Rice Research: Open Access

Research Article Open Access

Effect of Rice Peptide Beverage Combined with Light Walking Exercise on Body Fat Parameters in Japanese Subjects

Yasuko Oike[1], Takeshi Miyanaga[1], Yusuke Hori[2], Aya Takahashi[2], Mitsunori Kikuchi[3] and Hiroshi Shimoda[1,3]*

[1]*Oriental Occupational Health Association, Tokyo Branch, Oriental Ueno Detection Center, Tokyo, Japan*
[2]*TES Holdings Co. Ltd., Tokyo University Entrepreneurs Plaza, Tokyo, Japan*
[3]*Oryza Oil & Fat Chemical Co. Ltd., Ichinomiya, Japan*

Abstract

Various branched chain amino acids and peptides are thought to enhance physical performance or energy consumption. Rice peptide (RP) is a hydrolyzed peptide derived from rice endosperm protein. RP has been suggested to reduce body fat by enhancing fat metabolism in muscle, but the effects of RP on body fat parameters have not been evaluated in humans. We conducted a double-blind placebo-controlled study to investigate the influence of RP supplementation combined with light walking exercise on body fat parameters in Japanese women. Subjects ingested a beverage containing RP (600 mg) or a placebo beverage for 8 weeks (10 subjects each) and walked 1.3 times more steps daily than usual during the intervention period. The subjects had a body mass index (BMI) of 25 to 30 kg/m^2 and a waist circumference of at least 85 cm. Before intervention and after 4 and 8 weeks of intervention, the body weight, body fat composition, metabolic rate, abdominal fat area (on CT scans), and neck, arm, and thigh circumferences were measured. For evaluation of the safety of RP, hematology tests, blood chemistry tests, and urinalysis were also performed. After 8 weeks, the visceral fat area on CT was significantly reduced in both groups compared with before intervention, but there was no significant difference between the RP and placebo groups. Body weight, BMI, lean mass, muscle mass, and basal metabolic rate were all significantly reduced in the placebo group after 8 weeks, while these parameters did not change significantly in the RP group. There were no abnormalities of blood and urine data suggesting adverse effects in either group. In conclusion, intake of RP did not enhance body fat reduction in Japanese women performing light walking exercise.

Keywords: Rice peptide; Body mass index; Body weight; Visceral fat; CT scan

Introduction

Under the Japanese regulations governing claims about the health benefits of foods, several natural ingredients have been approved with regard to health-promoting effects such as weight loss and fat reduction. As Japanese Foods for Specified Health Use (FOSHU), it is permitted to state that green tea catechins and chlorogenic acids in green coffee beans promote weight loss [1,2]. These polyphenols enhance hepatic fat metabolism by promoting mitochondrial β-oxidation or by enhancing mitochondrial uptake of free fatty acids in hepatocytes [3,4]. Since 2015, similar weight loss claims have been permitted under the Japanese Functional Foods Claims system for other polyphenols, including pueraria flower isoflavonoids, licorice flavonoids, rosehip tiliroside, ellagic acid from African mango, proantocyanidin B_2 from apples, and polymethoxyflavones from black ginger [5-10]. Under this regulatory system, it is also permitted to state that vinegar facilitates weight loss [11]. Despite such weight loss claims being allowed for several low molecular weight compounds, no peptide has been confirmed to achieve clinically useful weight loss.

Rice peptide (RP) is derived from rice albumen by hydrolysis. There have been several reports with regard to the anti-obesity effect of rice. Chung reported that adding brown rice to a high-fat diet (HFD) reduced weight gain in mice compared to mice fed the HFD alone [12]. As clinical evidence of weight loss promotion, partial replacement of white rice with brown rice was reported to reduce body fat parameters in Vietnamese women and a similar result was reported in Koreans [13,14]. Based on these findings, the anti-obesity factor seems to be contained in rice germ or bran rather than rice albumen, but there have been no reports about identification of anti-obesity compounds in rice germ or bran. RP derived from rice albumen or bran consist of albumin, globin, gluterin, and prolamin [15,16]. RP has been reported to exhibit antimicrobial, hypotensive and glutathione-promoting effects, but there have been no studies on its anti-obesity effect [17-19]. Accordingly, we conducted a clinical trial to assess the effects of RP on body weight and body fat in moderately overweight Japanese women, in order to evaluate its weight loss-promoting effect.

Materials and Methods

Participants

To recruit 20 subjects for this study, persons registered with the monitor bank of TES Holdings Co., Ltd. completed a questionnaire sheet covering the inclusion criteria. Candidate subjects were questioned about the exclusion criteria via telephone. The inclusion and exclusion criteria were as follows.

