— **Regular Article** —

Preliminary Research for the Anti-obesity Effect of Puerariae Flos Extract in Humans

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This research is preliminary study to examine whether puerariae flos extract (PFE) makes any action to body fat of man or not. We conducted the double-blind placebo controlled study on eighty mildly obese subjects for 8 weeks. The subjects were randomly divided into 4 groups: I, II, III, and IV. Subjects in groups I, II, and III consumed test food containing 100, 200, and 300 mg PFE, respectively, while those in group IV were given placebo food for 8 weeks. All subjects were instructed to restrict their total-energy intake to within 2650 kcal/day in the case of males and 2300 kcal/day in the case of females during the test period. Haematological and biochemical markers of blood, urinary markers, and physical markers were examined at 0, 4, and 8 weeks during the test period. As a result, no adverse effects were noted in any of the groups. For physical examinations, we analysed only males whose initial body mass index (BMI) values were over 24. BMI value and body weights of the subjects in group III were significantly reduced, and total fat area and subcutaneous fat area of the subjects in groups II and III were significantly reduced over the 8-week test period. Moreover, as a result of comparison among groups by unpaired *t*-test, significant difference appeared between group III and group IV in BMI. Total fat area in group III tend to decrease compared with group IV. By these results, the reduction of BMI in group III may reflect fat area reduction. To confirm the anti-obesity effect of PFE, we need to research particularly by extensive studies hereafter.

Key words —— anti-obesity, puerariae flower extract, kudzu, isoflavone, abdominal fat area

INTRODUCTION

Obesity is a major public health issue, as it is closely related to mortality. Obesity is caused by a variety of factors, one of which is an imbalance between energy intake and expenditure. Therefore, it is important for the prevention of obesity to suppress energy intake and increase its expenditure. Diet therapy is generally advocated for suppressing energy intake, while exercise is recommended for ensuring adequate energy expenditure. In addition, the use of a dietary supplement that can reduce energy intake by inhibiting the absorption of sugar and fat and/or elevate energy metabolism in the liver and muscles via β-oxidation is recommended.

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Pueraria flos extract (PFE) is an extract from flowers of kudzu (*Puerariae thomsonii*). Kudzu, a leguminous plant distributed in Japan, China, and other areas, has long been used in folk medicine. In particular, the *Puerariae* flower is used in Japanese and Chinese folk medicine for curing hangovers.[1–3)]

Niiho *et al.* confirmed that the consumption of the *Puerariae lobata* flower reduces glutamic oxaloacetic transaminase (GOT) or glutamic pyruvic transaminase (GPT) levels in individuals with liver injury due to carbon tetrachloride (CCl_4) or a high-fat diet in animal study.[1, 2)] In East Asia, this flower has also recently been used in herbal medicines for the management of menopausal symptoms.[4, 5)]

Puerariae thomsonii is known to contain 7 isoflavones (4 isoflavone glucosides: tectoridin, tectorigenin 7-*O*-xylosylglucoside, 6-hydroxygenistein-6,7-diglucoside, and glycitin; and 3 aglycones: tectorigenin, glycitein, and genistein)

Table 1. Analysis Value of Nutrient Composition for Test Food (1.0 g per Packet)

	Test food (containing 100 mgPFE)	Test food (containing 200 mgPFE)	Test food (containing 300 mgPFE)	Placebo food
Energy[a] (kcal)	3.94	3.88	3.84	3.96
Moisture (g)	0.036	0.039	0.040	0.037
Protein[b] (g)	0.019	0.039	0.059	0.001
Fat (g)	0.033	0.036	0.040	0.031
Carbohydrate (g)	0.891	0.851	0.812	0.918

a) Calorie conversion factor: Protein 4, Fat 9, Carbohydrate 4. *b*) Nitrogen-to-protein conversion factor: 6.25.

and 3 saponins (soyasaponin I, kaikasaponin III, and kakkasaponin I).[6]

It has been reported that isoflavones such as genistein affect lipid metabolism in the liver and inhibit adipocyte differentiation,[7,8] and that saponins have an inhibitory effect on lipase activity.[9] Therefore, PFE consumption is expected to provide the dual benefits of reducing energy intake and increasing its expenditure.

Wang *et al.* confirmed that flavones derived from puerariae radix have inhibitory effects on body weight, abdominal fat, and lipids in the liver.[10] However, the impact of PFE on body fat is not known. This clinical study was preliminary conducted on eighty mildly obese subjects to view the body fat reducing action of PFE.

MATERIALS AND METHODS

Design and Subjects —— We had drew up a clinical protocol for this study. Then this study had been approved by Institutional Review Board of Kurume clinical pharmacology clinic (July 28, 2005) before its launch. Furthermore this study was conducted in accordance with the Helsinki Declaration, and informed consent was obtained from all subjects. We entrusted all affairs of this study to Iberica Holdings Co., Ltd. (Fukuoka, Japan). They carried it out at Kurume clinical pharmacology clinic (Kurume, Japan).

Volunteers who live in around Kurume city had a questionnaire related to previous disease and administration of drugs, blood tests, body checks and a medical interview by a doctor. Subjects aged between 20 and 65 years were recruited for the study, according to fixed inclusion and exclusion criteria. The inclusion criteria pertained to the following prospective subjects: those having a body mass index (BMI) value of 23–30 or a waist circumference larger than 85 cm for males or 90 cm for females, those who had visited a hospital for treatment and provided informed consent for study enrolment, those who were deemed to be suitable for the study by a doctor, those who had been certified as healthy by a doctor during a prior clinical inspection, and those who were not under any drug treatment.

