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Consumption of *Pueraria* Flower Extract Reduces Body Mass Index *via* a Decrease in the Visceral Fat Area in Obese Humans

Tomoyasu KAMIYA,[1,†] Akira TAKANO,[1] Yuki MATSUZUKA,[1] Nobutaka KUSABA,[1] Motoya IKEGUCHI,[1] Kinya TAKAGAKI,[1] and Kazuo KONDO[2]

[1]*Research and Development Division, Toyo Shinyaku Co., Ltd., 7-28 Yayoigaoka, Tosu, Saga 841-0005, Japan*
[2]*Institution of Environmental Science for Human Life, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan*

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In Japan, kudzu is a familiar plant, well-known as an ingredient in the Japanese-style confections kudzu-kiri and kudzu-mochi. In this study, we focused on the flower of kudzu (*Pueraria thomsonii*) and conducted a clinical trial to investigate the effects of *Pueraria thomsonii* flower extract (PFE) on obesity using obese Japanese males and females (BMI $\geq$ 25 kg/m^2). Eighty-one obese subjects were randomly divided into three groups and consumed test food containing 300 mg of PFE, 200 mg of PFE, and a placebo over 12 weeks. The results indicate that PFE intake reduces BMI and decreases, the visceral fat area, but not the subcutaneous fat area. In addition, the decrease in visceral fat area showed no sexual dimorphism. Consequently, we propose that PFE intake expresses its BMI reduction effects *via* a decrease in visceral fat area.

Key words: *Pueraria* flower; obesity; visceral fat area; *Pueraria thomsonii*; kudzu

Obesity is a well-established risk factor for developing hypertension, diabetes, dyslipidemia, and cancers, and it causes premature death.[1)] Most importantly, an increase in visceral fat area is responsible for many of the metabolic abnormalities, including impaired glucose tolerance, insulin resistance, and increased very low-density lipoprotein triglycerides (VLDL-TG) associated with abdominal obesity.[2–4)] Hence, a reduction in visceral fat has become a key therapeutic goal in the management of obesity.[5)]

Kudzu belongs to the Fabaceae family, Papollonoideae subfamily, Phaseoleae tribe, and Pueraria genus. It is known as kudzu vine, kudsu, wa yaka, and nepalem.[6,7)] It is a climbing, semi-woody, perennial vine with hairy, rusty brown stems. The flower is pea-like and is colored pink to purple with yellow center. It is highly fragrant with a sweet grape-like scent and is borne in long hanging panicles at nodes on the stem.[6)] The kudzu flower is also known to be a rich source of isoflavones.[7)] Recent studies have found that methanol and water extracts of the kudzu flower possess hypolipidiemic, hypoglycemic, anti-oxidant, and hepatoprotective properties *in vivo* and *in vitro*.[7–11)]

Previously, we investigated the effect of *Pueraria thomsonii* flower extract (PFE) on body weight in humans. The results indicated the possibility that oral intake of 300 mg of PFE reduces both body weight and abdominal fat in the mildly obese,[12)] but this preliminary study was conducted only on males. In the present study, we performed a long-term clinical study over 12 weeks in order to investigate the BMI reduction and visceral fat area decrease effects of PFE oral intake in obese males and females. In addition, we checked the subjects' blood biochemical parameters to observe other effects of PFE intake, if any.

Materials and Methods

Subjects. This study received the approval of the Institutional Review Board of C'est Lavie Shimbashi Clinic, Shinkokai Medical Corporation (Tokyo) in accordance with the ethical standards established in the Helsinki declaration, and informed consent was obtained from all subjects. All of the business side was entrusted to KSO Corporation (Tokyo), which carried out the study at C'est Lavie Shimbashi Clinic.

Candidate subjects were male and female volunteers aged 20 to 65 years, recruited by advertisement. A preliminary physical examination (the screening) was performed on all candidates. The study enrolled 90 candidates as test subjects, all of whom had a BMI over 25.0 kg/m^2, who did not fall under any of the following exclusion criteria:

1. Are taking drugs that might affect obesity, hyperlipidemia, or lipid metabolism;
2. Cannot stop taking supplements or functional foods that might affect obesity, hyperlipidemia, or lipid metabolism;
3. Have implanted metal in the abdominal area, as detected by computerized tomography (CT);
4. Have serious complications or have contracted a disease that requires urgent remedy;
5. Have been diagnosed with familial hyperlipidemia;
6. Have drug or alcohol dependency in the history of a present disease or medical history;
7. Are in pregnancy or lactation, or have plans to become pregnant during the study;
8. Are participating in other clinical studies, taking drugs, or applying cosmetics or drugs to the skin;
9. Are judged to be unsuitable test subjects by a physician.