Inclusion criteria:

1) Women aged from 30 to 54 years;
2) BMI from 25 to 30 kg/m^2;
3) Waist circumference ≥ 85 cm;
4) High blood pressure (self-diagnosed);
5) Women who take photographs of their bodies;
6) Women who were permitted to participate in the study by their physicians.

***Corresponding author:** Hiroshi Shimoda, Oryza Oil & Fat Chemical Co. Ltd., Ichinomiya, Aichi, Japan, Tel: +81-0586-86-5141; Email: kaihatsu@mri.biglobe.ne.jp

Received July 12, 2019; **Accepted** October 22, 2019; **Published** October 29, 2019

Citation: Oike Y, Miyanaga T, Hori Y, Takahashi A, Kikuchi M, et al. (2019) Effect of Rice Peptide Beverage Combined with Light Walking Exercise on Body Fat Parameters in Japanese Subjects. J Rice Res 7: 112.

Exclusion criteria:

1) Women on medication for chronic symptoms;
2) History of a severe allergic reaction to specific foods or medicines;
3) Current use of medicines or dietary supplements that could influence test results, such as treatment for metabolic syndrome or dietary supplements to promote weight loss;
4) Current or previous cardiovascular disease, nephritis, hepatitis, and other disorders;
5) Severe anemia;
6) Pregnancy or breastfeeding;
7) Regular alcohol intake ≥ 60 g on most days;
8) Women deemed to be unsuitable for this study by their attending physicians.

Finally, 10 subjects were allocated to each of 2 groups (20 in total) so that the mean values of total fat mass, visceral fat mass, subcutaneous fat mass, body weight, BMI, age, and systolic blood pressure were similar in both groups.

Intake of alcohol was prohibited on the day before the test. The subjects were told to avoid an irregular lifestyle (eg: insufficient sleep, overeating, or overdrinking) during the study period. Regarding their diet, subjects were asked to maintain a similar quantity and quality of food intake as before the study. During the study period, each subject was directed to walk a specified number of steps daily. Use of medicines and dietary supplements that could influence assessment of the effect of the test substance (eg: Lipid-lowering or weight loss-promoting agents) was prohibited. Blood donation was prohibited during the study period.

Preparation and Allocation of Test Samples

The test beverage (50 mL) contained 1 g of Oryza peptide-P60 (Oryza Oil & Fat Chemical Co., Ltd.). Oryza peptide-P60 is a rice peptide powder with a peptide content of 60% (600 mg of RP). The composition of the beverage is listed in Table 1. The placebo beverage had the same ingredients except for Oryza peptide-P60. Nutritional data for the two beverages are shown in Table 2. As the appearance of each beverage was indistinguishable, colored identification tags were attached to the bottles. Information about test beverage allocation was strictly protected by third-party treatment allocation controllers who were not directly involved in the study, and the information was not disclosed until handling of the participants was determined at a clinical conference after the study.

Study Protocol

This study (protocol No. HR-2010-OY02) was carried out at the Oriental Ueno Detection Center of the Oriental Occupational Health Association, Tokyo Branch. Statistical analysis was done by TES Holdings Co. Ltd. Placebo-controlled double-blind comparison was performed between the drink containing RP (RP group) and the placebo drink (placebo group). The primary outcomes were body weight, body composition, abdominal fat area on CT scans (total fat, visceral fat, and subcutaneous fat), and various body circumferences (waist, upper arms, neck, and thighs). Secondary outcomes were the blood pressure and pulse rate, hematological parameters, blood biochemical parameters, and urinalysis parameters. Analysis of blood and urine was performed by Health Science Research Institute Co., Ltd. During the study period, a bottle of the assigned beverage was ingested after breakfast once daily for 8 weeks. Each subject wore a pedometer (Power walker EX-700, Yamasa Co. Japan) from the time of getting up until retiring to bed. To set the individual target number of steps, each subject was asked to record the daily number of steps for 7 days from the day after the pre-test day. Using data on the number of steps and other exercises, the total number of steps was calculated and the target was set as 1.3 times the total number (Table 3).

Daily reports written by the subjects were collected, including a simplified dietary survey and questionnaire. The efficacy and safety of the rice peptide drink were evaluated from the data and survey items described above.