The exclusion criteria pertained to the following prospective subjects: those using functional foods, cosmetics, or instruments that may influence lipid metabolism (*e.g.*, hypolipidaemic compounds and antihypertensive agents), those with liver dysfunction, pregnant women or those suspected to be pregnant, those who were considered unsuitable for the study due to an illness or the possibility of developing serious side effects, and those who were deemed to be inappropriate subjects by a doctor.

Test Food —— Tablets containing PFE (Ohta's Isan Co. Ltd., Ushiku, Japan), reduced palatinose, cellulose, fatty acid esters of sucrose, and silicon dioxide were used as the test food. The placebo food consisted of tablets containing caramel as a dye, instead of PFE. Table 1 shows analysis value of nutrient composition. The test food and the placebo food were distributed with plain aluminium packets sealed individually containing 1.0 g per packet. The subjects in each group were required to consume the allotted tablet once per day.

Experimental Design —— Double-blind placebo controlled study was conducted in this case. Figure 1 shows the test schedule. Following a 2-week pre-observation period (*i.e.*, at "week 0"), haematological, urine, and physical examinations and blood biochemical analyses were conducted at the hospital. Further, 80 subjects with mild obesity were randomly divided into 4 groups using block randomization. Subjects in groups I, II, and III were required to ingest 100, 200, and 300 mg of PFE, respectively, while those in group IV were required to ingest the placebo food once per day for 8 weeks. Subjects were restricted to take medicines and supplements as a general rule and had dietary instruction

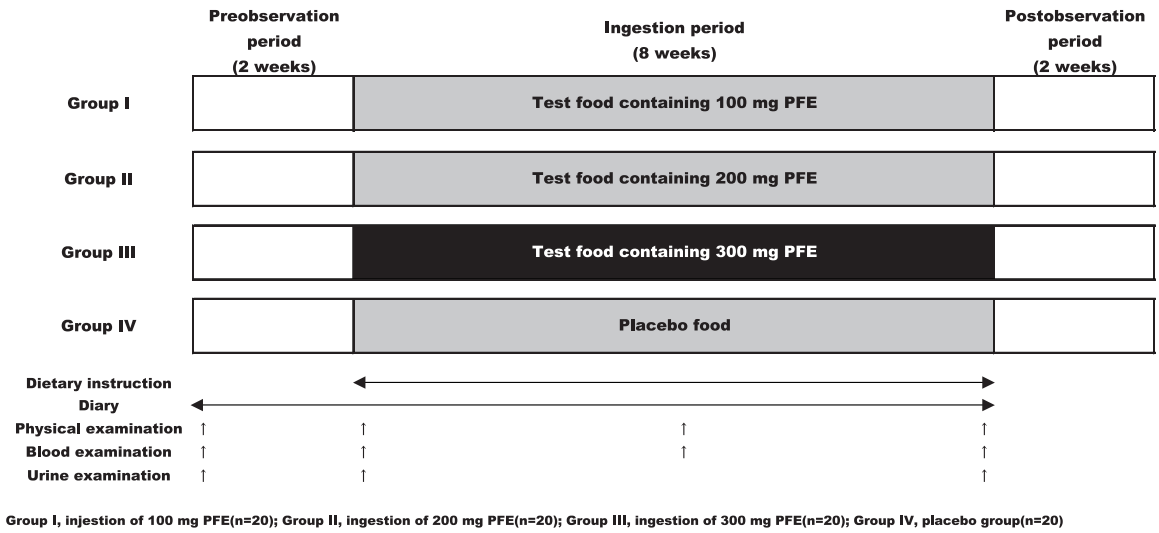


Fig. 1. Test Schedule

to prevent from rapid change of calories consumed during the intake of PFE. We distributed pedometers to the subjects to instruct not to make rapid change to their amount of exercise. Regarding other daily habit, we instructed to have a life as usual. Subjects were also asked to keep the daily record of test food consumption, rational symptoms, all foods consumed including articles of taste, amount of exercise and intake of prohibited medicines or supplements.

Examinations were conducted at 4 and 8 weeks after week 0 (*i.e.*, during week 4 and week 8). Blood biochemical analyses and physical examinations were conducted at week 4 and additional haematological and urine examinations and blood biochemical analyses were conducted at week 8.

Furthermore, subjective symptoms were recorded 2 weeks after the study period.

Dietary Instruction —— The subjects were instructed to limit their total energy intake to under 2650 kcal/day in the case of males, and 2300 kcal/day in the case of females, and also instructed not to reduce food intake substantially basing on the Dietary Reference Intakes for Japanese, 2005. The details of their meal intake are provided as followings. Breakfast: bread or rice, a side dish, and milk, Lunch: nutritious foods for which the total energy intake could be measured and the nutrients classified, and Dinner: a home-delivered meal (main dish, 300 kcal; side dish, 320 kcal).

With regard to alcohol consumption, the subjects were permitted less than 1 large bottle of beer or the equivalent. Alcohol consumption was forbidden on the day before a blood examination or medical interview.

