

Plant Proteins Differently Affect Body Fat Reduction in High-fat Fed Rats

– Research Note –

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Abstract

This study examined the effects of corn gluten (CG), wheat gluten (WG), and soybean protein isolate (SPI), as well as their hydrolysates, on weight reduction in rats fed a high-fat diet. Eight-month-old male Sprague–Dawley rats (n=70) were fed a high-fat diet (40% of the calories were fat) for 4 weeks. Rats were then randomly divided into seven groups and were fed isocaloric diets with different protein sources for 8 weeks. The protein sources were casein (control group), intact CG (CG group), CG hydrolysate (CGH group), intact WG (WG group), WG hydrolysate (WGH group), intact SPI (SPI group), and SPI hydrolysate (SPIH group). Body weight gain, adipose tissue weights, lipid profiles in plasma and liver; and hepatic activities of carnitine palmitoyl transferase, fatty acid synthase (FAS), malic enzyme, and glucose-6-phosphate dehydrogenase were assessed. The CGH group showed significant weight reduction compared with the other groups. Epididymal fat pad and plasma triglycerides in the CGH group were the lowest and were significantly different than those in the control group. FAS activity in the CGH group was significantly lower than that in the other groups. In conclusion, the CGH diet of these experimental animals demonstrated a weight-reducing effect by lowering the adipose tissue weight and by affecting the activities of hepatic lipogenic enzymes.

Key words: corn gluten, hydrolysate, soybean protein isolate, weight reduction, wheat gluten

INTRODUCTION

The prevalence of overweight and obesity is increasing worldwide, which is leading to dramatic increases in the prevalence of hyperlipidemia, heart disease, and type II diabetes mellitus (1). Obesity is defined as the accumulation of excess adipose tissue resulting from an imbalance between energy intake and expenditure. Overeating is one of the major causes of obesity and restriction of energy intake is the basis of dietary therapy. However, simple food restriction reduces both body fat and body protein, and the low-energy diet consumed by obese people decreases the protein efficiency ratio. Thus, treating obesity requires both the reduction of excess body fat and the maintenance of adequate body protein, which should be achieved through an appropriate diet (2,3).

Much attention has been focused on components in several plant foods and dietary peptides, namely the functional protein hydrolysates, which might have beneficial effects on nutrient metabolism. It is thought that these substances have antiobesity, antihypertensive, antithrombotic, and anticarcinogenic actions (4-6). Plant proteins usually have inferior functional qualities compared with

animal proteins, but particular attention has been given to the enzymatic hydrolysis products of plant proteins. There are many examples of biologically active food proteins that have physiological actions beyond the pure nutritional activity of providing nitrogen for normal growth and maintenance. Peptides might be useful as health-enhancing foods and as functional ingredients in dietary supplements; however, few studies have investigated the effect of plant protein hydrolysates on obesity. Therefore, this study examined the effect of plant proteins, particularly corn gluten (CG), wheat gluten (WG), and soybean protein isolate (SPI), as well as their hydrolysates, on weight reduction in rats fed a high-fat diet.

MATERIALS AND METHODS

Animals

Eight-month old male Sprague–Dawley rats (Jung-Ang Lab. Animal, Inc., Seoul, Korea) were placed in individual stainless steel wire-mesh cages in a climate-controlled room. The room had a 12:12 hr light-dark cycle, a temperature of 22 ~ 24°C, and a relative humidity of 45 ± 5%. The experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC)

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Experimental design and diets

The rats were fed a pellet diet (Samyang Co., Seoul, Korea) for the first 6 days (adaptation period). At the end of the adaptation period, the rats weighed 564.03 ± 5.12 g (\pm standard error). The rats were then fed the modified American Institute of Nutrition (AIN)-93M diet (7) with high fat (40% of energy as fat) for 1 month. The lipid sources in the high fat diet were lard and soybean oil. The amounts of protein, fiber, minerals, and vitamins per total calories in the high fat diet were equalized to those of the AIN-93M diet (8). The rats weighed 628.38 ± 4.79 g after this period. They were then stratified according to body weight, randomly blocked into seven treatment groups, and raised for 2 months. The rats were allowed free access to the experimental diets and deionized water during the experimental period. The experimental diets were formulated according to the nutrient contents of the AIN-93M diet with different pro-

tein sources (Table 1). The protein sources were casein (control group), intact CG (CG group), CG hydrolysate (CGH group), intact WG (WG group), WG hydrolysate (WGH group), intact SPI (SPI group) and SPI hydrolysate (SPIH group). CG was obtained from Doosan Food Company (Seoul, Korea), WG was from Yangji Corp. (Seoul, Korea), and soybean protein isolate (SPI) was from IC Food Company (Daejeon, Korea). All other materials were purchased from Dyets Inc. (Bethlehem, PA, USA).

