

Anti-obese effects of chitosan and psyllium husk containing vitamin C in Sprague-Dawley (SD) rats fed a high fat diet

Eun Young Jung^{1†}, Yang Hee Hong^{2†}, Un Jae Chang³, Hyung Joo Sub⁴

¹Department of Home Economics Education, Jeonju University, Jeonju 560-759, Korea; ²Department of Beauty Art, Suwon Women's University, Suwon 441-748, Korea; ³Department of Food and Nutrition, Dongduk Women's University, Seoul 136-714, Korea; ⁴Department of Public Health Science, Graduate School, Korea University, Seoul 07249, Republic of Korea - E-mail: suh1960@korea.ac.kr; [†]Eun Young Jung and Yang Hee Hong contributed equally to this study

Summary. We investigated whether vitamin C would enhance the anti-obese effects of chitosan and psyllium husk. The study was carried out with male Sprague-Dawley (SD) rats for 6 wk using the following diet groups. Normal diet (control), high fat diet (F-control), 5% chitosan (chitosan-1), 5% chitosan containing 1% vitamin C (chitosan-2), 10% psyllium husk (psyllium-1), and 10% psyllium husk containing 1% vitamin C (psyllium-2). Chitosan-2 (1.5%) showed a significant ($p < 0.05$) decrease in epididymal fat pad weight as compared to F-control (2.3%) and chitosan-1 (2.1%). The epididymal fat pad weight of psyllium-2 (1.9%) was significantly ($p < 0.05$) lower than that of F-control (2.3%). In conclusion, additions of vitamin C to chitosan and psyllium husk may be useful for reducing body fat.

Key words: chitosan, psyllium husk, vitamin C, body weight, body fat

Introduction

Obesity, characterized by excess accumulation of body fat, is closely related to the development of diabetes, dyslipidaemia, arteriosclerosis, hypertension, and other metabolic diseases (1). Obesity has become an important public health problem that demands urgent therapeutic attention and interventional approaches. The essential emphasis in the management of obesity is to mitigate modifiable risk factors such as physical inactivity and an inappropriate atherogenic diet through lifestyle changes (2). Although the ideal dietary pattern for obese patients is yet to be ascertained, there are growing opinions among nutritionists and scientists about the benefits of increased dietary intakes of fruits, legumes, vegetables, and cereals, which are considered important sources of dietary fiber.

Dietary fiber appears to be particularly beneficial with regard to its putative role in weight control. A large number of studies in humans and experimental

models have indicated the efficacy of dietary fiber in regulating body weight (3, 4). Moreover, a number of randomized studies have described the positive effects of foods rich in dietary fiber on obesity (5, 6).

Psyllium (*Plantago psyllium*) husk is a dietary fiber rich in soluble components (7). Psyllium husk is safe and well tolerated when used as an adjunct to a low-fat diet. Although by itself it produces a modest effect in lowering body weight, consistent results in clinical trials and meta-analyses have encouraged physicians to recommend its use in combination with dietary interventions (8, 9).

Although chitosan is not derived from plants, it shares the characteristics of dietary fiber such as psyllium husk by being a polysaccharide that is indigestible by mammalian digestive enzymes. Chitosan is the deacetylated form of chitin, an aminopolysaccharide found in the exoskeleton of arthropods and certain fungi (10). Several studies have shown chitosan to have anti-obese effects in animal models by strong inhibition of fat digestion (11). Chitosan is dissolved in the stomach and

then changes to a gelled form, entrapping fat in the intestine. Kanauchi et al., (12) reported that inhibition of fat digestion by chitosan was improved in the presence of vitamin C.

Therefore, the addition of vitamin C to chitosan might be more effective than chitosan alone in the inhibition of fat digestion. We hypothesized that vitamin C might also improve the hypolipidemic effect of psyllium husk, which has similar activities as chitosan. Based on these considerations, we investigated whether vitamin C enhanced the anti-obese effects of chitosan and psyllium husk on body weight gain and fat loss in male Sprague-Dawley (SD) rats fed a high fat diet. We also determined the antioxidant activities of chitosan and psyllium husk mixtures containing vitamin C, a free radical scavenger with strong antioxidant properties.