Haematological Examinations and Biochemical Examinations of Blood —— Haematological examinations were conducted at weeks 0 and 8. Blood biochemical analyses were conducted at weeks 0, 4, and 8.

The parameters assessed in the haematological examinations were the white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) level, and haematocrit (Ht) value. Those assessed in the blood biochemical analyses performed at week 4 were the triglyceride, total cholesterol (T-Cho), low-density-lipoprotein cholesterol (LDL-Cho), high-density-lipoprotein cholesterol (HDL-Cho), remnant-lipoprotein cholesterol (RLP-Cho), leptin, fasting blood glucose (FBG), and insulin levels. In addition, the parameters assessed in the blood biochemical analyses performed at week 8 were the total proteins, and the GOT, GPT, γ-glutamyl transpeptidase (γ-GTP), albumin, creatinine, total bilirubin (TB), blood urea nitrogen (BUN), uric acid, creatine kinase (CPK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglyceride, T-Cho, LDL-Cho, HDL-Cho, RLP-Cho, leptin, FBG, insulin, and haemoglobin A1c (HbA1c) levels.

Urine Examinations —— Urine examinations were conducted at weeks 0 and 8. The parameters assessed were the urine sugar and protein content and the presence of blood in the urine.

Rational Symptoms —— Subjective symptoms, which occurred during the study, were tallied. Symptoms, which confirmed a relation to the test food by a doctor, were also tallied.

Table 2. Subject Characteristics

	Group I (n = 18)	Group II (n = 15)	Group III (n = 18)	Group IV (n = 18)
Number	Male: 16	Male: 12	Male: 15	Male: 15
	Female: 2	Female: 3	Female: 3	Female: 3
Age (year-old)	40.3 ± 7.9	37.9 ± 9.1	39.3 ± 12.4	36.7 ± 9.4
Body weight (kg)	75.2 ± 9.1	74.2 ± 11.8	72.0 ± 9.4	76.1 ± 8.6
BMI (kg/m^2)	26.7 ± 2.6	26.4 ± 3.2	25.5 ± 2.9	26.5 ± 2.5
Waist circumference (cm)	95.2 ± 8.5	95.2 ± 10.1	93.3 ± 7.6	96.8 ± 8.4
Hip circumference (cm)	99.9 ± 5.4	98.6 ± 5.7	99.8 ± 4.4	101.4 ± 4.2
Total fat area (cm^2)	343.0 ± 82.4	323.0 ± 126.4	306.6 ± 92.0	331.4 ± 85.4
Visceral fat area (cm^2)	128.5 ± 38.1	133.8 ± 115.6	111.3 ± 44.2	122.8 ± 53.5
Subcutaneous fat area (cm^2)	214.5 ± 65.1	189.2 ± 52.4	195.3 ± 65.2	208.6 ± 59.4
Systolic blood pressure (mmHg)	128.9 ± 11.4	128.5 ± 17.3	120.1 ± 9.4	126.1 ± 15.4
Diastolic blood pressure (mmHg)	82.2 ± 7.3	80.3 ± 15.2	74.9 ± 9.6	80.8 ± 12.6

See Fig. 1 for Groups. Values are means ± S.D.

Physical Examinations —— Physical examinations were conducted at weeks 0 and 8, and the parameters assessed were height (data not shown), body weight, BMI, waist circumference, hip circumference, total fat area, visceral fat area, subcutaneous fat area, systolic blood pressure, and diastolic blood pressure.

The hip circumference was measured using a calibrated tape. The waist circumference, total fat area, visceral fat area, and subcutaneous fat area were measured by performing computerized tomography (CT) while the subjects held their breath following expiration. The CT scan data were analysed using the Fat scan software (East Japan Institute of Technology Co. Ltd., Hitachi, Japan).[11)]

Statistical Analysis —— We performed a repeated-measures one-way analysis of variance (ANOVA) to investigate the changes over the time course. When statistically significant differences were detected, a post-hoc pairwise comparison across treatments was performed using Fisher's probability of least significant difference (PLSD) test for multiple comparisons. For the comparison among groups, analyses were performed by Tukey-Kramer test on all test items. However, this study is preliminary. Therefore, distinctions of weight index relating to body fat, BMI, waist circumference, hip circumference, total fat area, subcutaneous fat area and visceral fat area between the group consumed test food (100, 200, and 300 mg) and the group consumed placebo food were analysed by unpaired t-test.

All statistical analyses were performed using Statview ver. 5.0 (SAS Institute Japan Ltd., Tokyo, Japan), and significance was set at $p < 0.05$.

RESULTS

Exclusion from the Study

One subject in group II withdrew his consent for participation and was excluded from the study; however, this was not due to any problem with the test food.

Subjects who had not eaten home-delivered meals for more than 10% of all meals were excluded from the study. The number of subjects was 18, 15, 18, and 18 for groups I, II, III, and IV, respectively. Subject characteristics are listed in Table 2.

Haematological Examinations and Blood Biochemical Analyses

The results of the haematological examinations are shown on Table 3. At week 8, the WBC count in group III and the Ht value in group I had changed significantly from the initial values. However, the WBC count remained within the normal range. Although the average Ht values recorded for group I at week 8 exceeded the normal values for females, the values recorded for individual female subjects in this group were all within the normal range. Thus, no abnormalities were noted during the haematological examinations (RBC, WBC, Ht, and Hb).

