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Feeding of buckwheat protein extract reduces hepatic triglyceride concentration, adipose tissue weight, and hepatic lipogenesis in rats

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The objective of this study was to examine effects of feeding buckwheat protein extract (BWPE) on hepatic and plasma lipids, fat pad weights and activities of enzymes relating to lipid metabolism in tissues of rats. Rats were fed a semipurified diet containing either buckwheat protein extract or casein for 3 weeks. Food consumption and growth rate were unaffected by dietary treatment. Hepatic triglyceride concentration and the weights of epididymal and perirenal fat pad were significantly lower in rats fed BWPE compared with those fed casein diet. BWPE feeding caused lower activities of hepatic glucose-6-phosphate dehydrogenase and fatty acid synthase, but did not affect the activity of hepatic carnitine palmitoyltransferase I. In addition, BWPE caused increases in fecal fat and nitrogen. These results demonstrated that feeding BWPE causes reductions in hepatic triglyceride concentration and fat pad weights, and suggested that these reductions might be ascribed to lower activities of hepatic lipogenic enzymes and to lower digestibility of fat and protein. Furthermore, analysis of plasma free amino acids showed marked increases of glycine and arginine in rats fed BWPE compared with rats fed casein, raising the possibility that these alterations in plasma amino acids lead to reduction in body fat. © Elsevier Science Inc. 1996 (J. Nutr. 555–559, 1996.)

Keywords: buckwheat; protein; fat; lipogenesis; rat

Introduction

Buckwheat protein contains a higher amount of essential amino acids than any other plant proteins examined and its amino acid score is 92.[1] However, little is known about other nutritional aspects of this protein. Recently we have established a method for extraction of buckwheat protein from buckwheat flour and found a strong hypocholesterolemic effect of buckwheat protein extract (BWPE) in rats fed a cholesterol-enriched diet.[2] In a preliminary experiment, we have further observed that this protein extract caused not only a reduction in plasma cholesterol but also a reduction in adipose tissue weight as compared to casein in the rats fed cholesterol-free diet (Kayashita et al. unpublished data). It has been reported that soy protein diet[3] and fish oil diet,[4] reduce body fat accretion and plasma lipids in rats. In this report, we provide the evidence for lower concentration of hepatic triglyceride and fat pad weights in rats by feeding BWPE compared with feeding casein. Further, these alterations were found to be associated with reduced activities of hepatic lipogenic enzymes and reduced digestibility of dietary fat and protein.

Methods and materials

Materials

BWPE was prepared according to the method of our previous report.[2] The preparation method was as follows. Ten liters of deionized water were added to 1 kg of buckwheat flour (Manju A, Nikkoku Flour Mill, Nagano, Japan), stirred, and adjusted to pH 8.0 with solid sodium hydroxide. After centrifugation at 5000 × g for 20 min, the supernatant was adjusted to pH 4.5 with concen-

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Received January 25, 1996; accepted July 9, 1996.

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0955-2863/96/$15.00
PII S0955-2863(96)00110-6

trated hydrochloric acid to precipitate protein. The precipitate was washed with distilled water, neutralized with solid sodium hydroxide, and sterilized. Then the buckwheat protein extract was treated with spare drying. Composition of BWPE was: water, 8.0%; protein, 52.5%; lipid, 11.3%; crude fiber, 0.2%; ash, 5.2%; and carbohydrate, 22.8%. Composition of fatty acids in the lipid of BWPE is indicated in the footnotes.[a] Casein was purchased from Oriental Yeast Co. (Tokyo, Japan).

Animals and diets

Male Sprague-Dawley rats (3-week old, Japan SLC Co., Shizuoka, Japan) were housed individually in metal cages in a room with controlled temperature (21 to 23°C), humidity (40 to 50%) and a 12-hr light (8:00 to 20:00) dark cycle. Diets and deionized water were provided ad libitum. For 1 week, rats were fed a diet according to the formula recommended by the American Institute of Nutrition.[5] Subsequently, the rats (75 to 95 g) were fed on experimental diets (eight animals per diet group in experiment 1 and 2) containing either casein or BWPE (*Table 1*) for 3 weeks. Diets were made isoenergetic and isonitrogenous. Because BWPE contains some lipids, adjustment of dietary level of fat (100 g/kg diet) for the lipids of BWPE (42 g/kg diet) was made by reducing dietary corn oil to 58 g/kg of diet. Diets were stored at −35°C until used. Food consumption and body weight were recorded every morning. In experiment 1, feces were collected for the last 5 days of the experimental period, dried to a constant weight, and ground to a fine powder. At the end of experimental period (experiment 1 and 2), the diets were removed from cages (8:00). Blood was collected into a syringe with EDTA · Na_2 (3 mM/L) from the vena cava under anesthesia with diethyl ether (13:00–15:00), and plasma was obtained by centrifugation at 3,000 × g for 10 min. In experiment 1, organs were immediately removed, weighed, and stored at −80°C until analysis, and carcass samples were obtained by removing head, tail, digestive tract, lungs, kidneys, heart, testes, and abdominal adipose tissues (epididymal, perirenal, and mesenteric adipose tissues).