The subjects were randomly categorized into three groups with equal distributions in terms of gender and BMI by a controller who was not directly involved in the trials.

† To whom correspondence should be addressed. Fax: +81-942-81-3554; E-mail: kamiyat@toyoshinyaku.co.jp

Abbreviations: PFE, *Pueraria thomsonii* flower extract; VLDL-TG, very low-density lipoprotein triglycerides; CT, computerized tomography; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, asparatate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase; HbA_{1c}, hemoglobin A_{1c}; *t*-BHP, *tert*-butyl hyperoxide

Table 1. Compositions of Test Foods (per set of 12 Tablets)

	Test food (containing 300 mg of PFE)	Test food (containing 200 mg of PFE)	Placebo (containing no PFE)
Energy (kcal)[1)]	11.8	11.9	11.8
Water (g)	0.090	0.087	0.090
Ptotein (g)[2)]	0.057	0.039	≤0.003
Lipid (g)	0.105	0.111	0.096
Ash (g)	0.093	0.078	0.072
Carbohydrate (g)	2.66	2.69	2.74

[1)]Calorie conversion factor: protein 4, fat 9, carbohydrate 4
[2)]Nitrogen protein conversion factor: 6.25

Test foods. PFE, a hot-water extract of *Pueraria thomsonii* dry flowers, was purchased from Ohta's Isan (Ushiku, Japan). It contains seven isoflavones (four isoflavone glucosides, tectoridin (4.65%), tectorigenin 7-*O*-xylosylglucoside (8.48%), 6-hydroxygenistein-6,7-diglucoside (3.53%), and glycitin (0.13%), and three aglycones, tectorigenin (0.88%), glycitein (0.07%), and genistein (0.06%)). All isoflavone standards for quantification were purchased from Nagara Science (Gifu, Japan) and Tokiwa Phytochemical (Chiba, Japan). We prepared tablets as test foods, containing 300 mg PFE, 200 mg PFE, and no PFE (placebo) per set of 12 tablets. The tablets also contained reduced palatinose, cellulose, fatty acid esters of sucrose, silicon dioxide, and caramel dye to render the tablet types indistinguishable. The nutritional compositions of the test foods are shown in Table 1.

Study design. A double-blind, placebo-controlled parallel group study was conducted over 18 weeks, consisting of a 2-week pre-observation period (−2 w to 0 w), a 12-week test period with consumption of test foods (0 w to 12 w), and a 4-week post-observation period without test-food consumption (12 w to 16 w). The test subjects took each test food (12 tablets) 1 time per day during the test period. A physician conducted interviews, physical examinations, and blood sampling at each examination, including the pre- and post-observation periods (see below).

Examination items. The general physical examinations (except for total, visceral, and subcutaneous fat area) and biochemical blood tests were done at −2 w (for screening), 0 w (just before the test period), 4 w, 8 w, 12 w (after each test period), and 16 w (after the post-observation period). Total, visceral, and subcutaneous fat areas were detected at 0 w, 8 w, and 12 w *via* CT scan. All the test subjects were instructed to avoid raw fruits and vegetables, as well as sparkling drink intake the day before CT was performed (0 w, 8 w, and 12 w). In addition, they were prohibited from engaging in alcohol consumption 2 d before an examination, and from dietary consumption (except for water) after 21:00 on the day before an examination.

Physical examination. Height, body weight, waist circumference, and hip circumference were measured. BMI was then calculated as the body weight divided by the height squared (kg/m^2). Total, visceral, and subcutaneous fat areas were detected by CT (ProSpeed II; GE, NY). CT scan data were analyzed using conventional software (Fat Scan ver3.0; N2SM, Tokyo).

Biochemical blood test. Blood samples were collected from the subjects after overnight fasting. Triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were assessed as parameters of lipid metabolism, and leptin and adiponectine as adipocytokines, asparatate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γGTP) as hepatopathy markers, and glucose, hemoglobin A_{1c} (HbA_{1c}), and insulin as carbohydrate metabolism markers.

Management. A uniform dinner (approximately 800 kcal) was prepared for all test subjects each day (except for Sunday) during the test period with test food consumption. All the test subjects were instructed to take dinner before 21:00, and to set an interval of 3 h or more between daily meals (breakfast, lunch, and dinner). In addition, the subjects were allowed to consume snacks at up to 200 kcal per day, and alcohol consumption was limited to less than one large bottle of beer (500 mL), or its equivalent.

The daily diet, test food ingestion, rational symptoms, amount of exercise, and drug, functional food, and supplement intake of the test subjects were surveyed by asking them to describe each of these factors in a subject diary every day. The amount of exercise was checked by pedometer. Seven d before an examination (at 0, 4, 8, 12, and 16 w), a nutritionist checked the diets of all the test subjects and estimated the total energy, protein, fat, carbohydrate, and dietary fiber for each.