Material and methods

Animals and diets

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee. The study was carried out with male Sprague-Dawley rats aged 6 wk (Central Lab Animal Inc., Seoul, Korea) for 6 wk. The rats were acclimatized for 5 days

under conditions of controlled temperature ($24\pm 1^\circ\text{C}$), relative humidity (55%), and lighting (dark/light 12/12 h) in a room with low background noise. In the preliminary period, they were given *ad libitum* access to water and diet. After an adaptation period, they were divided into 6 groups (8 rats/group): normal diet (control), high fat diet (F-control), high fat diet with 5% chitosan (chitosan-1), high fat diet with 5% chitosan containing 1% vitamin C (chitosan-2), high fat diet with 10% psyllium husk (psyllium-1), high fat diet with 10% psyllium husk containing 1% vitamin C (psyllium-2) (Table 1).

Measurements of body weight, food intake, organ weights, plasma lipids, and fat pads

Food intake and body weight were monitored every 2 d for 6 wk. The food was removed for the last 12 h of the experimental term, and the rats were anesthetized with ethyl ether, sacrificed, and dissected. Blood was collected from the heart with a heparinized syringe. Plasma was separated by centrifugation at $3,000\times g$ for 15 min at 4°C and then stored at -70°C until analysis. The levels of triacylglycerol, total cholesterol, and HDL-cholesterol in plasma were measured using a FUJI DRI-CHEM 3500 (Fuji Photo Film Co., Tokyo, Japan). LDL-cholesterol was estimated using the method validated by Friedewald et al., (13).

Table 1. Composition of the experimental diets for rats (g/100 g diet)

Ingredient	Control	F-control	Chitosan-1	Chitosan-2	Psyllium-1	Psyllium-2
Casein	19.6	26.2	26.2	26.2	26.2	26.2
Corn starch	56.6	27.6	27.6	27.6	22.6	22.6
Cellulose	5	5	0	0	0	5
Sucrose	9.95	9.95	9.95	9	9.95	9
Lard	0	22.4	22.4	22.4	22.4	22.4
Soybean oil	4	4	4	4	4	4
Mineral mixture ^a	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mixture ^b	1	1	1	1	1	1
L-methionine	0.3	0.3	0.3	0.3	0.3	0.3
Chitosan	0	0	5	5	0	0
Psyllium husk	0	0	0	0	10	10
Vitamin C	0.05	0.05	0.05	1	0.05	1

^aMineral mixture (g/kg diet): $\text{CaPO}_4\cdot 2\text{H}_2\text{O}$, 145.6; KH_2PO_4 , 257.2; NaH_2PO_4 , 93.5; NaCl , 46.6; calcium lactate, 350.9; ferric citrate, 31.8; MgSO_4 , 71.7; ZnCO_3 , 1.1; $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$, 1.2; $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 0.3; KI , 0.1. ^bVitamin mixture: ICN Vitamin mixture (No 904654, 1999).

The liver, kidney, spleen, ileum, jejunum, duodenum, colon, cecum, epididymal fat, and perirenal fat were removed and the wet weights were measured. The liver was washed in 0.15 mol NaCl solution, blotted onto filter paper, weighed, and homogenized in 4.5 mL potassium phosphate buffer (pH 7.0). The homogenates were centrifuged at 13,000×g for 4 min at 4°C and the supernatant was stored at -70°C until analysis.

2,2-Azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) radical cation scavenging activity

The scavenging activity on ABTS radical cation was measured according to the method of Re et al., (14) with some modifications. ABTS radical cation was generated by adding 7 mmol ABTS to 2.45 mmol potassium persulfate solution and the mixture was left to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4–1.5 at 414 nm. The diluted ABTS radical cation solution (1 mL) was added to 50 µL of plasma or liver tissue. After 90 min, the absorbance was measured at 414 nm. ABTS radical cation scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC), and defined as mg of ascorbic acid equivalents per 100 g of sample (15).

Determination of lipid peroxidation level

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substance (TBARS) concentrations according to the method of Quintanilha et al., and using malondialdehyde (MDA) as a standard. The plasma or liver tissue solution (1 mL) was added to 2 mL of 17 µmol thiobarbituric acid plus 3.36 µmol butylated hydroxy toluene, and the solution was then incubated for 15 min at 100°C. After cooling, absorbance was measured at 535 nm. The data were expressed as TBARS µmol of produced/L of plasma or liver tissue.