Measurement of Body Fat Parameters

A body composition meter (MC-180, Tanita Co. Japan) was used for measurement of body weight and body composition. The subjects wore the specified test clothing and measurements were carried out with the meter set at 500 g. Body weight, percent body fat, fat mass, fat-free mass, muscle mass, BMI, and basal metabolic rate were measured. The abdominal area was determined from a CT scan obtained at the level of the umbilicus while holding the breath. For measurement of fat areas, CT scans were converted into BMP format images and were analyzed by using special software (Fat Scan N2 System, East Japan Institute of Technology Co. Ltd., Japan), which calculated the visceral fat area, subcutaneous fat area, and total fat area. The circumference of the waist, neck, upper arm, and thigh was measured as follows. Waist circumference was measured at the umbilicus in the standing position after the subject exhaled lightly. When the subject had a significant amount of abdominal fat and the umbilicus was dependent, the waist circumference was measured at the midpoint between the lowest rib and the anterior superior iliac spine. Neck circumference was measured

Component	Active (RP 600 mg)	Placebo
Oryza peptide-P60	1.91 (%)	-
Citric acid	1.91	1.91
Purified honey	13.37	13.36
White peach essence	0.19	0.19
Caramel K	-	0.06
Water	82.14	84.00
Lemon flavor	0.48	0.48

Table 1: Composition of the test beverages.

Nutrient	Active (RP 600 mg)	Placebo
Water (g)	92.41	93.34
Protein (g)	0.66	0.00
Fat (g)	0.00	0.00
Carbohydrate (g)	6.93	6.66
Sodium (mg)	0.36	0.00
Calories (kcal)	30.45	26.62

Table 2: Nutritional content of the test beverages.

Activity	Loading time (min)	Activity	Loading time (min)
Jog	4	Badminton	4
Cycling	7	Golf	8
Hiking	4	Ski	4
Baseball	8	Swimming	3
Football	4	Light sport	10
Basketball	4	Medium sport	7
Tennis	5	Heavy sport	4

Table 3: Exercise load equivalent to 1000 steps.

at the base of the neck. Upper arm circumference was measured at the midpoint of the left and right upper arms. Thigh circumference was measured at the proximal part of the left and right thighs. All measurements were done in the standing position.

Laboratory Tests

A fasting venous blood sample was collected after measurement of the fat parameters. Hematology parameters measured were the red blood cell count, white blood cell count, hemoglobin, hematocrit, platelet count, MCV, MCH and MCHC, while the biochemical parameters were protein, urea N, triglyceride, creatinine, uric acid, total cholesterol, LDL-cholesterol, HDL-cholesterol, blood glucose, free fatty acids, AST, ALT, and γ--GTP. After the blood sample was obtained, a urine sample was collected and the specific gravity and pH were determined.

Ethics, Adherence and Compliance

This study was performed according to the Helsinki Declaration (as revised at the Edinburgh General Assembly in 2000) and was carried out in compliance with ethical considerations. The rights and safety of each subject were taken into consideration, and it was confirmed that reliable data were obtained in compliance with Ministry of Health and Welfare Ordinance No. 28 "Standards for the Implementation of Clinical Trials of Pharmaceutical Products (GCP)" dated March 27, 1997. The Ethics Committee of TES Holdings was made up of professors from a pharmaceutical university, a medical doctor, a hospital consultant, and a lawyer. The Ethics Committee was convened to deliberate on the ethicality and appropriateness of the study protocol. This study was implemented according to the protocol approved by the Ethics Committee and any substantial deviations from the protocol required authorization by the committee.

Investigation of Adverse Events

Each adverse event was evaluated and the causal relationship with the test drink was determined. A decision about whether to continue the study was made by the doctor in charge, if necessary. Adverse events were defined as any unwanted symptoms/signs resulting from ingesting the test drink. If a subject asked to discontinue the study, the study was promptly stopped while taking care to avoid any disadvantage to the subject.

Exclusion Criteria for Data Analysis

If any of the following events occurred, discussion at the clinical conference was required and the subject in question was to be excluded from analysis after review.

1) The subject was at least 7 days late in attending for each scheduled visit.
2) The number of days without ingestion of the test drink (i.e., the specified daily dose was not reached) was >15% of the scheduled number of days.
3) It was confirmed that the obligations outlined in this document were markedly disregarded during the study period.
4) Major issues with data reliability were identified due to problems with testing or other reasons.
5) It was considered appropriate to exclude the subject for other reasons.

Statistical Analysis

Results are reported as the mean and SE. The two-tailed paired *t*-test was used for comparisons between before and after ingestion of the test drink, while the two-tailed unpaired *t*-test was used comparison of the placebo group with the RP group. In all analyses, a probability of less than 5% was considered to indicate significance.

Results

Study performance

The study was performed from March 3 to May 16, 2010. During intervention period, no subject withdrew from either group. The (age mean ± SE) of the placebo group was 40.4 ± 6.3 years and that of the RP group was 41.4 ± 5.9 years.