The results of the blood biochemical analyses are shown in Tables 4 and 5. Although the T-Cho and HDL-Cho levels in all the groups had reduced significantly at week 4 compared to week 0, they returned to their original values at week 8. The LDL-Cho levels reduced significantly in groups I and III at week 4, but returned to their original normal valves at week 8. Although the LDL-Cho levels

Table 3. Hematological Examination

	Group	number	0 week	8 week
White blood cell count	I	18	6.644 ± 1.641	6.600 ± 1.721
($\times 10^3$/μl)	II	15	6.693 ± 2.045	6.513 ± 1.904
	III	18	6.967 ± 1.304	6.439 ± 1.501*
	IV	18	6.578 ± 1.196	6.694 ± 1.555
Red blood cell count	I	18	4.99 ± 0.38	5.05 ± 0.38
($\times 10^6$/μl)	II	15	4.85 ± 0.38	4.94 ± 0.34
	III	18	5.00 ± 0.39	5.00 ± 0.39
	IV	18	4.92 ± 0.44	4.93 ± 0.47
Hemoglobin level	I	18	15.24 ± 1.23	15.22 ± 1.26
(g/dl)	II	15	15.01 ± 1.49	15.14 ± 1.32
	III	18	15.16 ± 1.41	15.09 ± 1.40
	IV	18	14.98 ± 1.22	14.83 ± 1.31
Hematocrit	I	18	45.66 ± 3.23	46.65 ± 3.38**
(%)	II	15	44.62 ± 3.80	45.79 ± 3.42
	III	18	45.10 ± 3.77	45.55 ± 4.11
	IV	18	44.87 ± 3.18	45.39 ± 3.55

See Fig. 1 for Groups. Values are means ± S.D. * and ** indicate significantly different from the value obtained in week 0 at $p < 0.05$ and $p < 0.01$, respectively.

recorded for group III exceeded the normal range at week 8, these levels were higher than normal, even at week 0. Therefore, the changes in these levels were not considered abnormal. The leptin levels were significantly lower in group III at both of weeks 4 and 8, as compared to week 0. Group I significantly showed low value at weeks 8, as compared to week 0. Since leptin is secreted from fat tissue, this result may be attributable to a reduction in body fat. The TB level in group II; GOT and GPT levels in groups I, II, and III; uric acid levels in groups I and III; and the ALP level in group III were all significantly different from the corresponding values at week 0, but remained within the normal range. Although the average γ-GTP values of groups I and III were significantly lower than the week 0 values, they exceeded the normal range for females. Nevertheless, the values recorded for individual female subjects in these 2 groups were all within the normal range. No significant changes were noted in the other parameters for any of the groups.

Urinary Examinations

Sugar was not detected in the urine of any of the subjects throughout the study period. In addition, proteins were not detected in the urine of any of the subjects in groups II and III throughout the study period. For 1 subject belonging to group I, the status of urinary proteins was detected (±) at week 8; however, proteins were detected (+) in the urine of this subject even at week 0. In addition, in the case of 1 subject belonging to group IV, the status of urinary proteins was detected (±) at week 8; however, this group was the placebo group. Therefore, we considered that PFE consumption did not cause any of the abnormalities detected during the urine examinations.

Blood was detected (+) in the urine of 1 subject belonging to group III in week 8, but this subject exhibited bloody urine throughout the study period. In group II, the status of blood in the urine was assessed as (2+) in 1 subject, (+) in 1 subject, and (±) in 1 subject at week 8. In the case of the subject for whom the status was found to be (2+), the blood in the urine was confirmed to be related to menstruation, according to the recordings in a diary that had been maintained for this patient. With regard to the subject for whom the status was assessed as (+), a status ranging from (±) to (+) was noted throughout the study period. Further, in the case of the subject for whom the status of blood in the urine was assessed as ±, a similar status had been noted during prior examinations. Thus, our observations may have been due to an inherent characteristic of this subject.

In 1 subject belonging to group IV, the status of blood in the urine was found to be (2+) at week 8, which was identical to the status found at week 0. Therefore, we consider that our findings may be attributed to the inherent characteristics of this subject.

Table 4. Biochemical Examination (Liver and Renal Function)