Statistical analysis. Values were expressed as mean ± SD. The degree of change in the fat area data from the CT scans was calculated for three study groups: total, male, and female. Repeated-measures analysis of variance (ANOVA) was used to compare differences among the groups. When significant differences were detected, Dunnett's test was used for multiple comparisons (*vs.* the placebo). In addition, a repeated-measures analysis of variance (ANOVA) was used to compare differences among periods (in each group). When significant differences were detected, Dunnett's test was used for multiple comparisons (*vs.* 0 w). A p-value ($p < 0.05$) was used as the criterion for statistically significant differences. All statistical analyses were performed using SPSS ver 16.0 (SPSS Japan, Tokyo).

Table 2. Baseline Characteristics of Study Subjects (0 week)

Parameter		300 mg	200 mg	Placebo
	Total	28	28	25
	Male	14	13	12
	Female	14	15	13
Age (years)	Total	43.4 ± 7.8	44.2 ± 7.5	44.0 ± 10.6
	Male	43.7 ± 8.9	43.2 ± 5.9	40.5 ± 9.8
	Female	43.0 ± 6.7	45.1 ± 8.8	47.3 ± 10.7
Height (cm)	Total	165.2 ± 7.7	164.3 ± 7.0	165.8 ± 9.0
	Male	170.3 ± 5.1	169.8 ± 5.2	173.1 ± 6.8
	Female	160.1 ± 6.5	159.5 ± 4.5	159.1 ± 4.2
Body weight (kg)	Total	75.3 ± 6.9	74.3 ± 7.6	75.7 ± 9.4
	Male	77.8 ± 5.3	78.7 ± 7.8	82.6 ± 7.6
	Female	72.9 ± 7.6	70.5 ± 5.2	69.3 ± 5.6
BMI	Total	27.6 ± 1.5	27.5 ± 1.5	27.4 ± 1.5
	Male	26.8 ± 1.2	27.3 ± 1.9	27.6 ± 1.7
	Female	28.4 ± 1.5	27.7 ± 1.3	27.3 ± 1.3
Visceral fat area (cm^2)	Total	116.4 ± 35.0	108.0 ± 49.1	95.3 ± 31.9
	Male	124.6 ± 40.7	127.4 ± 62.6	105.5 ± 39.8
	Female	108.3 ± 27.3	91.2 ± 25.1	86.0 ± 19.5
Subcutaneous fat area (cm^2)	Total	210.9 ± 60.1	219.5 ± 66.8	228.2 ± 45.0
	Male	165.4 ± 40.1	184.3 ± 37.4	218.4 ± 55.2
	Female	256.4 ± 37.8	250.1 ± 72.5	237.2 ± 32.9
Total fat area (cm^2)	Total	327.3 ± 59.8	327.5 ± 71.3	323.5 ± 58.4
	Male	290.0 ± 48.7	311.7 ± 75.1	323.9 ± 73.7
	Female	364.7 ± 45.2	341.3 ± 67.3	323.2 ± 43.0
Waist circumference (cm)	Total	95.9 ± 4.2	96.2 ± 6.3	96.0 ± 3.9
	Male	93.9 ± 4.1	95.0 ± 6.9	96.0 ± 4.1
	Female	98.0 ± 3.5	97.3 ± 5.6	95.9 ± 3.9
Hip circumference (cm)	Total	100.6 ± 4.5	100.1 ± 4.2	100.9 ± 4.4
	Male	99.0 ± 3.4	99.1 ± 3.7	100.8 ± 4.0
	Female	102.3 ± 4.9	100.9 ± 4.5	101.0 ± 4.9

Data are expressed as mean ± SD.

Results

One subject dropped out of the study due to personal problems not related to the study. In addition, among the 89 remaining subjects, eight subjects were excluded from analysis for evaluation, due to BMI < 25 kg/m^2 at 0 weeks (two subjects), irregular diet (five subjects), and