Body composition parameters

Table 4 shows the data on body composition parameters. After ingestion of the placebo drink for 8 weeks, it was found that the body weight, BMI, lean mass, muscle mass, and basal metabolic rate all decreased significantly compared to before ingestion. On the other hand, these parameters were not changed by ingesting the RP drink for 8 weeks. With regard to CT parameters, visceral fat area decreased significantly in both the placebo group and the RP group compared to before ingestion (Table 5). The reduction was slightly greater in the RP group, but there was no significant difference between the two groups.

When body circumference parameters were assessed, the neck circumference and right upper arm circumference showed a significant decrease in both groups after intake of the test drink, with no significant difference between the two groups (Table 6).

Safety Parameters

After 8 weeks, assessment of hematology parameters showed a significant change in hemoglobin in the placebo group, as well as changes in MCV and MCHC in both groups (Table 7). Among biochemical parameters, triglyceride was decreased significantly in the RP group compared to the baseline level, although there was no significant difference between the RP and placebo groups (Table 8). In addition, creatinine was significantly increased in both groups and uric acid was significantly increased in the RP group, but these changes were within the standard ranges. Urine specific gravity and pH did not change significantly in either group (Table 9).

Adverse Events

According to the questionnaire data, the physical condition of the subjects improved in both groups, with no difference between the 2 groups. No severe adverse events occurred. Base on the daily reports from the subjects, there were no abnormalities of the physical or mental condition, as well as no fatigue, back/shoulder pain, sleep problems, digestive dysfunction, or skin abnormalities related to ingestion of the placebo drink or RP drink.

Discussion

Rice is a staple food in the diet of people from many countries. We previously found that oral administration of RP (100 mg/kg) to mice on a high-fat diet slightly suppressed weight gain and enhanced the hepatic activity of carnitine palmitoyl transferase, which is important for lipid metabolism. These findings suggested that RP might reduce body weight or body fat in humans. In the present clinical study of RP beverage in Japanese women, body composition parameters tended to improve in both the RP group and the placebo group, probably because the subjects walked 1.3 times further than usual during the study period. On CT scans, visceral fat area was significantly reduced in both groups compared to before the study, with the reduction being 1.5 times larger

	Before	After 4 weeks of ingestion (Relative value)	△	After 8 weeks of ingestion (Relative value)	△
Body weight (kg)					
Placebo	68.5 ± 2.5	67.8 ± 2.3	-0.7	67.5 ± 2.5*	1
		(99.1 ± 0.7)	(-0.9)	(98.5 ± 0.6*)	(-1.5)
RP	67.5 ± 2.2	66.7 ± 1.9	-0.8	66.1 ± 2.0	-1.4
		(99.1 ± 1.4)	(-0.9)	(98.1 ± 1.5)	(-1.9)
BMI (kg/m^2)					
Placebo	27.7 ± 0.8	27.5 ± 0.	-0.2	27.3 ± 0.8*	-0.4
		(100.1 ± 0.3)	-0.1	(100.1 ± 0.3)	-0.1
RP	26.8 ± 0.5	26.6 ± 0.5	-0.2	26.3 ± 0.5	-0.5
		(99.2 ± 1.4)	(-0.8)	(98.1 ± 1.4)	(-1.9)
Body fat ratio (%)					
Placebo	37.8 ± 1.0	37.2 ± 1.0	-0.8	37.6 ± 1.2	-0.2
		(98.4 ± 0.9)	(-1.6)	(99.3 ± 0.8)	(-0.7)
RP	36.0 ± 1.2	35.8 ± 1.2	-0.2	35.3 ± 1.4	-0.7
		(99.7 ± 1.7)	(-0.3)	(98.2 ± 2.1)	(-1.8)
Fat mass (kg)					
Placebo	26.1 ± 1.6	25.4 ± 1.4	-0.7	25.6 ± 1.7	-0.5
		(97.5 ± 1.5)	(-2.5)	(97.8 ± 1.4)	(-2.2)
RP	24.4 ± 1.5	24.0 ± 1.3	-0.4	23.5 ± 1.5	-0.9
		(99.1 ± 2.9)	(-0.9)	(96.6 ± 3.2)	(-3.4)
Lean body mass (kg)					
Placebo	42.3 ± 1.1	42.4 ± 1.1	0.1	41.8 ± 1.1*	-0.5
		(100.2 ± 0.3)	-0.2	(98.8 ± 0.4*)	(-1.2)
RP	43.0 ± 1.0	42.6 ± 0.9	-0.4	42.6 ± 1.0	-0.4
		(99.3 ± 0.6)	(-0.7)	(99.1 ± 0.7)	(-0.9)
Muscle mass (kg)					
Placebo	39.8±1.0	39.9 ± 1.0	0.1	39.4 ± 1.0*	-0.4
		(100.2 ± 0.3)	-0.2	(98.9 ± 0.4*)	(-1.1)
RP	40.4 ± 0.9	40.1 ± 0.9	-0.3	40.0 ± 0.9	-0.4
		(99.3 ± 0.6)	(-0.7)	(99.1 ± 0.7)	(-0.9)
Basal metabolic rate (kcal)					
Placebo	1294 ± 40	1292 ± 39	-2	1278 ± 40*	-16
		(99.9 ± 0.4)	(-0.1)	(98.7 ± 0.4)	(-1.3)
RP	1300 ± 35	1289 ± 31	-11	1284 ± 32	-16
		(99.2 ± 0.9)	(-0.8)	(98.8 ± 0.9)	(-1.2)

Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ± S.E. (n=10). An asterisk denotes a significant difference from before ingestion at *: $p<0.5$.

Table 4: Changes in body composition parameters.

	Before ingestion	After 8 weeks of ingestion (Relative value)	△
Total fat area (cm^2)			
Placebo	346.0 ± 23.6	328.5 ± 23.7	-17.5
		(94.9 ± 2.3)	(-5.1)
RP	343.4 ± 27.2	327.9 ± 29.7	-15.5
		(95.4 ± 3.5)	(-4.6)
Visceral fat area (cm^2)			
Placebo	78.9 ± 9.0	72.4 ± 8.6*	-6.5
		(91.8 ± 3.4*)	(-8.2)
RP	77.9 ± 8.8	68.8 ± 10.4*	-9.1
		(86.4 ± 4.5*)	(-13.6)
Subcutaneous fat area (cm^2)			
Placebo	267.0 ± 17.3	256.0 ± 17.7	-11
		(95.8 ± 2.2)	(-4.2)
RP	265.4 ± 22.2	259.1 ± 22.8	-6.3
		(98.2 ± 3.9)	(-1.8)

Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ± S.E. (n=10). An asterisk denotes significant difference from before ingestion at *: $p<0.05$.

Table 5: Changes in CT parameters.

	Before ingestion	After 4 weeks of ingestion (Relative value)	Δ	After 8 weeks of ingestion (Relative value)	Δ
Waist circumference (cm)					
Placebo	90.2 ± 2.1	90.7 ± 2.1	0.5	89.8 ± 2.3	-0.4
		(100.6 ± 0.8)	-0.6	(99.6 ± 1.2)	(-0.4)
RP	88.3 ± 1.5	90.0 ± 2.2	1.7	90.1 ± 2.0	1.8
		(101.9 ± 1.9)	-1.9	(102.1 ± 1.7)	-2.1
Neck (cm)					
Placebo	36.6 ± 0.3	35.1 ± 0.5**	-1.5	34.4 ± 0.4**	-2.2
		(95.8 ± 1.0**)	(-4.2)	(94.0 ± 1.1)	(-6.0)
RP	35.3 ± 0.7	34.3 ± 0.6*	-1	34.3 ± 0.5*	-1
		(97.2 ± 0.9)*	(-2.8)	(97.3 ± 1.1*)	(-2.7)
Right upper arm (cm)					
Placebo	32.0 ± 0.7	31.5 ± 0.7	-0.5	30.7 ± 0.6**	-1.3
		(98.5 ± 0.8)	(-1.5)	(96.0 ± 1.0**)	(-4.0)
RP	32.2 ± 0.7	31.5 ± 0.7	-0.7	30.8 ± 0.6*	-1.4
		(98.0 ± 1.1)	(-2.0)	(95.9 ± 1.6*)	(-4.1)
Left upper arm (cm)					
Placebo	31.2 ± 0.7	31.0 ± 0.7	-0.2	30.6 ± 0.7	-0.6
		(99.6 ± 0.8)	(-0.4)	(98.3 ± 1.1)	(-1.7)
RP	31.6 ± 0.8	31.2 ± 0.7	-0.4	30.2 ± 0.6	-1.4
		(98.6 ± 1.2)	(-1.4)	(95.8 ± 1.9)	(-4.2)
Right thigh (cm)					
Placebo	59.5 ± 1.5	59.7 ± 1.1	0.2	60.0 ± 1.4	0.5
		(98.8 ± 1.0)	(-1.2)	(98.2 ± 1.2)	(-1.8)
RP	61.1 ± 1.2	60.9 ± 0.9	-0.2	59.9 ± 0.9	-1.2
		(100.5 ± 0.9)	-0.5	(101.0 ± 1.0)	-1
Left thigh (cm)					
Placebo	59.5 ± 1.2	59.7 ± 1.3	0.2	59.4 ± 1.3	-0.1
		(100.4 ± 0.5)	-0.4	(98.8 ± 1.4)	(-1.2)
RP	60.7 ± 1.2	60.2 ± 0.7	-0.5	59.8 ± 0.7	-0.9
		(99.3 ± 1.1)	(-0.7)	(98.8 ± 1.4)	(-1.2)
Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ± S.E. (n=10). Asterisks denote significant differences from before ingestion at *: $p<0.05$ and **: $p<0.01$.					

