Group B Soyasaponin Aglycone Suppresses Body Weight Gain and Fat Levels in High Fat-Fed Mice

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Summary Group B soyasaponins, found in soy, have various health-promoting properties, but it is unclear whether they have an anti-obesity effect. The aim of this study was to evaluate the anti-obesity effect of group B soyasaponin glycosides and aglycone in mice fed a high-fat diet. Six-week-old C57/BL6 mice were divided into three groups (each $n=10$) and orally administered a high-fat diet for 35 d; two of the groups also received group B soyasaponin glycosides or aglycone. Although there was no significant difference among the three groups in consumption, the weight of fat adipose tissue at autopsy was more than 30% lower in the group B soyasaponin aglycone group than in the control group, but X-ray computed tomography showed no significant difference in muscle weight between these two groups. The ratio of muscle to whole body weight was higher in the group B soyasaponin aglycone group than in the control group. These results suggest that group B soyasaponin aglycone has a stronger anti-obesity effect than group B soyasaponin glycosides, without a loss in muscle weight, and that it increases the ratio of muscle to whole body weight. To our knowledge, this is the first report showing the anti-obesity effect of soyasaponin aglycone in vivo using animal models.

Key Words soyasaponin aglycone, soyasaponin glycosides, anti-obesity, visceral fat, muscle

Soyasaponins, bioactive compounds found in soy and other legumes, are triterpene glycosides with one or two carbohydrate chains bound to an oleanane aglycone. They are mainly classified as group A or group B according to their aglycone structure: group A soyasaponins have a carbohydrate chain bound at two locations (C-3 and C-21) on soyasapogenol A, whereas group B soyasaponins have a carbohydrate chain bound to a single location (C-3) on soyasapogenol B (Fig. 1).

Soyasaponins are reported to promote health in various ways. Reported characteristics include antioxidant activity (1, 2), cholesterol lowering (3), colitis improvement (4), renin-inhibiting activity (5, 6), hepatoprotective activity (7, 8), neuroprotective effects (9, 10), cancer prevention (11), the downregulation of inflammation induced by toll-like receptors (12), enhanced zinc absorption via increased levels of the zinc transporter ZIP4 (13), and inhibition of contact hypersensitivity induced by 2,4-dinitrofluorobenzene (14). Although the cholesterol-lowering effect of soyasaponin with respect to lipid metabolism has been reported, it is not known whether soyasaponin glycosides or aglycone inhibit lipid metabolism-related weight gain (i.e., have an anti-obesity effect). In our previous report, the bioavailability of soyasaponins was shown to be higher for

group B than for group A, and higher for aglycone than for glycoside (15). These bioavailability characteristics suggest that the effect of the group B aglycone soyasapogenol B would be stronger than that of group B soyasaponin glycosides when administered in equivalent molar quantities, but as yet there has been no report of this. Group B soyasaponins are reported to exert various specific functions (16, 17), so these are what we focused on in the present study. The aim of this study was to investigate the degree of suppression of weight gain and fat levels by administering group B soyasaponins or aglycone to mice fed a high-fat diet.

MATERIALS AND METHODS

Reagents. Group B soyasaponin glycosides used for the study were produced by J-Oil Mills, Inc. (Tokyo, Japan) and contained 51.0% soyasaponin I, 38.2% soyasaponin V, and 1.1% diacetylsoyasaponin A1. Soyasapogenol for the group B soyasaponin aglycone group was prepared in our laboratory as described previously (15) and contained 2.3% soyasapogenol A and 77.9% soyasapogenol B.