	Group	Number	Week 0	Week 8
Total protein	I	18	7.41 ± 0.36	7.38 ± 0.36
(g/dl)	II	15	7.29 ± 0.25	7.25 ± 0.32
	III	18	7.41 ± 0.25	7.31 ± 0.33
	IV	18	7.41 ± 0.26	7.29 ± 0.32
Albumin	I	18	4.57 ± 0.24	4.62 ± 0.25
(g/dl)	II	15	4.50 ± 0.21	4.55 ± 0.24
	III	18	4.59 ± 0.17	4.56 ± 0.18
	IV	18	4.62 ± 0.22	4.61 ± 0.22
Total bilirubin	I	18	0.67 ± 0.17	0.68 ± 0.27
(mg/dl)	II	15	0.72 ± 0.23	0.61 ± 0.22*
	III	18	0.72 ± 0.35	0.64 ± 0.27
	IV	18	0.68 ± 0.23	0.65 ± 0.22
GOT	I	18	26.4 ± 7.8	22.0 ± 5.3**
(IU/l)	II	15	25.5 ± 13.6	19.9 ± 7.0*
	III	18	24.0 ± 11.3	20.4 ± 7.6*
	IV	18	24.8 ± 8.4	24.9 ± 15.9
GPT	I	18	43.7 ± 24.4	35.1 ± 22.2*
(IU/l)	II	15	37.3 ± 31.1	25.9 ± 16.8*
	III	18	38.4 ± 26.5	29.2 ± 19.7***
	IV	18	36.2 ± 24.1	37.2 ± 29.8
ALP	I	18	221.1 ± 39.8	226.7 ± 50.4
(IU/l)	II	15	221.2 ± 52.1	224.4 ± 55.3
	III	18	195.4 ± 47.9	206.6 ± 50.7*
	IV	18	213.4 ± 62.6	220.5 ± 65.3
LDH	I	18	195.7 ± 34.4	186.4 ± 25.7
(IU/l)	II	15	188.0 ± 33.6	186.7 ± 27.7
	III	18	177.0 ± 28.1	170.3 ± 24.1
	IV	18	190.5 ± 27.7	191.6 ± 26.5
γ-GTP	I	18	55.6 ± 33.6	42.9 ± 21.7**
(IU/l)	II	15	51.3 ± 46.4	41.3 ± 28.1
	III	18	43.7 ± 37.7	34.4 ± 24.2*
	IV	18	45.4 ± 32.5	48.9 ± 39.7
BUN	I	18	14.6 ± 4.3	12.7 ± 2.5
(mg/dl)	II	15	12.5 ± 2.2	12.1 ± 2.9
	III	18	12.8 ± 2.1	13.3 ± 3.0
	IV	18	13.1 ± 2.5	12.8 ± 2.5
Creatinine	I	18	0.80 ± 0.13	0.79 ± 0.13
(mg/dl)	II	15	0.81 ± 0.14	0.80 ± 0.14
	III	18	0.79 ± 0.12	0.79 ± 0.11
	IV	18	0.77 ± 0.10	0.78 ± 0.10
Uric acid	I	18	6.55 ± 1.70	5.94 ± 1.55***
(mg/dl)	II	15	6.21 ± 1.51	6.05 ± 1.09
	III	18	5.98 ± 1.60	5.68 ± 1.55*
	IV	18	6.09 ± 1.29	6.07 ± 1.52
CPK	I	18	155.3 ± 81.9	156.6 ± 101.7
(IU/l)	II	15	118.0 ± 52.4	118.4 ± 47.7
	III	18	110.9 ± 43.2	104.6 ± 33.6
	IV	18	127.4 ± 52.9	124.1 ± 74.3

See Fig. 1 for Groups. Values are means ± S.D. *, **, and *** indicate significantly different from the value obtained in week 0 at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Table 5. Biochemical Examination (Lipid and Glucose Levels)

	Group		Number	Week 0	Week 4	Week 8
Total cholesterol	I		18	197.2 ± 25.1	186.8 ± 26.9*	198.1 ± 27.7
(mg/dl)	II		15	202.1 ± 54.3	191.0 ± 45.0**	202.3 ± 54.4
	III		18	215.4 ± 29.9	194.0 ± 23.2***	208.1 ± 29.9
	IV		18	208.6 ± 43.7	194.1 ± 33.5**	209.2 ± 30.8
LDL cholesterol	I		18	135.7 ± 24.1	126.2 ± 25.9**	134.0 ± 26.5
(mg/dl)	II		15	139.4 ± 54.4	127.9 ± 43.9	133.8 ± 51.6
	III		18	146.7 ± 27.1	132.0 ± 18.0**	139.1 ± 25.5
	IV		18	141.0 ± 34.8	131.5 ± 31.0	137.1 ± 28.5
HDL cholesterol	I		18	48.8 ± 9.4	43.6 ± 6.8***	49.1 ± 8.1
(mg/dl)	II		15	52.6 ± 8.3	49.5 ± 8.7*	52.9 ± 10.2
	III		18	50.2 ± 10.7	47.7 ± 9.9*	50.7 ± 11.4
	IV		18	49.7 ± 13.1	45.8 ± 7.9*	51.2 ± 9.9
RLP cholesterol	I		18	6.4 ± 3.2	6.6 ± 3.6	5.1 ± 2.9
(mg/dl)	II		15	4.9 ± 2.4	4.9 ± 2.8	4.9 ± 2.1
	III		18	6.8 ± 5.0	5.6 ± 3.3	6.4 ± 3.9
	IV		18	7.1 ± 3.6	5.6 ± 2.4	5.8 ± 2.1
Triglycerides	I		18	139.0 ± 71.9	160.7 ± 67.2	127.9 ± 71.3
(mg/dl)	II		15	104.4 ± 57.3	119.9 ± 63.8	109.6 ± 42.0
	III		18	148.8 ± 84.6	135.4 ± 66.1	137.5 ± 62.2
	IV		18	154.5 ± 61.2	138.2 ± 47.2	138.1 ± 44.2
Fasting blood glucose	I		18	90.3 ± 5.8	90.7 ± 7.2	91.9 ± 6.4
(mg/dl)	II		15	91.1 ± 6.0	91.2 ± 5.9	92.7 ± 8.9
	III		18	89.9 ± 4.8	91.4 ± 8.9	91.1 ± 4.2
	IV		18	90.1 ± 4.5	91.3 ± 8.0	89.7 ± 6.6
Insulin	I		18	9.2 ± 3.5	9.3 ± 4.2	9.9 ± 5.5
(mu/ml)	II		15	7.0 ± 3.0	7.3 ± 3.6	8.1 ± 4.0
	III		18	8.1 ± 3.3	8.2 ± 3.6	9.1 ± 4.5
	IV		18	11.6 ± 8.4	11.5 ± 8.7	11.1 ± 5.6
HbA1c	I		18	4.81 ± 0.22	—	4.76 ± 0.20
(%)	II		15	4.81 ± 0.20	—	4.83 ± 0.25
	III		18	4.77 ± 0.35	—	4.81 ± 0.32
	IV		18	4.82 ± 0.23	—	4.84 ± 0.23
Leptin	I	male	16	6.9 ± 2.4	6.0 ± 2.7	5.8 ± 3.0*
(ng/dl)		female	2	14.9 ± 8.1	9.9 ± 0.1	7.6 ± 0.8
	II	male	12	7.3 ± 4.2	4.8 ± 2.0	4.9 ± 2.4
		female	3	14.9 ± 8.1	12.8 ± 6.0	12.3 ± 4.2
	III	male	15	6.4 ± 4.6	4.7 ± 2.9**	4.5 ± 2.3**
		female	3	14.2 ± 5.0	10.7 ± 2.1	10.1 ± 3.5
	IV	male	15	7.0 ± 3.4	6.6 ± 3.9	6.2 ± 3.8
		female	3	14.6 ± 5.7	10.6 ± 2.8	10.2 ± 2.7