Table 3. Dietary Composition and Exercise

Parameter	Group	n	0 w	4 w	8 w	12 w	16 w
Energy (kcal)	300 mg	28	1908 ± 271	1940 ± 252	1987 ± 287	1918 ± 248	1846 ± 270
	200 mg	28	1938 ± 341	2029 ± 318	1998 ± 284	1996 ± 276	1894 ± 313
	Placebo	25	1989 ± 380	2030 ± 287	2026 ± 378	2033 ± 347	1954 ± 344
Protein (g)	300 mg	28	68.1 ± 10.5	67.6 ± 9.0	68.7 ± 10.1	68.4 ± 9.4	65.1 ± 10.3
	200 mg	28	70.3 ± 13.7	71.4 ± 10.5	69.4 ± 9.7	71.2 ± 9.5	69.5 ± 13.6
	Placebo	25	71.6 ± 14.9	72.4 ± 11.1	72.3 ± 14.9	74.6 ± 14.3	72.6 ± 15.2
Fat (g)	300 mg	28	65.8 ± 14.1	61.4 ± 10.7	62.2 ± 10.5	60.5 ± 10.5	62.4 ± 12.5
	200 mg	28	68.6 ± 16.6	62.7 ± 14.6	63.3 ± 11.6	64.5 ± 12.1	63.0 ± 16.6
	Placebo	25	68.3 ± 15.9	61.9 ± 12.8	64.3 ± 15.7	66.2 ± 14.1	65.3 ± 14.9
Carbohydrate (g)	300 mg	28	247.3 ± 37.5	266.6 ± 42.4**	274.9 ± 42.3***	261.5 ± 36.8	242.7 ± 44.6
	200 mg	28	245.1 ± 41.9	281.3 ± 42.8***	275.4 ± 39.7***	269.7 ± 42.5***	249.3 ± 40.9
	Placebo	25	258.0 ± 53.6	283.2 ± 40.9***	276.9 ± 44.8*	270.7 ± 45.8	253.8 ± 45.8
Dietary fiber (g)	300 mg	28	11.6 ± 2.5	13.0 ± 1.8**	14.4 ± 2.0***	13.8 ± 2.2***	10.9 ± 2.4
	200 mg	28	11.9 ± 2.6	14.3 ± 1.8***	15.3 ± 1.8***	14.6 ± 1.6***	11.6 ± 2.0
	Placebo	25	12.1 ± 3.3	14.1 ± 2.4***	14.8 ± 2.8***	14.2 ± 2.4***	11.5 ± 3.1
Pedometer count (steps)	300 mg	28	7818 ± 2764	8062 ± 2955	8139 ± 2962	8152 ± 2969	8338 ± 3052
	200 mg	28	9283 ± 3750	9230 ± 3567	9679 ± 4223	9600 ± 3546	9291 ± 3488
	Placebo	25	8976 ± 2320	8993 ± 2422	9297 ± 2694	9525 ± 2730	9268 ± 3191

Data are expressed as means ± SD.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* 0 w

taking supplements related to lipid metabolism (one subject). The final subject numbers in the three study groups were as follows: placebo group (n = 25; male = 12, female = 13), 200 mg PFE intake group (n = 28; male = 13, female = 15), and 300 mg PFE intake group (n = 28; male = 14, female = 14). The general characteristics of the study subjects are shown in Table 2. There were no significant differences between either of the PFE intake groups and the placebo group.

Table 3 shows the average values for dietary composition (total energy, protein, fat, carbohydrates, and dietary fiber intake) and pedometer count at 0, 4, 8, 12, and 16 weeks. There were no significant differences between either of the PFE intake groups and the placebo group, on any parameter. These data indicate that the study groups did not differ in daily energy, nutrient intake, or amount of exercise during the study.

First we investigated the effects of PFE intake on physical parameters including BMI, and hip and waist circumferences. In addition, the visceral, subcutaneous, and total fat areas were also detected by CT. Figure 1 shows the degrees of change, value at 0 w (baseline) = 0.0, in visceral fat area, subcutaneous fat area, total fat area (0, 8, and 12 w), BMI, waist circumference, and hip circumference (0, 4, 8, 12, and 16 w) for 300 mg PFE, 200 mg PFE, and placebo intake. As for BMI and visceral fat area at 300 mg of PFE intake, the degrees of change in BMI were −0.5, −0.7, and −0.8 at 8, 12, and 16 w respectively, while those for visceral fat area were −8.9 and −15.3 cm^2 at 8 and 12 w, a significant difference as compared to 0 w. In addition, there were significant differences between the 300 mg PFE intake group and the placebo intake group at 4 w and 16 w (BMI only), and at 8 w and 12 w (both BMI and visceral fat area). In contrast, no significant decrease in subcutaneous fat area relative to 0 w and to the placebo group was observed. These data indicate that 300 mg of PFE intake reduces BMI *via* a decrease in visceral but not subcutaneous fat area. As for waist and hip circumference for the PFE intake groups, although significant reductions were observed chronologically, these were not significant as compared to the placebo group.

Table 4 shows the results of a gender stratification analysis of visceral fat area, subcutaneous fat area, and total fat area. In both the male and female 300 mg PFE intake groups, significant decreases as compared to 0 w and the placebo group were observed in visceral fat area at 12 w. These data indicate that the visceral fat area decrease effect of PFE intake displays no sexual dimorphism.