Table 6: Changes in circumference parameters.

	Before ingestion	After 8 weeks of ingestion	Δ	Standard value
Red blood cells (×10^4 cells/mL)				
Placebo	473 ± 16	479 ± 16	6	327-500
RP	451 ± 9	453 ± 9	2	
Leukocytes (cells/mL)				
Placebo	5760 ± 550	5880 ± 423	120	3500-9100
RP	6220 ± 413	5640 ± 574	-580	
Hemoglobin (g/dL)				
Placebo	13.7 ± 0.4	13.9 ± 0.4*	0.2	11.3-15.2
RP	13.1 ± 0.4	13.3 ± 0.5	0.2	
Hematocrit (%)				
Placebo	42.6 ± 1.3	42.1 ± 1.2	-0.5	33.4-44.9
RP	41.3 ± 1.2	40.6 ± 1.2	-0.7	
Platelet (×10^4 cells/mL)				
Placebo	29.2 ± 2.0	27.8 ± 1.9	-1.4	13.0-36.9
RP	26.9 ± 2.4	26.2 ± 2.2	-0.7	
MCV (fL)				
Placebo	90.4 ± 1.5	88.1 ± 1.5*	-2.3	79-100
RP	91.6 ± 1.5	89.6 ± 1.8**	-2	
MCH (pg)				
Placebo	28.9±0.6	29.2 ± 0.6	0.3	26.3-34.3
RP	29.1 ± 0.6	29.4 ± 0.8	0.3	
MCHC (%)				
Placebo	32.1 ± 0.2	33.1 ± 0.2**	1	30.7-36.6
RP	31.8 ± 0.3	32.8 ± 0.3**	1	
Data are represented as the mean ± S.E. (n=10). Asterisks denote significant differences from before ingestion at *: $p<0.05$, **: $p<0.01$.				

Table 7: Changes in blood laboratory parameters.

	Before ingestion	After 8 weeks of ingestion	Δ	Standard value
Protein (g/dL)				
Placebo	7.4 ± 0.1	7.4 ± 0.1	-	6.7-8.3
RP	7.6 ± 0.1	7.5 ± 0.1	-0.1	
Urea N (mg/dL)				
Placebo	10.8 ± 0.6	10.8 ± 0.6	-	8-22
RP	9.6 ± 0.8	11.5 ± 0.6*	1.9	
Triglyceride (mg/dL)				
Placebo	109 ± 17	82 ± 10	-27	35-149
RP	146 ± 20	81 ± 7	-65**	
Creatinine (mg/dL)				
Placebo	0.58 ± 0.02	0.63 ± 0.02**	0.05	0.47-0.79
RP	0.55 ± 0.01	0.64 ± 0.01**	0.09	
Uric acid (mg/dL)				
Placebo	4.8 ± 0.4	5.0 ± 0.5	0.2	2.5-7.0
RP	4.6 ± 0.2	5.2 ± 0.3**	0.6	
Total cholesterol (mg/dL)				
Placebo	217 ± 14	196 ± 10**	-21	130-219
RP	238 ± 10	230 ± 13	-8	
LDL-cholesterol (mg/dL)				
Placebo	135 ± 12	125 ± 8	-10	70-139
RP	144 ± 9	149 ± 13	5	
HDL-cholesterol (mg/dL)				
Placebo	58 ± 3	55 ± 3	-3	40-96
RP	66 ± 3	65 ± 3	2	
Blood glucose (mg/dL)				
Placebo	93 ± 2	90 ± 1	-3	70-109
RP	95 ± 3	94 ± 2	-1	
Free fatty acid (mEq/L)				
Placebo	0.41 ± 0.05	0.51 ± 0.07	0.1	0.10-0.85
RP	0.45 ± 0.06	0.59 ± 0.04	0.14	
AST (U/L)				
Placebo	22.3 ± 1.7	22.6 ± 2.3	0.3	10-40
RP	21.2 ± 2.2	19.9 ± 2.6	-1.3	
ALT (U/L)				
Placebo	32.7 ± 6.3	30.3 ± 8.8	0.6	5-45
RP	26.9 ± 4.2	25.0 ± 5.1	-1.9	
Gama-GTP(U/L)				
Placebo	37.4 ± 14.4	21.4 ± 4.4	-16	<45
RP	44.0 ± 20.6	38.7 ± 17.9	-5.3	
Data are represented as the mean ± S.E. (n=10). Asterisks denote significant differences from before ingestion at *: $p<0.05$ and **: $p<0.01$.				