Analysis of plasma parameters

Plasma concentrations of triglyceride, cholesterol, and phospholipids were measured using enzymatic kits (Triglyceride G-Test Wako, Total cholesterol C-Test Wako, and Phospholipid B-Test Wako, respectively, Wako Pure Chemical, Osaka, Japan). In experiment 1, plasma concentrations of free fatty acid, insulin, 3-β-hydroxybutyrate, and acetoacetate were measured using enzymatic kit (NEFA C-Test Wako and Insulin-EIA TEST respectively, Wako Pure Chemical, Osaka and Ketone Test Sanwa, Sanwa Chemical Laboratory, Nagoya, Japan). Concentrations of free amino acids in plasma were measured using an amino acid analyzer (Hitachi 8500, Hitachi, Tokyo, Japan). In experiment 2, 2 mL plasma of two rats were pooled and subjected to preparative ultracentrifugation. The lipoproteins, including chylomicrons plus very-low-density lipoproteins (chylomicrons + VLDL, d < 1.006), low-density lipoproteins (LDL, 1.019 < d < 1.063), and high-density lipoproteins (HDL, 1.063 < d < 1.21), were isolated by flotation.[6] Concentrations of lipids in lipoproteins were measured by kits described previously.

Analysis of liver and fat pad

In experiment 1, extraction of lipids from liver was performed using chloroform-methanol (2:1 v/v) by the method of Folch et al.[7]

[a] Composition of fatty acids of lipid in BWPE 16:0, 14.0%; 18:0, 2.3%; 18:1, 39.7%; 18:2, 33.6%; 18:3, 2.0%; 20:0, 1.7%; 20:1, 3.6%; 22:0, 1.9%; 24:0, 1.2%.

Table 1 Composition of experimental diets

Ingredients	Casein	BWPE
	g/kg	
Casein[1]	230	
BWPE[2]		381
DL-Methionine	3	3
Corn oil	100	57
Mineral mixture[3]	35	35
Vitamin mixture[3]	10	10
Choline chloride	2	2
Cellulose	50	50
α-Corn starch	370	262
Sucrose	200	200

[1]Protein component of casein is 87.0% (w/w) (N × 6.25).
[2]Protein component of BWPE is 52.5% (w/w) (N × 6.25).
[3]The mineral mixture and vitamin mixture are prepared according to the AIN-76 mixture (5).

Hepatic concentrations of lipids were measured using the kits described. Tissues were homogenized with a Potter-Elvehjem apparatus in 4 volumes of ice-cold 0.14M KCl. The homogenate was centrifuged at 35,000 × g for 20 min at 4°C, and the supernatant was used for the assay of glucose-6-phosphate dehydrogenase (G6PD) and fatty acid synthase (FAS). For the assay of carnitine palmitoyltransferase I (CPT), tissues were homogenized with 0.25M sucrose, 3 mM HEPES, and NaEDTA (pH 7.4). G6PD and FAS activities were assayed spectrophotometrically as described by Freedland[8] and Martin et al.,[9] respectively. Activity of CPT was measured using L-carnitine, palmitoyl CoA, and 5,5′-dithio-bis (2-nitrobenzoic acid) according to the method of Bieber et al.[10] Carcass fat was gravimetrically determined after lipid extraction by the method of Folch et al.[7]

Fecal analysis

In experiment 1, fecal nitrogen content was determined by the method of Kjeldahl.[12] Fecal fat was gravimetrically determined after lipid extraction by the method of Folch et al.[7] Apparent digestibilities of protein and fat were calculated as percentage of intake [100 × (intake-feces)/intake].

Statistical analysis

Results were expressed as means ± SE. Statistical significance of differences between the two groups was evaluated by Student's *t* test. Results were considered significant at $P < 0.05$.

Results

Body weight and food intake (Experiment 1)

The body weight gains of rats fed BWPE and casein were 147 ± 3 g and 147 ± 2 g, respectively. Food intakes during experimental period (3 weeks) in rats fed BWPE and casein were 296 ± 6 g and 296 ± 6 g, respectively. There was no significant difference ($P > 0.05$) in these parameters between the two groups.