Determination of glutathione reduced form (GSH) level

GSH level was determined by the method of Tietz (17). Plasma or liver tissue was precipitated by addition of 5% sulfosalicylic acid and removed by centrif-

ugation at 2,000×g for 10 min at 4°C. GSH in the supernatant was assayed as follows: 100 µL of plasma or liver tissue, 800 µL of 0.3 mmol Na₂HPO₄ and 100 µL of 0.04% 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) in 0.1% sodium citrate. The absorbance of DTNB was monitored at 412 nm for 5 min. A standard curve of GSH was prepared and the sensitivity of measurements was determined to be between 1 and 100 µL.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) ver. 12.0 (IL, USA). The differences among groups were evaluated statistically by one-way analysis of variance (ANOVA) and Duncan's multiple tests. All data were two-sided with a 5% significance level and were reported as means±standard deviations (SD).

Results and discussion

Body weight gain, food intake and food efficiency ratio

Body weight gain, food intake and food efficiency ratio (FER) during experimental period are shown in Figure 1. Body weight gains were significantly decreased ($p < 0.05$) in dietary supplement groups (chitosan and psyllium husk groups) compared to F-control, and were similar to the normal diet group, control. The addition of vitamin C to chitosan and psyllium husk accelerated the reduction of body weight gain, but this was not significant. High vitamin C groups (chitosan-2 and psyllium-2) tended to exhibit decreased weight gains and FER compared to low vitamin C groups (chitosan-1 and psyllium-1), but the differences were not statistically significant.

The relative weights of internal organs in relation to body weight are presented in Table 2. The relative weights of the liver, kidney, spleen, jejunum, duodenum, and colon of the dietary supplement groups did not differ from those of F-control. Dietary supplement groups tended to exhibit increased ileum and cecum weights compared to F-control, and the ileum weights of chitosan-2 (0.58 g) and psyllium-1 (0.49 g) were significantly ($p < 0.05$) higher than that of F-control

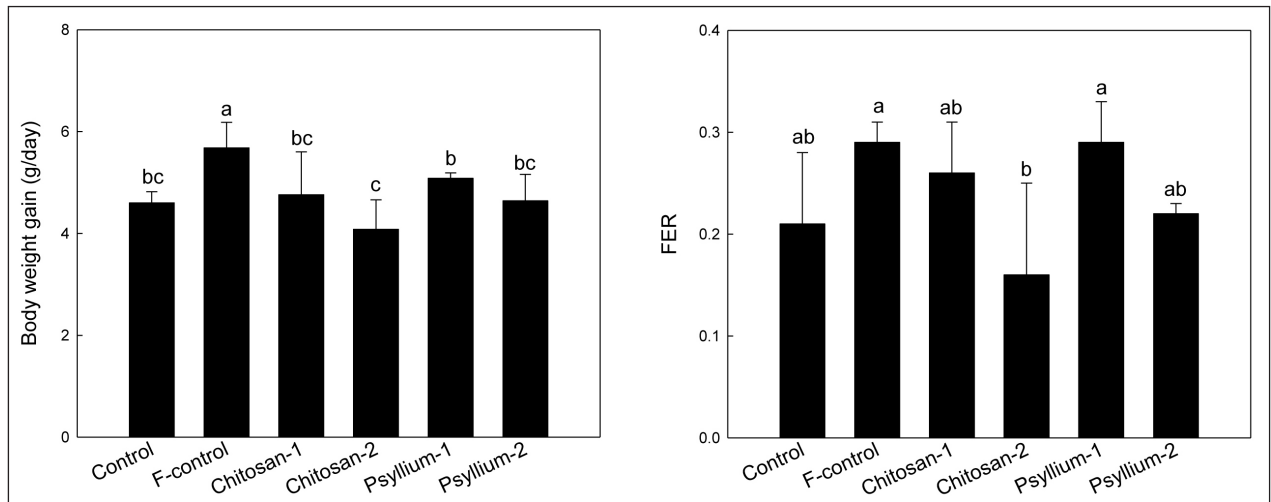


Figure 1. Body weight gain and food efficiency ratio (FER) in rats. FER, body weight gain/food intake.

Values are mean±SD for 8 rats. Different letters indicate significant differences ($p < 0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C.