Table 8: Changes in biochemical parameters.

Specific gravity	Before ingestion	After 8 weeks of ingestion	Standard value
Placebo	1.016 ± 0.003	1.020 ± 0.003	1.005-1.030
RP	1.018 ± 0.002	1.019 ± 0.003	
pH			
Placebo	6.30 ± 0.19	6.15 ± 0.17	5.0-8.5
RP	6.60 ± 0.34	6.35 ± 0.20	

Table 9: Changes in urine parameters.

in the RP group than in the placebo group. However, the subcutaneous fat area and total fat area were not significantly decreased. In addition, the body weight, BMI, body fat ratio, and fat mass did not change significantly in the RP group. These results suggested that intake of RP and light exercise may help to reduce visceral fat, but the effect is not strong enough to reduce subcutaneous fat or overall fat parameters, including body weight, BMI, and fat mass. Thus, concrete evidence of RP reducing body fat parameters was not obtained in Japanese women.

To further examine the effect of RP on body fat, several factors should be considered for in future studies such as the influence of gender, outcomes, quality of exercise, and RP dosage. For example, as it is generally more difficult for women to reduce body fat by exercise compared with men, male subjects with a BMI of 25 to 30 kg/m^2 may be more suitable for detecting weight loss due to RP [20,21]. Targeting muscle parameters could also be important, because the RP group maintained better lean body mass and muscle mass compared with

the placebo group. RP may be utilized to produce muscle protein and muscle tissue consumes fat for energy. Joy reported that consumption of RP increased lean mass and muscle thickness, and decreased body weight [22]. Thus, RP may exhibit a positive effect on body fat reduction in male subjects who have a higher amount of muscle tissue compared to female subjects. RP may maintain muscle mass, resulting in consumption of fat and enhancement of metabolism. Evaluation of "cerebral and vastus lateralis oxygenation" or dual-energy X-ray absorptiometry may be useful as muscle performance parameters [23,24]. On the other hand, walking exercise might not be effective for reducing body fat, suggesting more intense exercise should be performed that causes slight muscle damage such as resistance training (leg presses, hyperextension, and calf presses) or aerobic interval training [24,25].

Another point is that the dosage of RP is important to detect a fat-reducing effect. In a clinical study of branched-chain amino acids, approximately 10 g/day was administered to evaluate the effect on recovery from muscle damage [26,27]. Hence, a higher dose of RP (more than 10 times the dose in this study) may be required.

No abnormal changes in blood and urine parameters were observed in the RP group. Daily reports by the subjects also did not indicate any adverse events during the study period. In conclusion, oral intake of RP (600 mg/day) did not enhance fat reduction compared to placebo in Japanese women performing 1.3 times more walking exercise than usual. However, no adverse effects of RP were observed. A higher dose of RP may promote reduction of the visceral fat area.

Acknowledgement

The study was supported by a 2009 Manufacturing Support Subsidy for Small and Medium Companies in Japan.