Next we investigated other effects of PFE intake. Table 5 shows the results of a biochemical blood test. They indicate that there were no significant differences between either of the PFE intake groups and the placebo group on any parameter. Triglyceride and γGTP levels were significantly lower than baseline (0 w) only in the 300 mg PFE intake group. It appears that PFE intake also has weak effects on the normalization of triglycerides and as a hepatoprotectant. In the case of HbA_{1c}, significant reductions relative to 0 w were observed in both PFE intake groups and in the placebo group. Considering the chronological reduction in the placebo group, it is difficult to draw conclusions as to the relationship between PFE intake and sugar metabolism. In addition, we checked hormone levels in the blood, including leptin and adiponectin, because PFE intake reduces body fat mass. Leptin levels chronologically decreased in the male 300 mg PFE intake group, but a similar reduction was observed in the placebo group. There was no significant increase in adiponectin levels in either PFE intake group.

Finally, throughout the entire period of the study, no adverse events directly related to PFE intake were reported.

Discussion

Flavonids are present in vegetables, fruits, tea, and wine, and have a diversity of functions, as anti-oxidants, for example. Isoflavone is categorized as a flavonid.

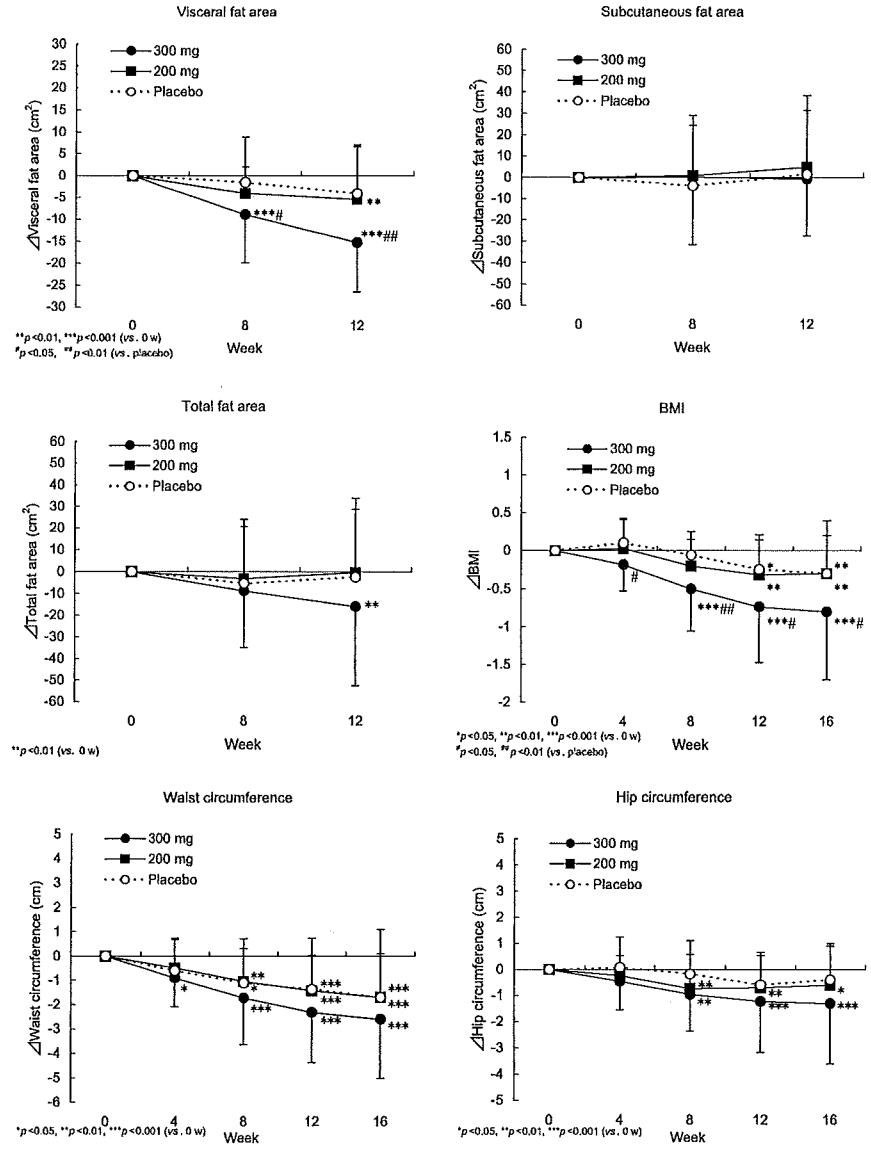

Fig. 1. Degrees of Change in Each of the Various Parameters.

The degrees of change in visceral fat area, subcutaneous fat area, total fat area (0, 8, 12 weeks), BMI, waist circumference, and hip circumference (0, 4, 8, 12, 16 weeks), and SD values are expressed at each point. Each value at 0 weeks indicates zero.