Materials

The hydrolysates of each intact protein were prepared by Sempio Foods Company (Seoul, Korea). The molecular weight distribution of dietary protein sources was analyzed by the gel permeation chromatography using High Performance Liquid Chromatography (Waters, Milford, MA, USA). A Bio-sil GPC column SEC-125 (7.5 mm \times 300 mm, Bio-Rad Laboratories, Hercules, CA, USA) was used and 0.05 M sodium phosphate buf-

Table 1. The composition of the experimental diet

(unit: g/kg diet)

Ingredients	Groups ¹⁾								
	High fat diet	Control	CG	CGH	WG	WGH	SPI	SPIH	
Corn starch	324.792	465.692	409.268	404.312	417.516	411.064	461.115	462.472	
Dextrinized cornstarch	110.0	155.0	155.0	155.0	155.0	155.0	155.0	155.0	
Sucrose	70.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Casein	174.0	140.000	—	—	—	—	—	—	
Intact CG	—	—	198.984	—	—	—	—	—	
CG hydrolysate	—	—	—	200.960	—	—	—	—	
Intact WG	—	—	—	—	189.656	—	—	—	
WG hydrolysate	—	—	—	—	—	194.208	—	—	
Intact SPI	—	—	—	—	—	—	144.157	—	
SPI hydrolysate	—	—	—	—	—	—	—	142.800	
Lard	100.0	—	—	—	—	—	—	—	
Soybean oil	100.0	40.000	37.440	40.420	38.520	40.420	40.420	40.420	
Fiber	60.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	
Mineral mix ²⁾	42.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	
Vitamin mix ³⁾	14.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	
L-Cystine	2.2	1.8	1.8	1.8	1.8	1.8	1.8	1.8	
Choline bitartrate	3.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
Tert-butyl hydroquinone	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	
Total amount	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	
Total calories (kcal)	4503.6	3776.4	3733.8	3759.9	3796.0	3763.7	3766.1	3774.9	
Energy ratio (%)	Carbohydrate	46.3	77.4	77.2	77.2	77.4	77.2	77.3	77.3
	Protein	13.6	13.0	13.1	13.1	13.0	13.1	13.1	13.1
	Fat	40.1	9.6	9.7	9.7	9.6	9.7	9.7	9.6

¹⁾Control, casein; CG, corn gluten; CGH, corn gluten hydrolysate; WG, wheat gluten; WGH, wheat gluten hydrolysate; SPI, soybean protein isolate; SPIH, soybean protein isolate hydrolysate.

²⁾AIN-93M mineral mixture (g/kg mixture): calcium carbonate 357.00, potassium phosphate monobasic 250.00, potassium citrate H₂O 28.00, sodium chloride 74.00, potassium sulfate 46.60, magnesium oxide 24.00, ferric citrate U.S.P. 6.06, zinc carbonate 1.65, manganese carbonate 0.63, cupric carbonate 0.30, potassium iodate 0.01, sodium selenate 0.01025, ammonium paramolybdate 4H₂O 0.00795, sodium metasilicate 9H₂O 1.45, chromium potassium sulfate 12H₂O 0.275, lithium chloride 0.0174 boric acid, 0.0815 sodium fluoride 0.0635, nickel carbonate 0.0318, ammonium vanadate 0.0066 and sucrose finely powdered 209.806.