References

1. Kobayashi M, Kawano T, Sagesaka YM, Fukuhara I (2016) Green tea beverages enriched with catechins with a galloyl moiety reduce body fat in moderately obese adults: a randomized double-blind placebo-controlled trial. Food Funct 7: 498-507.
2. Samadi M, Mohammadshahi M, Haidari F (2015) Green coffee bean extract as a weight loss supplement. J Nutr Disorders Ther 5: 180.
3. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I (2002) Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord 26: 1459-1464.
4. Shimoda H, Seki E, Aitani M (2006) Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. BMC Complement Altern Med 6: 9-15.
5. Kamiya T, Takano A, Matsuzuka Y, Kusaba N, Ikeguchi M, et al. (2012) Consumption of Pueraria flower extract reduces body mass index via a decrease in the visceral fat area in obese humans. Biosci Biotechnol Biochem 76: 1511-1517.
6. Mori N, Nakanishi S, Shiomi S, Kiyokawa S, Kakimoto S, et al. (2015) Enhancement of fat oxidation by licorice flavonoid oil in healthy humans during light exercise. J Nutr Sci Vitaminol (Tokyo) 61: 406-416.
7. Nagatomo A, Nishida N, Fukuhara I, Noro A, Kozai Y, et al. (2015) Daily intake of rosehip extract decreases abdominal visceral fat in preobese subjects: a randomized, double-blind, placebo-controlled clinical trial. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 8: 147-156.
8. Azantsa B, Kuate D, Chakokam R, Paka G, Bartholomew B, et al. (2015) The effect of extracts of *Irvingia gabonensis* (IGOB131) and *Dochrostachys glomerata* (DyglomeraTM) on body weight of participants. Function Foods Health Disease 5: 200-208.
9. Najima M, Miyata A, Wada H, Wada E (2016) Visceral fat-reducing effect of supplement containing apple polyphenol and oleanolic acid in healthy Japanese. A randomized, double-blind, placebo-controlled study. J New Remed Clinics 53: 237-244.
10. Yoshino S (2016) Effects of single oral intake of *Kaempferia parviflora* extract on energy metabolism. A randomized double-blind crossover study. Jpn Pharmacol Ther 44: 1757-1762.
11. Kondo T, Kishi M, Fushimi T, Ugajin S, Kaga T (2009) Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. Biosci Biotechnol Biochem 73: 1837-1843.
12. Chung SI, Rico CW, Lee SC, Kang MY. (2016) Instant rice made from white and pigmented giant embryonic rice reduces lipid levels and body weight in high fat diet-fed mice. Obes Res Clin Pract 10: 692-700.
13. Bui TN, Le TH, Nguyen do H, Tran QB, Nguyen TL, et al. (2014) Pre-germinated brown rice reduced both blood glucose concentration and body weight in Vietnamese women with impaired glucose tolerance. J Nutr Sci Vitaminol (Tokyo) 60: 183-187.
14. Kim JY, Kim JH, Lee DH, Kim SH, Lee SS (2008) Meal replacement with mixed rice is more effective than white rice in weight control, while improving antioxidant enzyme activity in obese women. Nutr Res 28: 66-71.
15. Okuda M, Miyamoto M, Joyo M, Takahashi K, Goto-Yamamoto N, et al. (2016) The relationship between rice protein composition and nitrogen compounds in sake. J Biosci Bioeng 122: 70-78.
16. Chen P, Shen Z, Ming L, Li Y, Dan W et al. (2018) Genetic basis of variation in rice seed storage protein (albumin, globulin, prolamin, and glutelin) content revealed by genome-wide association analysis. Front Plant Sci 9: 612.
17. Bundó M, Montesinos L, Izquierdo E, Campo S, Mieulet D (2014) Production of cecropin A antimicrobial peptide in rice seed endosperm. BMC Plant Biol 14: 102.
18. Kontani N, Omae R, Kagebayashi T, Kaneko K, Yamada Y, et al. (2014) Characterization of Ile-His-Arg-Phe, a novel rice-derived vasorelaxing peptide with hypotensive and anorexigenic activities. Mol Nutr Food Res 58: 359-364.
19. Moritani C, Kawakami K, Fujita A, Kawakami L, Shimoda H, et al. (2018) Isolation of activating factors of serotonin *N*-acetyltransferase from rice peptides. J Functional Foods 41: 148-154.
20. Kushiyama A, Yoshida Y, Kikuchi T, Suzawa N, Yamamoto M, et al. (2013) Twenty-year trend of increasing obesity in young patients with poorly controlled type 2 diabetes at first diagnosis in urban Japan. J Diabetes Investig 4: 540-545.
21. Christensen P, Meinert Larsen T, Westerterp-Plantenga M, Macdonald I (2018) Men and women respond differently to rapid weight loss: Metabolic outcomes of a multi-centre intervention study after a low-energy diet in 2500 overweight, individuals with pre-diabetes (PREVIEW). Diabetes Obes Metab 20: 2840-2851.
22. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO (2013) The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. Nutr J 12: 86.
23. Gelabert-Rebato M, Wiebe JC, Martin-Rincon M, Galvan-Alvarez V, Curtelin D (2019) Enhancement of exercise performance by 48 hours, and 15-day supplementation with mangiferin and luteolin in men. Nutrients 11: E344.
24. Joy JM, Vogel RM, Shane Broughton K, Kudla U, Kerr NY, et al. (2018) Daytime and nighttime casein supplements similarly increase muscle size and strength in response to resistance training earlier in the day: a preliminary investigation. J Int Soc Sports Nutr 15: 24.
25. Guadalupe-Grau A, Fernández-Elías VE, Ortega JF, Dela F, Helge JW, et al. (2018) Effects of 6-month aerobic interval training on skeletal muscle metabolism in middle-aged metabolic syndrome patients. Scand J Med Sci Sports 28: 585-595.
26. Ra SG, Miyazaki T, Kojima R, Komine S, Ishikura K, et al. (2018) Effect of BCAA supplement timing on exercise-induced muscle soreness and damage: a pilot placebo-controlled double-blind study. J Sports Med Phys Fitness 58: 1582-1591.
27. VanDusseldorp TA, Escobar KA, Johnson KE, Stratton MT, Moriarty T, et al. (2018) Effect of branched-chain amino acid supplementation on recovery following acute eccentric exercise. Nutrients 10: E1389.