Animal study. The animal study was approved by the Animal Experiment Ethics Review Board of Kindai University (KAAG-25-002). Six-week-old male C57/BL6 mice (Charles River, Inc., Japan) were maintained in an air-conditioned room at a temperature of $20\pm 2^\circ\text{C}$, with a 12-h light–dark cycle. The mice had free access

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Group B soyasaponin glycosides

Group B soyasaponin aglycone
(soyasapogenol B)

Group B soyasaponin glycosides

	R ₁	R ₂
soyasaponin Ba (V)	CH ₂ OH	β-D-Glc
soyasaponin Bb (I)	CH ₂ OH	α-L-Rha
soyasaponin Bc (II)	H	α-L-Rha
soyasaponin Bb' (III)	CH ₂ OH	H
soyasaponin Bc' (IV)	H	H

Glc, glucose

Rha, rhamnose

Fig. 1. Chemical structures of group B soyasaponin glycosides and group B soyasaponin aglycone (soyasapogenol B).

Table 1. Composition of experimental diets (g).

Ingredient	Control	Group B soyasaponin glycosides	Group B soyasaponin aglycone
Casein	200	200	200
Cornstarch	150	150	150
Sucrose	350	350	350
Soybean oil	200	200	200
Cellulose	50	50	50
AIN-93M mineral mix	35	35	35
AIN-93M vitamin mix	10	10	10
Methionine	3	3	3
Choline bitartrate	2	2	2
Group B soyasaponin glycoside	0	5.3	0
Group B soyasaponin aglycone	0	0	2.6

to food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water for 1 wk. The mice were divided into three groups (each $n=10$), ensuring that the mean body weight was the same for each group. The first group, the control group, was administered a high-fat diet; the second group (the “glycosides group”) was administered 0.53% group B soyasaponin glycosides; and the third group

(the “aglycone group”) was given 0.26% soyasapogenol (Table 1). The three groups of mice had free access to the experimental diet during the test period. The mice were weighed at the start and end of the diet, and on five occasions during the diet. On the 35th day, the mice were fasted and blood was collected under anesthesia from the cervical vein using a syringe and transferred to

Fig. 2. Effects of group B soyasaponin glycosides and group B soyasaponin aglycone on body weight in C57/BL6 mice fed high-fat diets. ■ Control group; ▲ Group B soyasaponin glycoside group; ● Group B soyasaponin aglycone group. Values are presented as mean $\pm$ SE ($n=10$). Values labeled "a" are significantly different from those labeled "b" ($p<0.05$).

Table 2. Liver weight, visceral fat weight, and visceral fat weight as a percentage of whole body weight for the C57/BL6 mice fed high-fat diets.

	Control	Group B soyasaponin glycosides	Group B soyasaponin aglycone
Liver (g)	1.48 $\pm$ 0.05 ^a	1.56 $\pm$ 0.07 ^a	1.42 $\pm$ 0.04 ^a
Epididymal adipose tissue (g)	1.63 $\pm$ 0.09 ^a	1.35 $\pm$ 0.15 ^a	0.94 $\pm$ 0.04 ^b
Perirenal fat (g)	0.63 $\pm$ 0.04 ^a	0.48 $\pm$ 0.06 ^{ab}	0.34 $\pm$ 0.04 ^b
Mesenteric fat (g)	0.59 $\pm$ 0.04 ^a	0.45 $\pm$ 0.06 ^{ab}	0.39 $\pm$ 0.06 ^b
Total visceral fat (g)	2.84 $\pm$ 0.15 ^a	2.27 $\pm$ 0.26 ^{ab}	1.68 $\pm$ 0.12 ^b
Epididymal adipose tissue (%)	4.67 $\pm$ 0.17 ^a	4.00 $\pm$ 0.35 ^a	3.07 $\pm$ 0.13 ^b
Perirenal fat (%)	1.80 $\pm$ 0.11 ^a	1.41 $\pm$ 0.15 ^{ab}	1.11 $\pm$ 0.11 ^b
Mesenteric fat (%)	1.69 $\pm$ 0.09 ^a	1.33 $\pm$ 0.15 ^a	1.27 $\pm$ 0.15 ^a
Total visceral fat (%)	8.16 $\pm$ 0.28 ^a	6.74 $\pm$ 0.61 ^{ab}	5.45 $\pm$ 0.31 ^b

Values are presented as mean $\pm$ SE ($n=10$).