Table 2. Relative weights of liver, kidney, spleen, ileum, jejunum, duodenum, colon and cecum (weight/100 g of body weight) of rats

Relative weights (g)	Control	F-control	Chitosan-1	Chitosan-2	Psyllium-1	Psyllium-2
Liver	3.29±0.33	3.33±0.37	3.34±0.14	3.25±0.15	3.15±0.48	3.02±0.19
Kidney	0.62±0.24	0.66±0.06	0.70±0.06	0.75±0.08	0.68±0.05	0.70±0.03
Spleen	0.18±0.01	0.16±0.02	0.17±0.03	0.17±0.02	0.17±0.02	0.17±0.02
Ileum	0.32±0.10 ^{bc}	0.28±0.03 ^c	0.41±0.14 ^{abc}	0.58±0.10 ^a	0.49±0.22 ^{ab}	0.41±0.15 ^{abc}
Jejunum	3.57±0.16	3.42±0.09	4.15±0.83	3.31±0.90	3.20±0.44	4.32±1.21
Deodenum	0.20±0.09	0.20±0.05	0.24±0.05	0.23±0.06	0.22±0.05	0.24±0.04
Colon	0.33±0.09 ^c	0.53±0.14 ^{ab}	0.50±0.33 ^{ab}	0.76±0.32 ^a	0.71±0.21 ^a	0.75±0.30 ^a
Cecum	1.17±0.32 ^{ab}	0.94±0.16 ^b	1.15±0.06 ^b	1.46±0.19 ^a	1.10±0.22 ^b	1.44±0.28 ^a

Values are mean±SD for 8 rats. Different letters indicate significant differences ($p < 0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C.

(0.28 g). Cecum weight was significantly ($p < 0.05$) increased by the addition of vitamin C.

Plasma lipid levels are summarized in Table 3. Plasma glucose, total-cholesterol and HDL-cholesterol levels in dietary supplement groups did not differ from those of F-control. Psyllium-2 (86.7 mg/dL) showed a significantly ($p < 0.05$) decreased triacylglycerol level compared to F-control (135.0 mg/dL). The LDL-cholesterol levels of chitosan-1, psyllium-1 and psyllium-2 were 17.9, 19.2, and 23.2 mg/dL, respec-

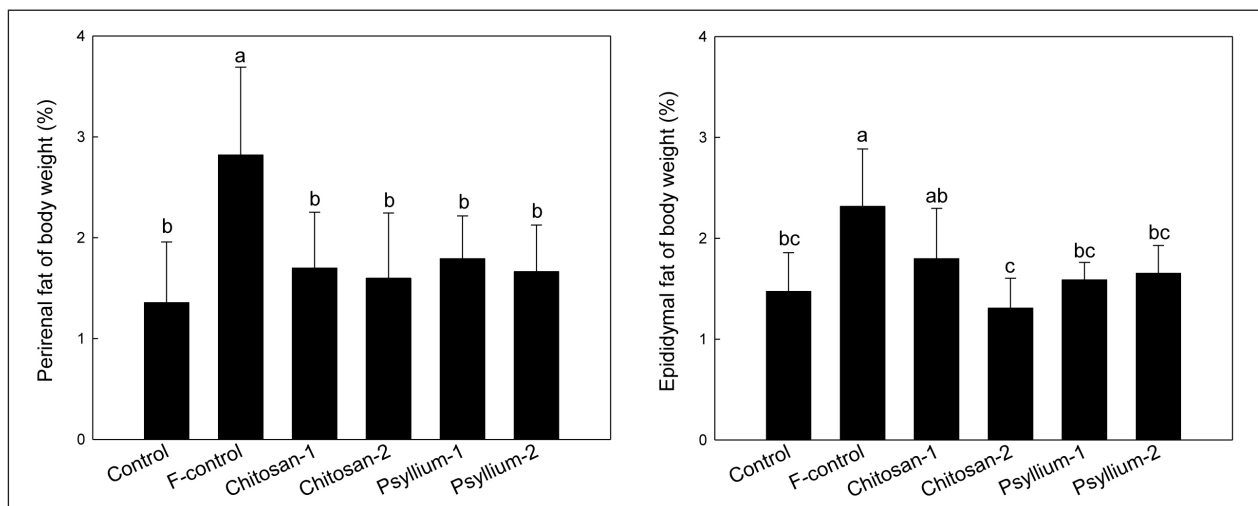
tively, and differed significantly ($p < 0.05$) from that of F-control (34.8 mg/dL).

