論　文

Effects of Soy Peptide on Lipid Metabolism and Energy Expenditure.

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Abstract

The present study evaluated the effect of dietary soy peptide, administered over two weeks, on hepatic lipid metabolism, abdominal fat tissues, and energy expenditure in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Relative weights of epididymal, peritoneal, and omental adipose tissues were tended to reduce by approximately 14.4%, 14.4% and 7.7%, respectively, in the soy peptide group compared with the casein group, although there was no significant difference. The soy peptide group showed a significant reduction in hepatic triglyceride (TG) compared with the casein group. Fatty acid synthase and phosphatidate phosphohydrolase activities were significantly reduced in rats fed soy peptide compared with those fed casein. Conversely, carnitine palmitoyltransferase activity was significantly higher in the soy peptide group compared with the casein group. Peroxisomal β-oxidation enzyme activity was comparable among both groups. For energy expenditure, after 10 days of feeding, the soy peptide diet tended to enhance lipid degradation rate, whereas carbohydrate and protein expenditure tended to reduce. Overall, this study suggests that soy peptide diet decreased hepatic TG concentration and enhanced lipid expenditure in OLETF rats.

Key words: soy peptide, lipid metabolism, energy expenditure, obesity

1 Introduction

Life-style related diseases, such as obesity, diabetes, hyperlipidemia, and hypertension, are increasingly prevalent in developed countries and contribute to increasing cardiovascular morbidity and mortality[1,2]. The pathogenesis of life-style related diseases is complicated and mechanisms have not yet been elucidated, although obesity has emerged as one of the major cardiovascular risk factors[3-5]. Obesity is defined as an increased mass of adipose tissue and both the prevalence and severity of obesity are increasing in developed countries.

The role of dietary components and their metabolites in prevention and treatment of a wide variety of human diseases has been well recognized. Soy bean (*Glycine max*) is an important foodstuff and contains valuable constituents of proteins, isoflavones, saponins, and phytosterols, is low in fat, and free of cholesterol. Previous studies have reported the potential benefits of soy protein and its components for the treatment of cardiovascular diseases, cancers[4], diabets[5]and kidney diseases[6-8].

Soy protein isolate contains several bioactive peptides that have distinct physiological activities involved in lipid metabolism[9,10]. Several studies have shown that dietary soy protein reduces triglyceride (TG) and cholesterol levels and lowers blood pressure in humans and animals[11-14]. In addition, peptides, or protein hydrolysates, exhibit greater bioactivities than intact proteins or amino acid mixtures[15]. In the previous study, we evaluated the effect of the soy protein hydrolysates (soy peptide) on lipid metabolism in HepG 2 cells and experimental rats[16]. Rats that were fed soy peptide diet for four weeks, significantly reduced liver weight and TG concentration. Interestingly, the study revealed three peptides in soy peptide that may be responsible for lowering TG concentration.

However, it is not known whether the soy protein hydrolysates affect abdominal tissue weights or energy metabolism. The present study evaluated the effect of a two-week administration of dietary soy peptide on hepatic lipid metabolism, abdominal fat tissues and energy expenditure in Otsuka Long-Evans Tokushima Fatty (OLETF) rats.

2 Materials and Methods

2.1 Preparation of soy crude peptide

Soy peptide was made by hydrolysis of soy protein isolate with endo-type protease extracted from Bacillus sp. It was donated from Fuji Oil Company (Osaka).

2.2 Animal experiment

Four-week-old male OLETF rats obtained from Tokushima Research Institute (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) were housed individually in stainless steel cages in a room maintained at constant temperature with a 12 h light/dark cycle. The rats had free access to an adequate stock pallets diet for one week. After the adaptation period, rats were assigned to three groups (6 per each group). The experimental rats were fed with semi-synthetic diet supplemented with 20 g/100 g casein or 19.1 g/100 g soy peptide. The composition of experimental diets is shown in Table 1. Food and water were freely available. Food intake and body weight were measured every day. Rats were fasted for 10 h and they were anesthetized with diethyl ether prior to blood collection, after 14 days of dietary treatment. Blood was collected from vena cava. All the experimental animal care and use of laboratory animals were conducted according to the guideline provided by the Ethical Committee of experimental Animal Care at Saga University.