Within a row, the values with different superscript letters indicate significant difference ($p<0.05$). Visceral fat weight as a percentage of whole body weight was calculated using the body weight on the last (35th) day of the diet.

a test tube containing serum-separating gel. The tubes were centrifuged for 5 min at 3,000 rpm and the serum obtained was stored at -18°C until the analysis. The liver, epididymal adipose tissue, perirenal fat, and mesenteric fat were collected from the mice the same day.

Measurement of blood parameters. Serum glucose was measured with a Glucose CII-Test Wako kit, serum triglycerides with a Triglyceride E-Test Wako kit, serum total cholesterol with a Cholesterol E-Test Wako kit, serum high-density lipoprotein cholesterol with an HDL-Cholesterol E-Test Wako kit, and serum free fatty acids with a Lab Assay NEFA kit (all from Wako Pure Chemical Industries, Ltd., Japan). Serum leptin was measured by using a Mouse/Rat Leptin Measurement Kit (Moriguchi Institute of Biological Science, Inc., Japan).

Measurement of liver lipids. The livers (50 mg) from mice were homogenized in 200 μL of buffer (10 mM

Tris-HCl, 1 mM EDTA, pH 8.0). The resultant liver homogenates were mixed with chloroform and distilled water for lipid extraction, after which the lipid fraction was dried and resuspended in Triton X-100 : methanol (1 : 4). The triglyceride and cholesterol contents were determined using the Triglyceride E-Test Wako kit and Cholesterol E-Test Wako kit (both from Wako Pure Chemical Industries, Ltd.).

Computed tomography. Transaxial X-ray computed tomography (CT) scans of the mice were taken using an LA Theta LCT-100 M experimental animal CT system (Hitachi Aloka Medical, Ltd., Japan) on the 35th day. Tomography measurements of muscle and fat-related parameters (visceral fat, mesenteric fat, and subcutaneous fat) were taken at 2-mm intervals from the 12th rib to the base of the femur.

Data analysis. The data are expressed as mean $\pm$ stan-

Table 3. Muscle and fat weight ratio measured using X-ray computed tomography in C57/BL6 mice fed high-fat diets.

	Control	Group B soyasaponin glycoside	Group B soyasaponin aglycone
Muscle (g)	11.3±0.3 ^a	12.3±0.2 ^b	11.8±0.2 ^a
Visceral fat (g)	2.53±0.13 ^a	2.54±0.27 ^a	1.80±0.17 ^b
Mesenteric fat (g)	0.59±0.04 ^a	0.48±0.06 ^{ab}	0.45±0.06 ^b
Subcutaneous fat (g)	3.64±0.30 ^a	2.34±0.37 ^b	1.37±0.17 ^b
Total fat (g)	6.17±0.39 ^a	4.88±0.60 ^a	3.18±0.30 ^b
Muscle (%)	32.8±1.0 ^a	37.4±0.8 ^b	38.7±0.7 ^b
Total fat (%)	35.1±1.5 ^a	27.5±2.4 ^b	20.9±1.5 ^c

Values are presented as mean±SE (*n*=10).

Within a row, the values with different superscript letters indicate significant difference (*p*<0.05). Visceral fat weight as a percentage of whole body weight was calculated using the body weight on the last (35th) day of the diet.

Table 4. Effects of group B soyasaponin glycosides and group B soyasaponin aglycone on plasma glucose, lipid, and adipocytokine levels in mice fed high-fat diets.

	Control	Group B soyasaponin glycoside	Group B soyasaponin aglycone
Glucose (mg/dL)	324±21 ^a	272±29 ^a	246±19 ^a
Triglyceride (mg/dL)	80±6 ^a	73±5 ^{ab}	57±5 ^b
Total cholesterol (mg/dL)	115±4 ^a	112±6 ^a	115±7 ^a
HDL cholesterol (mg/dL)	83±3 ^a	71±4 ^a	74±4 ^a
Phospholipid (mg/dL)	283±8 ^a	282±16 ^a	273±16 ^a
Leptin (ng/mL)	24.3±1.9 ^a	18.7±3.5 ^a	9.2±1.1 ^b
Resistin (ng/mL)	15.1±1.0 ^a	16.2±1.3 ^a	15.6±1.5 ^a
Adiponectin (μg/mL)	13.4±1.0 ^a	12.4±1.0 ^a	11.8±1.3 ^a
Non esterified fatty acid (mEq/L)	1.93±0.09 ^a	1.64±0.10 ^a	1.83±0.09 ^a

Values are presented as mean±SE (*n*=10).