Daidzein, genistein, glyctin are well-known as soy isoflavones, and many clinical and animal studies have reported that intake of soy isoflavones improves the blood lipid profile and glucose metabolism, and thus is beneficial in several chronic disorders associated with obesity and diabetes.[13] Recently, animal studies have evaluated the effects of soy isoflavones on body weight and lipid profile. It has been reported that daidzein and genistein suppressed weight gain and altered hepatic gene expression profiles, as for lipolysis and lipogenesis, as well as adipocyte metabolism.[13–15] We have reported that PFEs possess anti-obesity and anti-fatty liver effects through suppressing lipogenesis in the liver and promoting lipolysis in white adipose tissue and thermogenesis in brown adipose tissue, similarly to soy isoflavones such as daidzein.[13,16] In addition, it has been reported that *Pueraria* flowers are a rich source of isoflavones. *Pueraria thomsonii* contains seven isoflavones, tectoridin, tectorigenin 7-*O*-xylosylglucoside, 6-hydoroxygenistein-6,7-diglucoside, glycitin (glucosides), and tectorigenin, glycitein, and genistein (aglycones).[7] As a quantitative analysis using HPLC, the PFEs used in this study contained all these isoflavones, and the total amount of isoflaveone in the PFE was approximately 18% (see "Materials and Methods"). Moreover, the isoflavone-rich fraction (total isoflavone amount, 63%) of *Pueraria thomsonii*, as well as the PFE, had a body weight reduction effect in the mice (unpublished data). Hence, we speculate that the body weight reduction effect of PFE observed in this study is due primarily to isoflavones, and that the mechanism of fat reduction is an alteration of the hepatic or adipocytic gene profile.

Table 4. Effects of PFE on Abdominal Fat Area (Sex Stratificated Analysis)

Parameter	Group	Sex	n	8 w	12 w
ΔVisceral fat area (cm^2)	300 mg	Total	28	$-8.9 \pm 11.0^{***\#}$	$-15.3 \pm 11.1^{***\#\#}$
		Male	14	$-12.3 \pm 13.0^{**}$	$-16.7 \pm 9.0^{***\#}$
		Female	14	-5.6 ± 7.5	$-13.9 \pm 13.1^{***\#}$
	200 mg	Total	28	-4.0 ± 6.1	$-5.4 \pm 12.1^{**}$
		Male	13	$-5.3 \pm 4.4^{*}$	$-5.3 \pm 6.4^{*}$
		Female	15	-2.9 ± 7.1	-5.5 ± 15.7
	Placebo	Total	25	-1.5 ± 10.4	-4.1 ± 11.2
		Male	12	-2.6 ± 12.7	$-9.1 \pm 9.6^{*}$
		Female	13	-0.5 ± 8.2	0.5 ± 10.9
ΔSubcutaneous fat area (cm^2)	300 mg	Total	28	0.1 ± 24.4	-0.7 ± 32.2
		Male	14	1.1 ± 20.5	-5.9 ± 32.4
		Female	14	-0.8 ± 28.6	4.5 ± 32.2
	200 mg	Total	28	0.9 ± 28.2	4.9 ± 33.5
		Male	13	-2.7 ± 14.8	-2.3 ± 23.1
		Female	15	3.9 ± 36.4	11.2 ± 40.2
	Placebo	Total	25	-3.9 ± 27.7	1.5 ± 29.0
		Male	12	-9.2 ± 19.4	-10.6 ± 21.4
		Female	13	1.0 ± 33.7	12.6 ± 31.4
ΔTotal fat area (cm^2)	300 mg	Total	28	-8.8 ± 26.0	$-16.0 \pm 36.5^{**}$
		Male	14	-11.2 ± 23.3	$-22.6 \pm 34.8^{**}$
		Female	14	-6.4 ± 29.2	-9.4 ± 38.2
	200 mg	Total	28	-3.2 ± 27.2	-0.5 ± 34.4
		Male	13	-8.0 ± 15.7	-7.6 ± 25.7
		Female	15	1.0 ± 34.3	5.7 ± 40.4
	Placebo	Total	25	-5.4 ± 26.2	-2.6 ± 31.4
		Male	12	-11.8 ± 19.6	$-19.7 \pm 23.8^{**}$
		Female	13	0.4 ± 30.7	13.2 ± 29.8

Data are expressed as means ± SD.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* 0 w
$^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* placebo

In this study, we found that PFE intake over 12 weeks reduced BMI *via* a decrease in visceral fat area, with no sexual dimorphism. The body weight and fat reduction effects were observed for a daily oral intake of 300 mg of PFE, converted to an intake of 54 mg of isoflavones per day. Considering that some of the Zutphen Elderly Studies have indicated that a daily flavonoid intake of more than 30 mg reduces the risk of death from coronary heart disease for elderly men,[17,18] a dose of 300 mg of PFE might be correct. In contrast, although a dose of 200 mg of PFE also induced sequential reduction of BMI and visceral fat area as compared to 0 w, there were no significant differences as compared to the placebo group. These data suggest that 300 mg of PFE is the optimal effective dose for reduction of visceral fat.