³⁾AIN-93 vitamin mixture (g/kg mixture): niacin 3.00, calcium pantothenate 1.60, pyridoxine HCl 0.70, thiamine HCl 0.60, riboflavin 0.60, folic acid 0.20, biotin 0.02, vitamin E acetate (500 IU/g) 15.00, vitamin B12 (0.1%) 2.50, vitamin A palmitate (500,000 IU/g) 0.80, vitamin D₃ (400,000 IU/g) 0.25, vitamin K1/dextrose mix (10 mg/g) 7.50 and sucrose 967.23.

Table 2. Molecular weight distribution of dietary protein sources

(unit: g/100 g protein)

Dietary protein sources	Molecular weight (unit: Da)				
	<300	300~700	700~1,000	1,000~10,000	10,000<
Casein	<1	0	0	0	99<
Intact CG	<1	0	0	0	99<
CG hydrolysate	69.61	0	0	30.39	0
Intact WG	<1	0	0	0	99<
WG hydrolysate	17.06	82.55	0.39	0	0
Intact SPI	1.11	0	0	0	98<
SPI hydrolysate	5.15	94.85	0	0	0

fer with 0.15 M NaCl was used as the effluent. The molecular weights of intact proteins (casein, CG, WG and SPI) were more than 10,000 Da. More than 80% of WGH and SPIH were composed of 300~700 Da. About 70% of CGH was composed of less than 300 Da (Table 2).

Measurement

Body weight was recorded weekly. To determine food intake, the amount of food offered was weighed and the weights of scraps and waste were recorded three times per week. Blood samples were collected directly from the heart using syringes treated with heparin, centrifuged at 2,800 rpm for 30 min at 4°C and frozen at -80°C. The liver was removed, weighed, and cut into small pieces, which were frozen in liquid nitrogen and stored at -80°C until analysis. Perirenal and epididymal fat pads were removed and weighed after sacrificing the animals. Plasma activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), plasma concentrations of total protein, albumin, triglycerides, total cholesterol and high-density lipoprotein cholesterol, and hepatic concentrations of triglycerides and total cholesterol were measured using commercial kits (Asan Pharmaceutical, Seoul, Korea). Hepatic activities of carnitine palmitoyl transferase (CPT) (9,10), fatty acid synthase (FAS) (11), malic enzyme (ME) (12), and glucose-6-phosphate dehydrogenase (G6PDH) (13) were measured.

Statistical analysis

All results are expressed as mean \pm standard error. The data were analyzed by one-way analysis of variance, and the differences between experimental groups were evaluated using Duncan's multiple range tests at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Body weight gain, body fat and the blood and hepatic parameters related to lipid metabolism were measured. Only the CGH group showed a significant body weight reduction (Fig. 1). Accumulative food intake tended to be lowest in the CGH group but it was not significantly

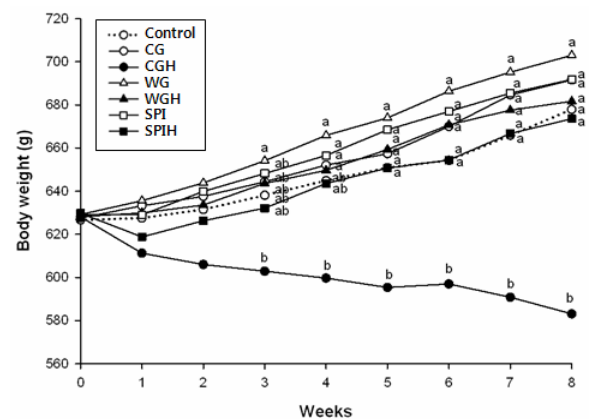


Fig. 1. Body weight changes during an 8 week treatment with different protein sources. Values with different letters are significantly different among the groups ($p < 0.05$).

different from that in the other groups (Fig. 2). The CGH group had the lowest perirenal and epididymal fat pad weights, and epididymal fat pad weight was significantly lower in the CGH group than that in the control, WG, and WGH groups ($p < 0.05$, Fig. 2). Though there were significant differences among the groups, plasma activities of AST and ALT and concentration of total protein and albumin were within the normal ranges for Sprague-Dawley rats (Table 3) (14,15). Plasma triglycerides were