Within a row, the values with different superscript letters indicate significant difference (*p*<0.05). HDL: high-density lipoprotein.

dard error (SE). Differences between the groups were compared by using the Tukey–Kramer multiple comparisons test, with *p*<0.05 considered significant.

RESULTS

No significant difference in mean food consumption was observed among the three groups: 8.32±0.18 g/d in the control group, 7.95±0.32 g/d in the glycosides group, and 8.26±0.11 g/d in the aglycone group. However, by the 21st day of the high-fat diet, weight gain had been significantly inhibited in the aglycone group compared to the other two groups (Fig. 2).

There was no significant difference in liver weight among the three groups at the end of the test period. The weight of epididymal adipose tissue, perirenal fat, and mesenteric fat, and the total weight of those fat regions, tended to be lower in the glycosides group than in the control group, although this did not reach statistical significance; however, the weights of each fat and tissue were significantly lower in the aglycone group compared to the control group (Table 2). Especially in

the aglycone group, there was a significant reduction in the total fat weight, which was approximately 40% lower than that of the control group and approximately 25% lower than that of the glycoside group. Similar trends were observed for the ratio of fat to total body weight and for the fat weight on the last day of the diet. Although the organ weights measured by X-ray CT differed from those measured in the autopsy, similar trends were observed, with fat weight gain being suppressed to a greater extent in the aglycone group than in the glycoside and control groups. Muscle weight was higher in the glycoside group than in the control group, but no significant difference in muscle weight was observed between the aglycone and control groups (Table 3). The ratio of muscle weight to total body weight on the last day of the diet was significantly higher in the glycoside and aglycone groups compared with the control group (Table 3).

Serum triglyceride levels were significantly lowered in the aglycone group compared with the control group, but there were no significant differences among the

Fig. 3. Effects of group B soyasaponin glycosides and group B soyasaponin aglycone on liver triglyceride and cholesterol levels in mice fed high-fat diets. a and b show liver triglyceride and cholesterol contents in mice fed high-fat diet. Values are presented as mean $\pm$ SE ($n=10$). Within a figure, values with different superscript letters indicate significant difference ($p<0.05$).

three groups for the other blood parameters (Table 4). Leptin was significantly lowered in the aglycone group, but there was no significant difference among the three groups in the other adipocytokines.

Liver triglyceride levels per liver weight were significantly lowered in the aglycone group than in the control group, but there was no significant difference among the three groups for liver cholesterol (Fig. 3).

DISCUSSION

Soyasaponins have been reported to have antioxidant and anti-inflammatory activities, antitumor and renin-inhibiting effects, and to inhibit the progression of renal disease. They have been reported to reduce cholesterol, but whether they inhibit whole body weight gain and the elevation of fat level has not been established.

The focus of this study was on soyasaponins. C57/BL6 mice were administered a high-fat diet that contained either group B soyasaponin glycosides or group B soyasaponin aglycone in equivalent molar amounts. We observed that the inhibition of fat weight gain and the reduction in blood triglyceride levels were stronger in the glycoside and aglycone groups than in the control group, with these effects strongest in the aglycone

group. However, there was no significant difference in muscle weight between the aglycone and control groups. The ratio of muscle weight to total body weight was significantly higher in the glycoside and aglycone groups compared with the control group. These findings suggest that the inhibition of the whole-body weight gain was mediated not through the loss of muscle weight but rather through the inhibition of fat-weight gain.

In our previous report (15), we observed that the bioavailability of group B soyasaponins was higher than that of group A soyasaponin and that of soyasaponin aglycone was higher than that of soyasaponin glycosides. We therefore speculated that the inhibition of weight gain would be more effective with group B soyasaponin aglycone due to its higher bioavailability.