It is also well-established that absorption of isoflavones from the diet requires deglycosylation (the conversion of glycosides to aglycones).[19] Tsuchihashi *et al.*, as well as our group have reported that the major glycosides of *Pueraria thomsonii* are metabolized to tectorigenin by anaerobic cultivation by human fecal or human intestinal bacterial strains.[20,21] It is likely that aglycone (tectorigenin), derived from *Pueraria thomsonii*, is a key factor in the reduction of body weight and fat observed in this study. In future work, we intend to investigate the active components of PFE in detail.

A metaanalysis has suggested that soy isoflavones lower total and LDL cholesterol in humans,[22] but in the present study no reduction in total or LDL cholesterol with PFE intake was observed. It has been reported that hormonal estrogen improves cholesterol homeostasis.[23] Tectorigenin, probably a main metabolite from PFE, had low binding activity to estrogen receptor α and β as compared to soy isoflavones such as genistein. Possibly due to this weak estrogenic effect of tectroigenin as compared to soy isoflavones, no cholesterol homeostasis was observed.

In this study, we detected a reduction in γGTP as compared to 0 w in the 300 mg PFE intake group. Tectoridin and tectorigenin reduced the ALT and AST values increased by *tert*-butyl hyperoxide (*t*-BHP).[24] Decreases in the γGTP concentration were observed in our previous studies.[12] Hence, PFE may also help in liver protection in humans. In addition, triglyceride reduction was observed as compared to 0 w in the 300 mg PFE intake group. In our previous animal study, PFE intake reduced ACC gene expression in the liver and increased UCP1 gene expression in the brown adipose tissue.[16] Some clinical studies have found that suppression of lipogenesis and mutation of UCP1 influence serum triglyceride levels.[25,26] The reduction of trigriceride observed in this study might have been due to changes in these gene profiles.

Previously we reported the effect on body weight reduction of PFE intake in males.[12] In the present study, we investigated the effect of PFE intake on obesity in both males and females. We found that 300 mg of PFE intake over 12 weeks reduced BMI *via* a decrease in visceral fat area, with no sexual dimorphism. Moreover, in this study, no serious adverse events directly related