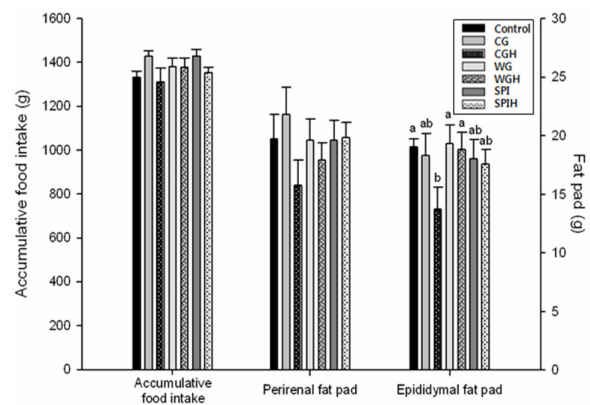


Fig. 2. Accumulative food intake and weights of the perirenal and epididymal fat pads of rats fed diets with different protein sources. Values are expressed as means \pm SE shown by vertical bars. Values with different letters are significantly different among the groups ($p < 0.05$).

Table 3. Plasma activities of AST and ALT and concentrations of protein and albumin of rats fed diets with different protein sources

Groups ¹⁾	AST (U/L)	ALT (U/L)	Total protein (g/dL)	Albumin (g/dL)
Control	49.54 ± 2.95 ^{2)ab3)}	27.11 ± 3.81 ^{ab}	6.54 ± 0.14 ^a	4.07 ± 0.07 ^a
CG	53.33 ± 2.39 ^{ab}	30.90 ± 4.06 ^{ab}	6.58 ± 0.12 ^a	4.04 ± 0.13 ^a
CGHA	43.25 ± 2.91 ^b	16.12 ± 5.30 ^b	5.90 ± 0.21 ^b	3.59 ± 0.08 ^b
WG	49.81 ± 3.07 ^{ab}	28.59 ± 5.56 ^{ab}	6.23 ± 0.21 ^{ab}	3.62 ± 0.18 ^b
WGH	50.88 ± 3.30 ^{ab}	29.19 ± 5.53 ^{ab}	6.21 ± 0.19 ^{ab}	3.67 ± 0.12 ^b
SPI	43.72 ± 3.03 ^b	24.56 ± 2.47 ^{ab}	6.16 ± 0.17 ^{ab}	3.57 ± 0.10 ^b
SPIH	46.66 ± 4.70 ^{ab}	22.22 ± 4.96 ^{ab}	6.46 ± 0.10 ^{ab}	3.81 ± 0.09 ^{ab}

¹⁾See Table 1.

²⁾Mean ± standard error (n=10).

³⁾Values with different letters within the cell are significantly different at $\alpha=0.05$ by Duncan's multiple range test.

significantly lower in the CGH ($p<0.05$, Fig. 3A), WGH, SPI and SPIH groups, whereas total cholesterol was significantly lower in the CGH, WGH and SPI treated groups ($p<0.05$, Fig. 3A). Although there were no significant differences in the hepatic total cholesterol and triglyceride levels (Fig. 3B), the hepatic CPT activity showed higher tendency in the CGH group compared to that in the control group. ME and G6PDH activities were inhibited by the all of the proteins treated in this study, whereas FAS activities were inhibited by only

CGH treatment ($p<0.05$, Fig. 4). Based on these results, we concluded that only CGH showed the most prominent anti-obesity activity among various plant protein sources and their hydrolysates.

Some studies have examined the effect of plant proteins and their hydrolysates on weight reduction in experimental animals. Some SPI and CGH showed a weight reduction effect (16,17) but other plant protein hydrolysates did not (18). We speculated that these different results might be explained by several factors, such as protein content in the experimental diet, kinds and compositions of peptides and amino acids from different sources, the manufacturing process of protein hydrolysates, and animal models. Among different plant protein sources and their hydrolysates, CG and CGH have a high number of branched chain amino acids (BCAAs), particularly leucine, which play an important role in body weight metabolism (19,20). The three BCAAs, leucine, valine, and isoleucine, support numerous metabolic processes ranging from a fundamental role as substrates for protein synthesis to metabolic roles as energy substrates, precursors for the synthesis of alanine and glutamine, and as a modulator of muscle protein synthesis through