Table 1. Composition of experiment diet.

Ingredients	Groups	
	Casein	Soy peptide
	(g / kg diet)	
Casein	200	0
Soy peptide	0	191※
Corn starch	150	150
Cellulose	50	50
AIN 76 Mineral Mix	35	35
AIN 76 Vitamin Mix	10	10
DL-Methionine	3	3
Choline Bitartrate	2	2
Corn oil	70	70
Sucrose	480	489

※Nitrogen content is same between casein and soy peptide.

2.3 Preparation of liver subcellular fractions

Liver homogenates and subcellular fractions were prepared as previously described by Yanagita et. al.[17]. In brief, a piece of liver from each rat was homogenized in ice-cold 10 mM Tris-HCl buffer (pH 7.4) containing 0.2 M sucrose solution and 1 mM EDTA. The homogenates were centrifuged at 1,000×g for 10 min to sediment nuclei and cell debris and the resulted surpernatant was then centrifuged at 20,000×g for 20 min at 4℃ to obtain mitochondria. The obtained supernatant was further centrifuged in 105,000×g for 45 min at 4℃ to sediment microsomes and the remaining supernatant was used as the cytosolic fraction. The pellets gently homogenized with a 10 mM Tris-HCl buffer (pH 7.4) that contained 0.2 M su-

crose solution and 1 mM EDTA. Protein concentration was measured by the method of Lowry et. al.[18] with bovine serum albumin used as the standard.

2.4 Lipid and protein analysis

Liver lipid was extracted with chloroform: methanol mixture(2:1 v/v) as described by Folch et. al.[19]. Hepatic triglyceride was determined by the acetyl acetone method described by Fletcher[20]. Protein concentration was determined by using the BCA protein assay kit (purchased from Pierce, Rockford, IL, USA)[21].

2.5 Assays of enzyme activities

The activity of fatty acid synthase (FAS) in the hepatic cytosol fraction was measured according to the method of Kelley et. al.[22]. The reaction mixture consisted of 0.5 M potassium phosphate buffer (PH 7.0) that contained 200 μM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 66 μM acetyl-coenzyme (CoA), and 200 μM malonyl-CoA. This mixture was placed in a curette and mixed with the enzyme. The reaction was observed spectrometrically at 340 nm for minute. A control reaction without substrate was also done. The plots of absorbance versus time were converted to nanomoles of NADPH reduction per minute, using a millimolar extinction coefficient of 6.22.

The activity of phosphatidate phosphohydrolase (PAP) was assayed by the method of Possmayer and Walton[23] with a slight modification. The reaction mixtures contained 0.05 M Tris-HCl (pH 7.0), 1 mM L-α-phosphatidate, and 1 mM phosphatidylcholine liposomes suspended in 1.55 M sodium choline, in the presence of 1.25 mM magnesium sulfate, and 50 to 100 μg of liver enzyme protein in a final assay volume of 0.2 ml. The mixture was incubated for 15 min at 25℃ and reaction was terminated by addition of 0.8 ml of solution containing 0.13% sodium dodecyl sulfate, 1.25% ammonium molybdate-4 H_2O, and 0.75 N H_2SO_4. The amount of liberated inorganic phosphate was measured. The phosphomolibdated color was developed at 45℃ for 20 min and the absorbance was measured at 820 nm. Non-enzymatic phosphate release was determined by inactivating the enzymes by boiling for 1 min without substrate. The enzyme activity expressed as nanomole in one minute per mg protein.

The activity of carnitine palmitoiltransferase (CPT) was assayed by measuring the CoA-SH formation as described earlier[24]. The reaction mixture contained 116 mM Tris-HCl (pH 8.0), 2.50 mmol EDTA neutralized to pH 8.0 with Tris, 2.50 mmol L-carnitine, 0.5 mmol 5,5'-dithio-bis. (2-nitrobenzoic acid), 75 mmol palmitoyl-CoA and 0.2% Triton-X 100. The whole solution was equilibrated at 25℃. The reaction was initiated by addition of enzyme source and then absorbance was monitored for 2-4 min. The L-carnitine independent rate was determined in a second curette without added L-carnitine. The difference between with and without carnitine gave the carnitine dependent rate for formation of CoA and was equated to csrnitine palmitoyltransferase. The assay was conducted in freeze-thawed homogenate.