See Fig. 1 for Groups. Values are means ± S.D. *, **, and *** indicate significantly different from the value obtained in week 0 at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Rational Symptoms

The findings with regard to the rational symptoms are shown in Table 6. Various subjective symptoms occurred in all groups. However, a doctor judged there were no relations between those symptoms and the test food.

Physical Examination

Here, we describe the rates of change in the parameters that were assessed during the physical examinations. To evaluate the effects of PFE consumption on body fat, we analysed 58 subjects with BMI values > 24, who were defined as obese ac-

Table 6. Number of Occurrence of Rational Symptoms

	Group I	Group II	Group III	Group IV
Cold	79 (0)	13 (0)	14 (0)	25 (0)
Vomiting	0 (0)	1 (0)	2 (0)	0 (0)
Skin eruption	0 (0)	0 (0)	0 (0)	0 (0)
Menstruation	2 (0)	4 (0)	21 (0)	20 (0)
Abdominal discomfort	0 (0)	0 (0)	0 (0)	2 (0)
Gastric pain	2 (0)	1 (0)	0 (0)	1 (0)
Abdominal pain	0 (0)	0 (0)	1 (0)	3 (0)
Abdominal tension	2 (0)	0 (0)	1 (0)	5 (0)
Constipation	0 (0)	0 (0)	0 (0)	9 (0)
Diarrhoea	25 (0)	16 (0)	20 (0)	36 (0)

Total sum of rational symptoms (the number of symptoms confirmed a relation to the test food by a doctor). See Fig. 1 for Groups.

Table 7. Subject Backgrounds (BMI > 24, Male)

	Group I (male) (n = 14)	Group II (male) (n = 11)	Group III (male) (n = 11)	Group IV (male) (n = 14)
Age (years)	40.3 ± 8.6	37.1 ± 8.0	37.8 ± 11.1	34.6 ± 9.0
Body weight (kg)	78.2 ± 7.9	78.4 ± 10.2	76.6 ± 8.5	78.5 ± 8.2
BMI (kg/m^2)	27.5 ± 2.4	27.1 ± 3.4	26.5 ± 3.2	26.8 ± 2.7
Waist circumference (cm)	97.2 ± 8.6	96.1 ± 11.5	97.3 ± 6.2	97.9 ± 8.5
Hip circumference (cm)	101.2 ± 5.3	99.4 ± 5.8	102.0 ± 3.9	102.2 ± 4.3
Total fat area (cm^2)	360.6 ± 83.6	330.6 ± 145.9	349.5 ± 80.2	335.5 ± 86.9
Visceral fat area (cm^2)	138.3 ± 37.1	151.3 ± 130.5	130.2 ± 40.5	131.5 ± 56.4
Subcutaneous fat area (cm^2)	222.4 ± 68.0	179.2 ± 39.1	219.4 ± 69.3	204.0 ± 54.2
Systolic blood pressure (mmHg)	130.0 ± 11.1	134.8 ± 12.2	118.5 ± 10.0	127.6 ± 16.8
Diastolic blood pressure (mmHg)	81.6 ± 8.0	84.5 ± 11.1	75.5 ± 10.4	81.1 ± 14.0

See Fig. 1 for Groups. Values are means ± S.D.

Table 8. Energy Intake and Amount of Exercise (BMI > 24, Male)

	Group I (male) (n = 14)	Group II (male) (n = 11)	Group III (male) (n = 11)	Group IV (male) (n = 14)
Total energy intake (kcal/day)	2132.5 ± 268.4	2186.3 ± 279.5	2086.2 ± 207.0	2065.1 ± 209.7
Number of steps (steps/day)	5436.2 ± 2346.5	7112.0 ± 2531.6	6126.7 ± 1635.4	6832.3 ± 2888.6

See Fig. 1 for Groups. Values are means ± S.D.

cording to the criteria calculated by the analysis of covariance (ANCOVA).[12] We analysed test items of physical examination on only male subjects because there were few female subjects. Thus, 14, 11, 11, and 14 subjects in groups I, II, III, and IV, respectively, were assessed as obese. The characteristics of these subjects are shown in Table 7, and energy intake and amount of exercise received during PFE intake are shown in Table 8. There were no significant differences among the groups with regard to these parameters.