As shown in Figure 2, the relative weights of perirenal fat pads were significantly ($p < 0.05$) reduced in dietary supplement groups compared to F-control, however, there were no significant differences between high vitamin C and low vitamin C groups. Chitosan-2 (1.5%) showed a significant ($p < 0.05$) decrease in epididymal fat pad weight compared to F-control (2.3%) and chitosan-1 (2.1%). The epididymal fat pad

Table 3. Plasma lipid levels of rats

Parameter (mg/dL)	Control	F-control	Chitosan-1	Chitosan-2	Psyllium-1	Psyllium-2
Glucose	188.0±168.1	196.7±92.7	340.3±36.5	270.7±102.9	291.7±78.6	255.3±124.7
Total-cholesterol	77.0±11.1	70.3±10.5	65.8±14.2	74.7±18.0	57.3±10.2	55.7±15.0
Triacylglycerol	120.7±9.0 ^{ab}	135.0±12.1 ^a	127.3±21.4 ^a	124.3±11.4 ^{ab}	119.7±33.1 ^{ab}	86.7±23.8 ^b
HDL-cholesterol	47.3±25.7	26.3±3.5	35.4±11.9	34.7±13.2	26.2±2.9	23.8±8.3
LDL-cholesterol	41.3±4.5 ^a	34.8±3.5 ^{ab}	17.9±5.2 ^d	27.6±4.2 ^{bc}	19.2±4.3 ^{cd}	23.2±6.2 ^{cd}

Values are mean±SD for 8 rats. Different letters indicate significant differences ($p<0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C

**Figure 2.** Effects of chitosan and psyllium husk with vitamin C on the relative weight of body fat in rats.

Values are mean±SD for 8 rats. Different letters indicate significant differences ($p<0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C.

weights of psyllium groups (psyllium-1: 1.7%, psyllium-2: 1.9%) were significantly ($p<0.05$) lower than that of F-control (2.3%), but there was no significant difference between psyllium-1 and psyllium-2.

The weight loss effect of chitosan has been studied both *in vivo* and *in vitro*. Remarkably, the data reported are conflicting. Although some trials, including this study, showed reducing effects of chitosan on body weight (18), others failed to show this effect (19, 20). It is considered that the reduced weight gain by chitosan was not due to lower food intake, because food intakes were similar among all groups.

The weight loss potential of chitosan has been generally attributed to the inhibition of fat absorption. It has been proposed that the hypolipidemic action of chitosan consists of the following (21). In the acidic environment of the stomach, chitosan swells and forms a positively charged gel. The gel attracts negatively charged molecules of fats, fatty acids, and bile acids, forming ionic complexes. In addition, chitosan can also interfere with emulsification of neutral lipids such as cholesterol by binding them through hydrophobic interactions, thus forming hydrophobic complexes (22). Passing further along the digestive system,

in the small intestine where pH is 7-8, chitosan-lipid ionic and hydrophobic complexes solidify and as aggregates are subsequently excreted in the feces (23). Thus, it is by binding to chitosan that lipid absorption from the small intestine to the bloodstream is thought to be prevented (24). The inhibition of fat absorption is closely associated with the reduction of plasma lipid concentrations and body fat accumulation. We observed chitosan decreased LDL-cholesterol levels in plasma and epididymal fat pads, supporting the hypolipidemic effects of chitosan.

The mechanism for the synergistic effect of vitamin C on the inhibition of fat digestion by chitosan is considered to be viscosity reduction in the stomach, which implies that chitosan mixed with a lipid is better than chitosan alone, by an increase in the oil-holding capacity of the chitosan gel, and the chitosan-fat gel being more flexible and less likely to leak entrapped fat in the intestinal tract (12, 25). The addition of vitamin C to chitosan has been found to cause a larger increase in the inhibition of fat digestion (12). In our previous study (26), it was recognized that the addition of vitamin C to chitosan influenced reductions in body weight gain and increases in total fecal weight and fecal fat excretion in guinea-pigs fed a high-fat diet, and we concluded these results may have been attributable to the synergistic effects of vitamin C on the fat-binding capacity of chitosan. The results of present study show chitosan itself did not influence FER, however, the addition of vitamin C to chitosan decreased FER significantly ($p < 0.05$) compared to F-control. We also observed that weight gains of rats fed chitosan and vitamin C mixtures were slightly less than those of rats fed chitosan alone, but it is thought that significant differences in body weight gain by the addition of vitamin C were not observed because the difference in dietary vitamin C level was $< 1\%$ (0.95%).