Plasma biochemical parameters

Plasma biochemical parameters are presented in *Table 2*. Concentrations of triglyceride, free fatty acid, phospholipids, 3-β-Hydroxybutyrate, acetoacetate, and insulin were

Table 2 Effects of experimental diets on plasma biochemical parameters (Experiment 1)

	Diet	
	Casein	BWPE
Triglyceride (mmol/L)	1.25 ± 0.09	1.17 ± 0.13
Free fatty acid (mmol/L)	0.61 ± 0.03	0.64 ± 0.03
Cholesterol (mmol/L)	2.39 ± 0.12	2.07 ± 0.09*
Phospholipids (mmol/L)	2.40 ± 0.10	2.33 ± 0.13
3-β-Hydroxybutyrate (μmol/L)	182 ± 29	125 ± 34
Acetoacetate (μmol/L)	51.2 ± 6.6	66.9 ± 7.0
Insulin (mU/L)	32.8 ± 3.8	34.2 ± 4.5

Values are means ± SE for eight rats per group.
*$P < 0.05$ significantly different between the two groups.

unaffected by the dietary manipulation. Cholesterol concentration was significantly lower in the BWPE group compared to the casein group. The data of plasma free amino acids (*Table 3*) indicated that BWPE feeding caused higher concentrations of Ser, Glu, Gly, Ala, Met, Ile, Arg, and Pro and lower concentrations of Thr and Lys compared with casein feeding. Concentrations of Asp, Val, Leu, Tyr, Phe, and His were unaffected by the dietary manipulation.

Hepatic lipids and enzymes

Hepatic weight; concentrations of hepatic lipids; and activities of G6PD, FAS, and CPT are presented in *Table 4*. Relative hepatic weight (% of body weight) was not significantly different between the two groups. Concentration of hepatic triglyceride was 38% lower in the BWPE group than that in the casein group ($P < 0.01$). Concentrations of cholesterol and phospholipids were unaffected by the dietary manipulation. Activities of G6PD and FAS were significantly lower in the BWPE group compared with the casein group, whereas CPT activity was unaffected by the dietary treatment.

Table 3 Concentrations of free amino acids in plasma of rats fed casein or BWPE (Experiment 1)

	Diet		
	Casein	BWPE	
	μmol/L		
Asp	13 ± 2	13 ± 1	
Thr	379 ± 22	275 ± 13**	−27%
Ser	181 ± 13	235 ± 7**	+30%
Glu	106 ± 5	126 ± 4*	+19%
Gly	143 ± 8	273 ± 11**	+91%
Ala	363 ± 19	460 ± 21**	+27%
Val	175 ± 10	181 ± 9	
Met	49 ± 3	57 ± 1*	+16%
Ile	94 ± 7	118 ± 8*	+26%
Leu	127 ± 9	127 ± 7	
Tyr	102 ± 7	88 ± 4	
Phe	57 ± 5	60 ± 2	
Lys	410 ± 19	357 ± 5*	−13%
His	71 ± 5	68 ± 3	
Arg	111 ± 7	204 ± 7**	+83%
Pro	174 ± 6	272 ± 11**	+56%
Total	2555 ± 141	2913 ± 89	

Values are means ± SE for eight rats per group.
*$P < 0.05$, **$P < 0.01$, significantly different between the two groups.

Table 4 Effects of experimental diets on hepatic metabolic parameters (Experiment 1)

	Diet	
	Casein	BWPE
Relative weight (g/100g BW)	4.3 ± 0.1	4.2 ± 0.1
Triglyceride (μmol/g tissue)	29.4 ± 1.8	18.2 ± 1.4**
Cholesterol (μmol/g tissue)	15.8 ± 1.0	13.7 ± 0.8
Phospholipids (μmol/g tissue)	29.4 ± 0.8	29.3 ± 1.0
G6PD (μmol/min · g tissue)	1.57 ± 0.09	0.98 ± 0.06**
FAS (μmol/min · g tissue)	0.72 ± 0.04	0.54 ± 0.04**
CPT (μmol/min · g tissue)	0.83 ± 0.05	0.74 ± 0.02

Values are means ± SE for eight rats per group.
**$P < 0.01$, significantly different between the two groups.

Fat pads and enzymes

As shown in *Table 5*, BWPE feeding caused a 23% reduction in relative weight of epididymal fat pad compared with casein feeding. Activities of G6PD, FAS, and CPT in epididymal fat pad were unaffected by the dietary treatment. A reduction (18%) in relative weight of perirenal fat pad was also observed with BWPE feeding. Carcass fat was 12% lower in the BWPE group than the casein group, but the difference was not significant ($P > 0.05$).