The results of the physical examinations are shown in Table 9. At weeks 4 and 8, the BMI and body weight were significantly reduced in group III only, and not in any of the other groups. The total fat area and subcutaneous fat area were significantly reduced in both groups II and III. At week 4, the hip circumference was significantly reduced in groups I, II, and III. The visceral fat area was not significantly different from that recorded at week 0 in any of the groups. As a result of comparison among groups by Tukey-Kramer test, no significant differences were noted among the groups for any of the abovementioned parameters.

Table 9. Change Ratio in Physical Examination (BMI > 24, Male)

	Group	Number	Week 0	Week 4	Week 8
Body weight	I	14	1.000 ± 0.000	0.992 ± 0.019	0.987 ± 0.027
(kg)	II	11	1.000 ± 0.000	0.998 ± 0.020	0.991 ± 0.023
	III	11	1.000 ± 0.000	0.989 ± 0.013**	0.975 ± 0.013***
	IV	14	1.000 ± 0.000	0.995 ± 0.013	0.993 ± 0.018
BMI	I	14	1.000 ± 0.000	0.992 ± 0.020	0.987 ± 0.027
(kg/m^2)	II	11	1.000 ± 0.000	0.998 ± 0.021	0.992 ± 0.023
	III	11	1.000 ± 0.000	0.988 ± 0.013**	0.975 ± 0.013***
	IV	14	1.000 ± 0.000	0.995 ± 0.013	0.993 ± 0.017
Waist circumference	I	14	1.000 ± 0.000	—	0.996 ± 0.024
(cm)	II	11	1.000 ± 0.000	—	0.993 ± 0.030
	III	11	1.000 ± 0.000	—	0.976 ± 0.021**
	IV	14	1.000 ± 0.000	—	0.990 ± 0.037
Hip circumference	I	14	1.000 ± 0.000	0.981 ± 0.029**	0.996 ± 0.023
(cm)	II	11	1.000 ± 0.000	0.984 ± 0.021**	0.999 ± 0.011
	III	11	1.000 ± 0.000	0.971 ± 0.036**	0.984 ± 0.036
	IV	14	1.000 ± 0.000	0.989 ± 0.021	1.007 ± 0.033
Total fat area	I	14	1.000 ± 0.000	—	0.971 ± 0.082
(cm^2)	II	11	1.000 ± 0.000	—	0.951 ± 0.060*
	III	11	1.000 ± 0.000	—	0.902 ± 0.070**
	IV	14	1.000 ± 0.000	—	0.968 ± 0.105
Subcutaneous fat area	I	14	1.000 ± 0.000	—	0.979 ± 0.131
(cm^2)	II	11	1.000 ± 0.000	—	0.928 ± 0.078*
	III	11	1.000 ± 0.000	—	0.886 ± 0.065***
	IV	14	1.000 ± 0.000	—	0.963 ± 0.122
Visceral fat area	I	14	1.000 ± 0.000	—	0.974 ± 0.166
(cm^2)	II	11	1.000 ± 0.000	—	0.958 ± 0.162
	III	11	1.000 ± 0.000	—	0.950 ± 0.140
	IV	14	1.000 ± 0.000	—	1.006 ± 0.211
Systolic blood pressure	I	14	1.000 ± 0.000	0.949 ± 0.063	0.966 ± 0.084
(mmHg)	II	11	1.000 ± 0.000	0.934 ± 0.056**	0.921 ± 0.066***
	III	11	1.000 ± 0.000	0.948 ± 0.082	0.965 ± 0.062
	IV	14	1.000 ± 0.000	0.976 ± 0.051	0.961 ± 0.086
Diastolic blood pressure	I	14	1.000 ± 0.000	0.970 ± 0.115	0.986 ± 0.133
(mmHg)	II	11	1.000 ± 0.000	0.982 ± 0.041	0.936 ± 0.078**
	III	11	1.000 ± 0.000	0.898 ± 0.106**	0.901 ± 0.103**
	IV	14	1.000 ± 0.000	0.952 ± 0.142	0.930 ± 0.140