In this study, psyllium husk appeared to affect reduction of weight gain in rats fed a high fat diet. It is believed that psyllium husk may provoke loss of body weight by acting as a bulking agent, thereby increasing satiety and reducing caloric intake. And weight loss by psyllium husk is believed to be associated with reduced plasma lipid concentrations. In our previous study (27), we tried to combine psyllium husk with hydroxycitrate (HCA) to improve anti-obesity effects. From

that study, we found that the combined use of psyllium with HCA had an enhanced anti-obesity effect through reduced body weight gain and accumulated body fat weight. Psyllium husk was reported to exhibit hypolipidemic effects in both animal and human studies (28). Marlett and Fischer (29) evaluated fractions of psyllium husk for their effects on stool output and blood cholesterol in rats, and concluded that the poorly fermented, gel-forming fraction of psyllium husk was more effective at holding moisture in stool and increasing bile acid excretion. Buhman et al., (30) reported that the hypocholesterolemic effects of psyllium husk in rats were due to the increased fecal excretion of bile acids and total sterols. In addition, this increased bile acid excretion was associated with increased hepatic cholesterol 7R-hydroxylase activity, which is the rate-controlling step of bile acid synthesis, and increased cholesterol 7R-hydroxylase mRNA abundance (30). Bile acids are the primary excretory route for cholesterol from the body, and increased conversion of cholesterol to bile acids is considered a potential mechanism for the hypocholesterolemic effect of dietary fibers (31). The results of reduced LDL-cholesterol in plasma and perirenal and epididymal fat pads by psyllium husk in this study agree with previously reported studies (32, 33). Total-cholesterol and triacylglycerol levels were decreased slightly in the rats fed psyllium husk; however, we did not observe a significant difference between the diets with psyllium husk and without psyllium husk. We hypothesized that vitamin C might improve the hypolipidemic effects of psyllium husk, which has similar activities as chitosan. Weight gain, FER, total-cholesterol and triacylglycerol levels were slightly less in rats fed the psyllium husk and vitamin C mixture than psyllium husk alone, but the difference in vitamin C ratio to total diet was too small, and it was considered that there were slight significant differences by the addition of vitamin C similar to the results of chitosan.

AEAC in plasma and liver tissue

The effects on AEAC in plasma and liver tissue are shown in Figure 3. AEAC in plasma was significantly ($p < 0.05$) higher in psyllium-2 than F-control. AEAC in liver tissue of dietary supplement groups did

