in male Sprague–Dawley rats. Ethanol-extracted soy protein also tended to increase tissue TG level compared with intact soy protein isolate in apo-E-deficient rats [41]. Thus, dietary isoflavones may exert hypolipidemic function, particularly in their aglycone. The modulations of peroxisome proliferator-activated receptors by the metabolites from dietary isoflavones may associate with hypolipidemic function. Soy isoflavones may exert hypolipidemic effect through the activation of PPARα and PPARγ gene expression, which is associated with an increase of β-oxidation as Mezei et al. [49] hypothesized using murine raw 264.7 cells. Nogowsky et al. [50] also indicated that plasma TG levels were lowered after the consumption of isoflavone-containing diets compared with the isoflavone-free diet in ovariectomized rats. Dietary genistein, among isoflavones, may have potent hypolipidemic function because Mezei et al. [49] suggest that genistein might have stimulated lipolysis and inhibited lipogenesis.

Dietary isoflavones, particularly isoflavone aglycone, lowered the hepatic Δ6 desaturase activity. Reflecting this effect, Δ6 desaturation indices of liver phospholipids in the rats fed isoflavones were significantly lower than those in the control group. Generally, soy protein isolate lowers the hepatic Δ6 desaturase activity compared with casein [51]. The modulation of Δ6 desaturase activity by dietary soy protein isolate appears to be mediated in part by coexisting isoflavones.

Moreover, the production levels of 6-keto-$PGF_{1\alpha}$ in the aorta and the levels of both PGE_2 and $PGF_{2\alpha}$ in the kidney also tended to be reduced by intake of soy isoflavones. Raso et al. [52] found that various naturally occurring flavonoids inhibited PGE_2 release and cyclooxygenase-2 enzyme expression in vitro. Isoflavone glucoside from immature fruits of *Sophora japonica* also inhibited cyclooxygenase-2 activity in mice [53]. Therefore, dietary isoflavone may lead to the possibility of inhibition on inflammatory response.

The difference of bioavailability of dietary isoflavone form may be attributed to the differences of regulative functions on lipid metabolism between isoflavones aglycone and glucoside, although they were not necessarily significant. The level of serum total isoflavones in the IF-A group was significantly higher than in the IF-G group, despite the same amount of aglycone level in each diet. Contrary to this observation, the excreted level of total isoflavones to feces was significantly lower in the IF-A group compared with the IF-G group. Isoflavone aglycones are firstly absorbed from the stomach [54] and secondarily from the small intestine [16]. However, isoflavone glucosides have to be hydrolyzed to aglycones by enterobacterial β-glycosidase in the intestines before absorption [55]. Richelle et al. [17] demonstrated that similar plasma and urine pharmacokinetics were observed for the aglycone and glycoside drinks in humans. Moreover, Piskula et al. [54] observed that no differences in absorption between isoflavones aglycone and glucoside were found except that aglycones, but not glucosides, were absorbed from the rat stomach. However, the absorptive rate and speed of isoflavone from the intestine in rats are still well unknown. On the other hand, some studies found that soy isoflavone aglycones are absorbed faster and in higher levels than isoflavone glucosides in humans [18]. Therefore, dietary isoflavone aglycones may be absorbed faster and effectively metabolized rather than isoflavone glucosides in rats, although the conclusive total amount of absorbed isoflavones may reach the same level, irrespective of isoflavone form, in long feeding term. Moreover, it is possible that dietary isoflavone aglycones, compared with isoflavone glucosides, may be retained in tissues for a comparatively long time and at high levels in rats, although we have no direct explanation for this. Thus, we speculate that the difference of absorption of soy isoflavone observed between the IF-A and IF-G groups may be responsible for the differences of regulative function of dietary isoflavones on lipid metabolism. Besides our experimental results, some studies found that isoflavone aglycones, compared with isoflavone glucosides, exerted effective beneficial effects on bone metabolism [8], antioxidative action [19] and anti-inflammatory properties [20].

In conclusion, our observations suggest that dietary soy isoflavones have regulative function on cholesterol and FA metabolism in rats fed cholesterol. These regulative functions were more effective in isoflavone aglycones than in isoflavone glucosides. Differences of bioavailability or speed of absorption between isoflavones aglycone and glucoside may be associated with differences of regulative function of dietary soy isoflavones on lipid metabolism. Therefore, the positive consumption of soy isoflavone aglycones may reduce the risk of some cardiovascular diseases or inflammatory reactions.

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