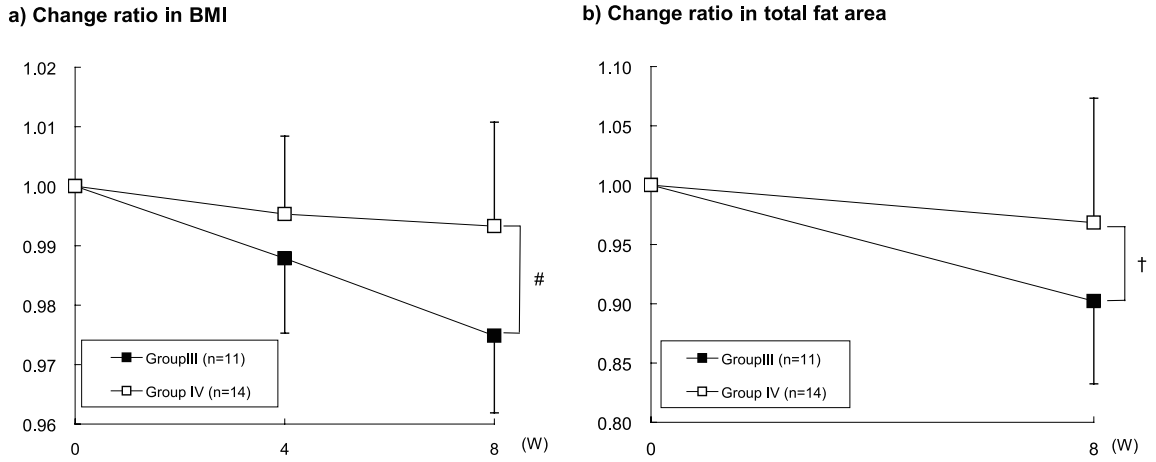
See Fig. 1 for Groups. Values are means ± S.D. *, **, and *** indicate significantly different from the value obtained in week 0 at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

The result of comparison among groups by unpaired *t*-test was appeared in Fig. 2. Regarding weight, BMI and total fat area, group III exhibited significantly lower value than group IV (weight data is not shown).

DISCUSSION

In Japan, the number of obese persons with BMI values from 25–30 has increased by 2- to 4-fold over the last 30 years. Based on BMI values, 18.9% of the female population and 21.4% of the male population are currently considered as preobese. This may be due to the westernization of the food culture in Japan, which has increased the fat intake to twice that in the 1960s.

Obesity is the cause of many adult diseases associated with lifestyle habits, including diabetes, hyperlipidaemia, and hypertension. The type-1 plasminogen activator inhibitor (PAI-1) and agents that promote thrombus formation are excessively secreted by the cells of visceral fat tissue. This results in adiponectin hyposecretion, and this condition in



Values are means ± SD
indicate significantly different from the value obtained in Group III and Group IV at $p < 0.01$. (unpaired t-test)
† indicate significant tencdency in Group III and Group IV at $p < 0.10$. (unpaired t-test)

Fig. 2. Change Ratio in BMI and Total Fat Area (Male)
Result of comparison between group III and group IV.

turn induces insulin resistance, abnormalities in glucose metabolism, hypertension, and arteriosclerosis. Therefore, visceral adiposity is considered a dangerous condition and is termed the 'metabolic syndrome'. To prevent its development, it is important to improve the status of obesity.

It is well known that catechin,[13–15] mannooligosaccharides,[16, 17] and coffee polyphenols[18] reduce body fat; however, it is unclear whether these ingredients improve liver function. The incidence of nonalcoholic steatohepatitis (NASH) is known to be strongly associated with obesity. Therefore, the consumption of ingredients that have effects in both reducing body fat and improving liver function is effective for the prevention of this condition.

The puerariae flower is used in Japanese and Chinese folk medicine for curing hangovers.[1–3] Additionally, Niiho *et al.* confirmed that its consumption reduces GOT or GPT levels in individuals with liver injury induced by CCl_4 or a high-fat diet in animal study.[2, 3] Further, Lee *et al.* have reported the hepatoprotective effects of tectoridin and tectorigenin, which are isoflavone constituents of puerariae flos.[19] Therefore, it is considered that the consumption of the puerariae flos may improve liver function. Since the ability of PFE to reduce body fat *in vivo* has been confirmed, it is expected to be efficient for improving the status of both body fat and liver function.

In this study, we preliminary investigated the effects of PFE on body fat in humans. Among those subjects with BMI values of more than 24, significant reductions were noted in BMI and body weight only in group III and in the total fat area and the subcutaneous fat area in groups II and III (Table 9). Although the visceral fat area was not significantly reduced in any of the groups, group III exhibited the highest reduction rate. No significant difference was confirmed on a comparison between placebo group and PFE group by Tukey-Kramer test. On the other hand, BMI value of male subjects in group III was significantly low compared with male subjects of group IV by unpaired *t*-test (Fig. 2). Although muscle mass was estimated, significant differences were not confirmed in any groups by impedance method (data not shown). In addition, significant differences were not confirmed on creatinine and red blood cell count that related to amount of water within the body. Therefore, BMI reduction may reflect the reduction of fat area. In fact, value of total fat area of male subjects in group III tended to be reduced compared with male subjects of group IV by unpaired *t*-test ($p < 0.10$).

GOT, GPT and γ-GTP as hepatic function markers were shown significantly low value on group I through group III at 8 weeks (Table 4). As described above, hepatoprotective effects of puerariae flos has been reported with the perception from *in vivo*.[2, 3] Therefore a possibility is considered that reduction of these makers may be attributable to hepatoprotective effect. This is very interesting.

In this study, we conducted the double-blind placebo controlled study to examine whether PFE has anti-obesity effect or not preliminary. As a

result, BMI value of male subjects in 300 mg/day group was significantly low compared with male subjects of placebo group. Consequently, PFE intake at 300 mg/day seems to have the possibility of anti-obesity effect on male. Since most subjects were male in this study, we could not confirm the actions on body fat of female. We need to confirm anti-obesity effect of PFE on female and the optimum dose for administration by a larger scale study. For the study, enough quantity of sample size should be ensured and equal numbers of male and female are required as subjects.

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