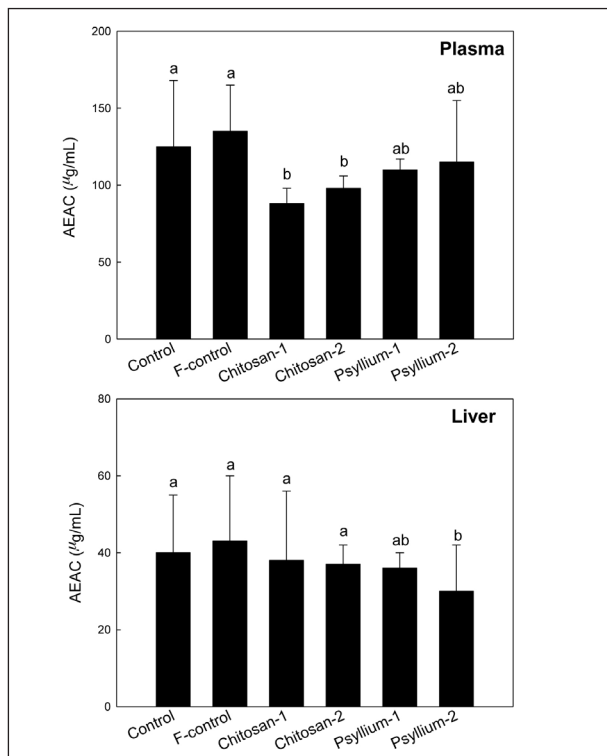


Figure 3. Effects of chitosan and psyllium husk with vitamin C on total antioxidant activity (AEAC) in rats. $AEAC = (\Delta A_{\text{sample}} / \Delta A_{\text{aa}}) \times C_{\text{aa}} \times V \times (100 / W_{\text{sample}})$ ΔA_{sample} : the change of absorbance in the presence of plasma or liver tissue solution; ΔA_{aa} : the change of absorbance after the addition of vitamin C standard solution; C_{aa} : the concentration of vitamin C standard solution (mg/mL); V : the volume of plasma or liver tissue solution (mL); W_{sample} : the weight of plasma or liver tissue solution (mL). Values are mean±SD for 8 rats. Different letters indicate significant differences ($p < 0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C.

not differ from that of F-control. Lipid peroxidation and GSH levels of the plasma and liver are presented in Table 4. TBARS content in plasma was significantly ($p < 0.05$) lower in psyllium-2 (2.40 μmol/L) compared to F-control (5.23 μmol/L). TBARS contents in liver tissue were reduced significantly ($p < 0.05$) in dietary supplement groups compared to F-control, but were not decreased by the addition of vitamin C. There were no significant differences in plasma GSH levels among the groups.

Vitamin C is a highly effective major nonenzymatic antioxidant, and capable of preventing lipid peroxidative damage (34). Vitamin C was reported to have antioxidant activity in both *in vivo* and *in vitro* studies. *In vivo* studies showed increased superoxide dismutase (SOD) activity and decreased catalase (CAT) activity, oxidized glutathione (GSSG), and GSH levels with 2 mmol of vitamin C. Lonrot et al., (35) reported that the antioxidant efficiency of vitamin C was concentration dependent. In other words, when vitamin C concentration decreases, it becomes a progressively more enduring antioxidant. In this study, GSH level in liver tissue was increased in the psyllium husk diet containing high vitamin C than low vitamin C, but other antioxidant activities in the plasma and liver tissue were not changed by the addition of vitamin C. This is considered to be due to the difference of vitamin C in the diets as too small, and antioxidant activities by vitamin C were not observed.

Although there is the possibility that additions of vitamin C to chitosan or psyllium husk are useful for reducing body weight, it is not clear whether they are effective for weight control. Broader research is needed

Table 4. Lipid peroxidation and glutathione reduced form (GSH) levels of plasma and liver in rats

Parameter		Control	F-control	Chitosan-1	Chitosan-2	Psyllium-1	Psyllium-2
TBARS	Plasma (μM/L)	2.10±0.77 ^b	5.23±2.41 ^a	5.03±2.55 ^a	3.68±0.86 ^{ab}	2.83±1.33 ^{ab}	2.40±0.66 ^b
	Liver (μM/g)	0.76±0.65 ^b	2.29±0.85 ^a	0.98±0.51 ^b	0.86±0.16 ^b	1.26±0.39 ^b	1.23±0.18 ^b
GSH	Plasma (μM/L)	141.7±46.6	104.9 ±7.5	163.8±73.7	176.4±15.2	138.6±82.9	143.9±36.4
	Liver (μM/g)	16.9±10.8 ^{ab}	8.5±3.1 ^b	11.2±3.1 ^b	16.6±9.9 ^{ab}	9.9±3.9 ^b	22.1 ±5.9 ^a

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substance (TBARS) concentrations. Values are mean±SD for 8 rats. Different letters indicate significant differences ($p < 0.05$) among groups by Duncan's multiple range test. Control, normal diet group; F-control, high fat diet group, Chitosan-1, high fat diet group with 5% chitosan; Chitosan-2, high fat diet with 5% chitosan containing 1% vitamin C; Psyllium-1, high fat diet with 10% psyllium husk; Psyllium-2, high fat diet with 10% psyllium husk containing 1% vitamin C.

to fully validate chitosan and psyllium husk containing vitamin C as dietary supplements to control obesity. m husk containing 1% vitamin C.

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Correspondence:

Hyung Joo Suh

Department of Public Health Science, Graduate School, Korea University, Seoul 07249, Republic of Korea

Tel. 82. 2. 3290. 5639

Fax 82. 2. 940. 2850

E-mail: suh1960@korea.ac.kr