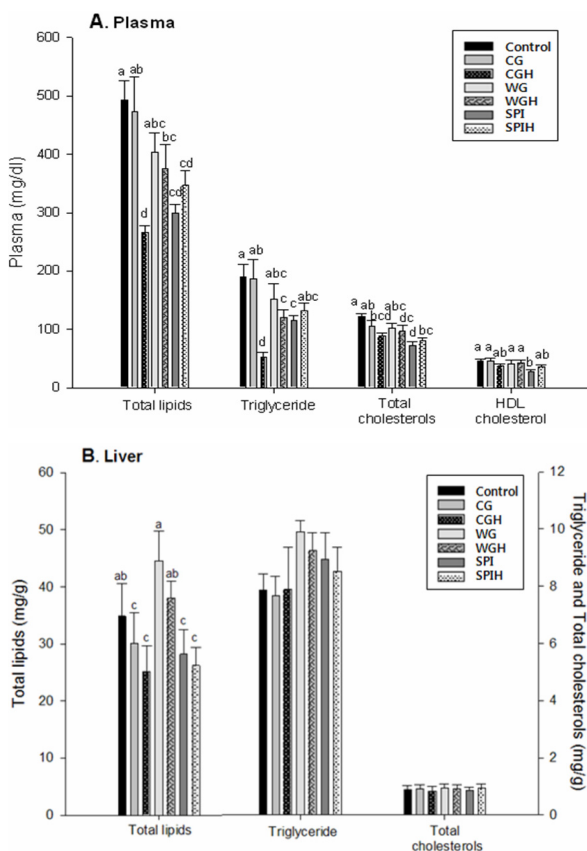


Fig. 3. Lipid concentrations in plasma (A) and liver (B) of rats fed diets with different protein sources. Values are expressed as means ± SE shown by vertical bars. Values with different letters are significantly different among the groups ($p<0.05$).

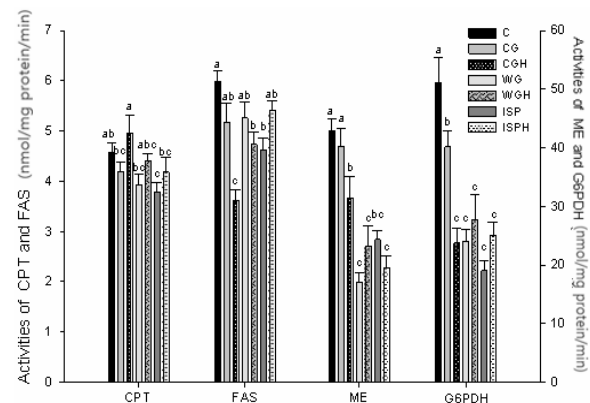


Fig. 4. Activities of hepatic enzymes in rats fed diets with different protein sources. Values are expressed as means ± SE shown by vertical bars. Values with different letters are significantly different among the groups ($p<0.05$).

the insulin-signaling pathway (21). In our previous study (22), we found that the body weight reduction in leucine-administered groups at 1.2 g/kg body weight was significantly higher than that of the control group. However, in another study (23), although BCAAs or leucine supplementation (about 4.6% of diet) tended to improve high fat induced metabolic disturbance, CGH mixed with BCAAs and various peptides showed greater effect on weight loss. In addition, in this study, we found that CGH was composed of smaller peptides than WGH and SPIH. More than 80% of WGH and SPIH were composed of 300~700 Da, whereas about 70% of CGH was composed of less than 300 Da. Therefore, besides BCAAs, we might postulate that smaller peptides which were di- or tri-peptides contained in CGH play an important role in anti-obesity activities of CGH. A recent study that compared low molecular yeast hydrolysate with high molecular yeast hydrolysate showed that the low weight hydrolysate significantly reduced body weight compared to the high weight hydrolysate, though the exact mechanism was not elucidated (24). These results indicate that this peptide mixture might signal energy homeostasis as well as insulin sensitivity.

Although we did not clarify the active anti-obesity compound in CGH, we confirmed that only the CGH diet had a weight reducing effect by lowering adipose tissue weight and affecting the activities of hepatic lipogenic enzymes compared with the other protein diets among various plant protein sources and their hydrolysates. However, further studies are needed to identify the active compounds and to determine the exact mechanism for the body weight reduction.

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