The lack of data to elucidate the mechanism for the suppression of body weight and adipose tissue weight gain following soyasapogenol B administration was the limitation of the current study. Therefore, further research is warranted to clarify this anti-obesity mechanism. We speculate that soyasapogenol B suppresses weight gain and inhibits adipose tissues accumulation through three different mechanisms.

The first mechanism is the suppression of lipid absorp-

tion in the intestine by inhibiting pancreatic lipase activity. Saponins derived from other plants have been reported to possess pancreatic lipase activity inhibition (18). Moreover, Karu et al. have reported that the consumption of ginseng saponins suppresses the expected increase in body weight and plasma triacylglycerols in high-fat diet-fed mice. Thus, soyasapogenol B might exert its anti-obesity effect through the reduction of intestinal fat absorption by inhibiting pancreatic lipase activity.

The second mechanism is the activation of AMP-activated protein kinase (AMPK). Both soyasapogenol A and soyasapogenol B isolated from cheonggukjang, a traditional Korean fermented soy food, have been reported to inhibit adipocyte differentiation in 3T3-L1 preadipocyte cells (19). Kim et al. have reported that the inhibition of triglyceride accumulation and the activation of AMPK were affected by soyasapogenol B, indicating that soyasapogenol B administration lowered adipose tissue accumulation by activating AMPK in C57BL/6 mice.

Finally, the third mechanism is the suppression of the transcriptional activity of peroxisome proliferator-activated receptor γ (PPAR γ). Saponins derived from other species of plants have been reported to exert anti-obesity effects by downregulating the PPAR- γ (20). In addition, Yang et al. (21) reported that soyasaponins Aa and Ab inhibited the accumulation of fat and the differentiation of adipocytes by downregulating the expression of PPAR γ and CCAAT-enhancer binding protein alpha. We previously observed that soyasapogenol B suppressed the expression of PPAR- γ in 3T3-L1 cells, suggesting the possibility that weight gain in vivo is suppressed through the pathway involved in PPAR- γ (in preparation).

In the present study, administration of group B soyasaponin aglycone not only increased the ratio of muscle tissue to the total body weight, but also suppressed the gain in body weight and fat weight while retaining muscle weight. It is not known whether soyasapogenol B directly increases the ratio of muscle tissue to body weight. Further studies are needed to clarify the mechanism by which soyasapogenol B retains muscle weight while suppressing the gains in whole body weight and fat weight.

Group B soyasaponin glycosides and aglycone have been observed to inhibit the elevation of blood glucose levels. Kitamura et al. (22) reported that the uptake of glucose in the skeletal muscle is enhanced by the conversion of *Panax notoginseng* saponin in a type 2 diabetic mouse model (KK-A^y mouse). In the present study, the blood glucose level was lowest in the aglycone group mice, which suggested that higher bioavailability possibly contributed to the inhibitory effect. Further study is needed to clarify the mechanism by which the blood glucose level was lowered by the administration of group B soyasaponin glycosides and aglycone.

Of the adipocytokines measured, leptin levels decreased in the glycoside group and significantly decreased in the aglycone group. Leptin is reported to act on the hypothalamus to control food intake and energy expenditure (23, 24). Leptin levels have been

shown to directly correlate with the white adipose tissue mass. A decrease in the visceral adipose tissue due to the administration of group B soyasaponin aglycone and glycosides may reduce the leptin levels.

In the present study, we observed that not only blood triglyceride levels but also liver triglyceride levels were significantly lowered in the group B soyasaponin aglycone group (Table 4, Fig. 3). Similarly, the degradation of triglycerides may be promoted by the administration of group B soyasaponin aglycone in hepatic cells. It remains unknown whether group B soyasaponin aglycone is involved in lowering the triglyceride levels both in the visceral fat and liver via the same mechanism. Further studies are warranted to clarify the mechanism through which triglyceride levels are lowered in both tissues.

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