Table 5. Changes in Blood Biochemical Parameters

Parameter	Group	n	0 w	4 w	8 w	12 w	16 w
Triglyceride (mg/dL)	300 mg	28	121.6 ± 59.1	111.5 ± 38.6	98.6 ± 37.3*	96.2 ± 33.0*	117.2 ± 52.0
	200 mg	28	112.6 ± 44.1	115.4 ± 71.8	104.1 ± 45.6	118.1 ± 56.7	110.4 ± 42.4
	Placebo	25	111.9 ± 54.0	117.6 ± 55.8	100.4 ± 43.6	113.8 ± 59.2	112.0 ± 45.6
Total cholesterol (mg/dL)	300 mg	28	204.1 ± 30.8	208.4 ± 32.0	204.6 ± 35.9	199.8 ± 29.8	205.0 ± 35.3
	200 mg	28	208.4 ± 37.8	212.9 ± 37.8	202.5 ± 33.2	204.1 ± 37.9	205.7 ± 40.3
	Placebo	25	204.0 ± 38.1	200.0 ± 33.4	199.1 ± 34.8	197.5 ± 33.4	205.7 ± 35.9
LDL cholesterol (mg/dL)	300 mg	28	136.8 ± 28.4	140.3 ± 30.0	137.4 ± 33.6	133.2 ± 28.7	134.2 ± 31.2
	200 mg	28	134.8 ± 34.7	136.4 ± 33.2	131.8 ± 31.5	130.8 ± 34.1	129.4 ± 34.1
	Placebo	25	129.6 ± 31.0	125.1 ± 26.1	128.4 ± 29.2	124.4 ± 25.5	130.3 ± 31.5
HDL cholesterol (mg/dL)	300 mg	28	50.5 ± 8.8	50.0 ± 7.7	50.4 ± 10.0	50.1 ± 9.2	49.6 ± 8.4
	200 mg	28	55.3 ± 10.8	54.5 ± 11.7	52.9 ± 10.4	52.8 ± 10.4	54.1 ± 9.8
	Placebo	25	55.5 ± 13.0	52.7 ± 11.9	52.3 ± 11.0	52.8 ± 11.7	53.6 ± 13.6
Glucose (mg/dL)	300 mg	28	91.0 ± 7.3	92.8 ± 9.2	92.2 ± 6.6	91.2 ± 9.4	92.3 ± 5.9
	200 mg	28	93.7 ± 7.5	93.1 ± 8.7	94.4 ± 8.8	93.8 ± 9.0	94.1 ± 8.3
	Placebo	25	90.8 ± 7.4	89.9 ± 7.2	90.9 ± 5.5	89.3 ± 5.5	92.3 ± 5.5
HbA_{1c} (%)	300 mg	28	5.20 ± 0.23	5.02 ± 0.23***	4.99 ± 0.22***	4.99 ± 0.19***	5.01 ± 0.22***
	200 mg	28	5.20 ± 0.31	5.06 ± 0.28***	5.03 ± 0.28***	5.01 ± 0.28***	5.03 ± 0.30***
	Placebo	25	5.12 ± 0.46	4.96 ± 0.44***	4.94 ± 0.44***	4.93 ± 0.44***	4.97 ± 0.42***
Insulin (μU/mL)	300 mg	27	6.65 ± 2.51	7.66 ± 6.83	7.14 ± 2.95	6.63 ± 2.59	7.14 ± 2.26
	200 mg	27	5.65 ± 2.07	5.40 ± 1.77	5.58 ± 2.40	6.16 ± 2.47	6.24 ± 2.22
	Placebo	25	5.58 ± 1.82	5.00 ± 1.70	5.95 ± 2.03	5.63 ± 1.66	6.18 ± 2.49
AST (IU/L)	300 mg	28	19.6 ± 3.9	18.9 ± 4.5	19.2 ± 5.8	20.0 ± 9.6	20.1 ± 5.9
	200 mg	28	19.1 ± 5.3	20.7 ± 7.9	17.9 ± 4.5	19.1 ± 7.4	19.1 ± 3.9
	Placebo	25	18.7 ± 3.5	18.6 ± 4.8	18.1 ± 4.6	18.0 ± 5.0	19.3 ± 5.7
ALT (IU/L)	300 mg	28	26.4 ± 11.2	23.4 ± 10.4	23.8 ± 11.0	25.0 ± 18.9	23.8 ± 11.5
	200 mg	28	21.1 ± 10.7	20.6 ± 10.2	18.5 ± 8.6	21.1 ± 15.8	18.9 ± 6.5
	Placebo	25	23.6 ± 12.6	23.4 ± 14.4	21.5 ± 13.6	20.4 ± 10.1	23.4 ± 12.7
γGTP (IU/L)	300 mg	28	31.4 ± 12.5	26.6 ± 8.5**	26.4 ± 10.5**	26.6 ± 9.3**	26.8 ± 7.6**
	200 mg	28	28.3 ± 15.3	28.2 ± 15.0	26.7 ± 14.8	32.1 ± 32.4	28.4 ± 15.7
	Placebo	25	28.3 ± 16.6	27.8 ± 18.1	26.4 ± 16.8	26.2 ± 15.4	29.3 ± 16.9
Leptin (ng/mL)	300 mg	Male14	4.84 ± 1.31	3.64 ± 1.80**	3.94 ± 1.99*	3.51 ± 2.50**	4.23 ± 1.83
		Female14	12.28 ± 2.81	12.19 ± 3.42	12.45 ± 3.81	10.46 ± 2.97	11.48 ± 3.81
	200 mg	Male13	5.38 ± 1.99	4.75 ± 1.66	4.75 ± 2.12	4.31 ± 1.83	4.98 ± 1.77
		Female15	12.03 ± 3.44	11.03 ± 3.28	12.08 ± 3.49	10.80 ± 3.80	13.14 ± 3.55
	Placebo	Male12	6.13 ± 2.72	5.00 ± 1.78*	4.73 ± 1.99**	4.29 ± 1.94***	5.07 ± 2.28
		Female13	11.38 ± 2.62	11.57 ± 2.71	11.30 ± 2.15	10.72 ± 3.32	11.21 ± 2.94
Adiponectin (μg/mL)	300 mg	28	5.35 ± 2.58	5.34 ± 2.88	5.60 ± 2.70	5.74 ± 3.26	
	200 mg	28	6.35 ± 2.62	6.41 ± 2.88	6.33 ± 2.95	6.28 ± 2.80	
	Placebo	25	7.35 ± 2.83	7.11 ± 2.99	7.99 ± 3.37	7.88 ± 3.51	

Data are expressed as means ± SD.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* 0 w
In the case of insulin, two people were eliminated from analysis because their values were too low to detect. Therefore, the numbers for the groups were 27 (300 mg PFE group), 27 (200 mg PFE group), and 25 (placebo group).

to PFE intake were reported. We propose that PFE extracted from flowers of *Pueraria thomsonii* might serve as a functional food promoting body fat reduction.

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