Fecal analysis

Fecal nitrogen and fat were significantly higher in the BWPE group compared with the casein group (*Table 6*). BWPE feeding caused 16% reduction in apparent protein digestibility ($P < 0.01$) and 3% reduction in apparent fat digestibility ($P < 0.01$).

Lipids in plasma lipoprotein fractions (Experiment 2)

Concentrations of plasma triglyceride, cholesterol, and phospholipids are presented in *Table 7*. Concentrations of

Table 5 Effects of experimental diets on metabolic parameters in fat pads (Experiment 1)

	Diet	
	Casein	BWPE
Epididymal fat pad		
Relative weight (g/100g BW)	1.36 ± 0.05	1.05 ± 0.04**
G6PD (μmol/min · g tissue)	0.19 ± 0.02	0.17 ± 0.01
FAS (nmol/min · g tissue)	24.5 ± 1.6	25.6 ± 1.8
CPT (nmol/min · g tissue)	24.9 ± 3.9	25.5 ± 3.4
Perirenal fat pad		
Relative weight (g/100g BW)	1.55 ± 0.07	1.26 ± 0.09*
Carcass fat (g/100g BW)	11.5 ± 0.3	10.1 ± 0.7

Values are means ± SE for eight rats per group.
*$P < 0.05$, **$P < 0.01$, significantly different between the two groups.

Table 6 Effects of experimental diets on fecal weight, nitrogen, and fat (Experiment 1)

	Diet	
	Casein	BWPE
Dry weight (g/5 days)	5.3 ± 0.5	11.5 ± 0.5**
Nitrogen (mgN/5 days)	111 ± 9	629 ± 31**
Apparent protein digestibility (%)	95.8 ± 0.4	80.6 ± 0.6**
Fat (mg/5 days)	432 ± 39	692 ± 34**
Apparent fat digestibility (%)	95.0 ± 0.2	92.1 ± 0.3**

Values are means ± SE for eight rats per group.
**$P < 0.01$, significantly different between the two groups.

Table 7 Effects of experimental diet on plasma lipoprotein distribution (Experiment 2)

	Diet	
	Casein	BWPE
Plasma		
Triglyceride (mmol/L)	1.72 ± 0.17	1.43 ± 0.13
Cholesterol (mmol/L)	2.36 ± 0.18	2.24 ± 0.92
Phospholipids (mmol/L)	2.34 ± 0.13	2.40 ± 0.10
Chylomicrons + VLDL		
Triglyceride (mmol/L)	1.34 ± 0.15	0.93 ± 0.31
Cholesterol (mmol/L)	0.29 ± 0.04	0.19 ± 0.03
Phospholipids (mmol/L)	0.40 ± 0.06	0.31 ± 0.04
LDL		
Triglyceride (mmol/L)	0.17 ± 0.05	0.36 ± 0.06*
Cholesterol (mmol/L)	0.49 ± 0.02	0.63 ± 0.05*
Phospholipids (mmol/L)	0.30 ± 0.01	0.43 ± 0.02*
HDL		
Triglyceride (mmol/L)	0.024 ± 0.004	0.039 ± 0.001*
Cholesterol (mmol/L)	1.50 ± 0.02	1.41 ± 0.08
Phospholipids (mmol/L)	1.04 ± 0.04	1.04 ± 0.06

Values are means ± SE for four samples from eight rats per group (2 mL plasma of two rats were pooled).
*$P < 0.05$, significantly different between the two groups.

plasma triglyceride, cholesterol, and phospholipids were not significantly affected by dietary treatment. The BWPE diet caused a trend to decreased triglyceride and cholesterol in chylomicrons plus VLDL fraction ($P > 0.05$). Concentrations of cholesterol, triglyceride, and phospholipids in LDL fraction were significantly higher in the BWPE group ($P < 0.05$). BWPE feeding caused a higher concentration of triglyceride in HDL fraction ($P < 0.05$). Concentrations of cholesterol and phospholipids in HDL fraction were unaffected by dietary treatment.

Discussion

It is well known that dietary treatments such as energy restriction,[12] fish oil diet,[4] and soy protein diet[3] reduce body fat. In this study, we demonstrated for the first time that BWPE intake reduces hepatic triglyceride and epididymal and perirenal fat pad weights. Because BWPE contained 11.3% (w/w) lipids as a minor component,[2] the influence of dietary addition to the lipids of BWPE on hepatic and plasma concentrations of lipids and fat pad weights has been studied in our recent study. The results showed no influence of the lipids on these parameters when compared with corn oil.[b]

The carcass samples in this study consisted of muscles, bones, skin, and subcutaneous fat. The fat content of the carcass sample in the BWPE group was not significantly different from that in the casein group, whereas the weight of abdominal adipose tissue including epididymal and perirenal adipose tissues were significantly affected by the dietary protein source. These results may indicate that dietary BWPE affect body fat more in the abdominal adipose tissues that in the subcutaneous adipose tissues.

To understand the difference in the effect of BWPE and casein on hepatic triglyceride and adipose tissue weight, activities of enzymes relating to lipid metabolism were examined in the two dietary groups. The results showed that BWPE reduced hepatic activities of lipogenic enzymes such as G6PD and FAS, but had no influence on the activity of CPT, a rate-limiting enzyme of mitochondrial fatty acid oxidation. Activities of these three enzymes in epididymal fat pad were unaffected by the dietary treatment. These results suggest that reduced hepatic lipogenesis in BWPE group might lead to decreased hepatic triglyceride and fat pad weights.

Another factor that might have contributed to lower fat deposition in BWPE-fed rats as compared to casein-fed rats was the lower fat and protein digestibilities. Recently we have observed that BWPE has dietary-fiber like activities such as enhanced excretion of tecal neutral sterols,[c] improvement of atropine-induced constipation,[13] and amelioration of amaranth toxicity.[14] We have speculated that these properties may be ascribed to lower digestibility of this protein. The lower digestibility of protein in BWPE may have contributed to the reduction in the digestibility or absorption of dietary fat. Possibly reduced absorption of these energy sources in the BWPE group may, at least in part, cause lower fat deposition.

Decreased activity of hepatic lipogenic enzymes and increased fecal excretion of fat by BWPE diet were similar to those by soy protein diet.[3,15] Recently, soy protein was reported to elevate the concentration of thermogenin in brown adipose tissue of rats.[16] Because thermogenin is responsible for diet-induced thermogenesis, elevated thermogenesis by soy protein may also partially lower body fat.[16] Further study is in progress in our laboratory to examine whether BWPE enhances diet-induced thermogenesis.

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Because the concentrations of plasma-free fatty acid and ketone bodies were unaffected by dietary treatment (*Table 2*), the possibility that BWPE enhances lipolysis of triglyceride in adipose tissue, leading to lower adipose tissue weight seems to have been eliminated.

Higher contents of glycine and arginine are characteristics of the amino acid composition in BWPE.[2] Analysis of plasma free amino acids also showed a marked increase of glycine and arginine in the BWPE group as compared with the casein group. Horigome and Cho reported that addition of glycine to a casein diet reduced the plasma triglyceride.[17] Sugano et al. demonstrated a trend of lower plasma triglyceride when arginine was supplemented to a casein diet.[18] The high contents of glycine and arginine in BWPE might have an effect on triglyceride metabolism. Further studies are necessary to elucidate the relationship between higher contents of these amino acids in BWPE and the alteration in lipid metabolism.

We have reported a marked hypocholesterolemic action of BWPE in rats fed a cholesterol-enriched diet.[2] In this study, we observed that in the rats receiving a cholesterol-free diet the reduction in plasma cholesterol by BWPE was only slight (13% [$P < 0.05$] and 5% [$P > 0.05$] reductions in experiment 1 and 2, respectively). Our previous study has suggested that the hypocholesterolemic action of BWPE is mediated through increased fecal excretion of neutral sterols when animals were fed cholesterol enriched diet (Kayashita et al. unpublished observations). It seems likely that the hypocholesterolemic effect of BWPE is prominent especially in the animals receiving exogenous cholesterol.

Analysis of plasma lipoproteins showed a trend to reduced cholesterol in the fraction of chylomicrons plus VLDL and a significant elevation of cholesterol in LDL fraction by BWPE intake. From these data it seems to hard to evaluate if BWPE is useful for the prevention of atherosclerosis in animals not receiving cholesterol. However, the finding that dietary BWPE caused a trend of lower concentration triglyceride in chylomicrons plus VLDL and higher concentration of triglyceride in LDL and HDL raises the possibility that BWPE enhances the use of plasma triglyceride by extrahepatic tissues through elevated activity of lipoprotein lipase. Further study is in progress to examine influence of BWPE on the activity of lipoprotein lipase in several tissues and to test the possibility that enhanced use of plasma triglyceride by some tissues including muscle and brown adipose tissue also causes lower weights of white adipose tissues in BWPE-fed rats.

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