Determination of the activity for peroxisomal β-oxidation was carried out with the method described in our previous papers[25]. The reaction mixture contained 940 μl 50 mM Tris-HCl buffer (pH 8.0), 10 μl of 20 mM NAD, 3 μl of 0.33 M dithiothreitol, 5 μl of 1.5 g/100 ml bovine serum albumin, 5 μl of 2 g/100 ml Triton X-100, 10 μl of 100 mM KCN. This mixture was placed in a curette and mixed with 5 μl of enzyme. The reaction was started by the addition of 2 μl of 5 mM palmitoyl-CoA and observed spectrometrically at 340 nm for 5 minutes. A control reaction without substrate was also done. The plots of absorbance versus time were converted to nanomoles of NAD reduction per minute, using a millimolar extinction coefficient of 6.22.

2.6 Respiratory gas analysis

Energy expenditure was measured 24 h after 10 days feeding period. The oxidation of carbohydrate (glucose), lipid and protein were computed from oxygen consumption (Vo_2), carbon dioxide production (Vco_2) and the respiratory quotient. Gas analysis was performed using an open-circuit metabolic gas analysis system connected directly to a mass spectrometer (model WSMR-1400, Arco system, Chiba, Japan). The gas analysis system is described in elsewhere[3,26,27]. Briefly, each metabolic chamber had a 210 cm^2 floor was 6.5 cm in height. Room air was pumped through the chambers at a rate of 1.8 L/min. Expired air was dried in a cotton thin column and then directed to an O_2/CO_2 analyzer. In addition, the oxygen production from protein metabolism (Vo_2-P, ml/min) was assed. Urine samples were collected during 24 h and urinary nitrogen concentration was calculated by using an assay kit (Urinary nitrogen test, Wako Pure Chemicals). Vo_2-P was calculated with the following formula: Vo_2-P = urea nitrogen × 5.923.

2.7 Data analysis

All values expressed as mean±standard error. Data was analyzed by Duncan's new multiple-range test[28]. Differences were considered statically significant at $p<0.05$.

3 Results

3.1 Effect of soy peptide on food intake and growth parameters in OLETF rats.

No significant differences in food intake or body weight were observed between rats fed a soy peptide diet or a casein diet (data not shown). Although relative liver weight was reduced in the soy peptide group (3.54 ± 0.13 g per 100 g body weight), compared with the casein group (3.68 ± 0.09 g per 100 g body weight), this was not statistically significant. Table 2 shows that relative weight of epididymal, peritoneal, and omental adipose tissues were also tended to reduce about 14.4%, 14.4% and 7.7%, respectively, in the soy peptide group compared with the casein group, although again, the difference was not significant.

Table 2. Effect of soy peptide on abdominal fat pads in OLETF rats.

	Groups	
	Casein	Soy peptide
	(g / 100 g B. W.)	
Epididymal	13.2 ± 1.2	11.3 ± 1.0
Peritoneal	22.2 ± 1.1	20.5 ± 1.5
Omental	8.77 ± 0.61	7.60 ± 0.63

Young male OLETF rats were fed a semi-purified diet supplemented with soy peptide or casein for two weeks. Data are mean±SE (n = 6).

3.2 Effects of soy peptide on hepatic lipid concentration and on energy activities related to lipid metabolism.

Figure 1 shows that the soy peptide group had significantly reduced hepatic TG compared with the casein group. Figure 2 shows the effect of soy peptide on enzyme activities that are related to lipid metabolism. FAS, and PAP activities were significantly reduced in rats fed with soy peptide compared with those fed casein. CPT activity was significantly higher in the soy peptide group compared with the casein group. However, peroxisomal β-oxidation enzyme activity was comparable among the two groups.

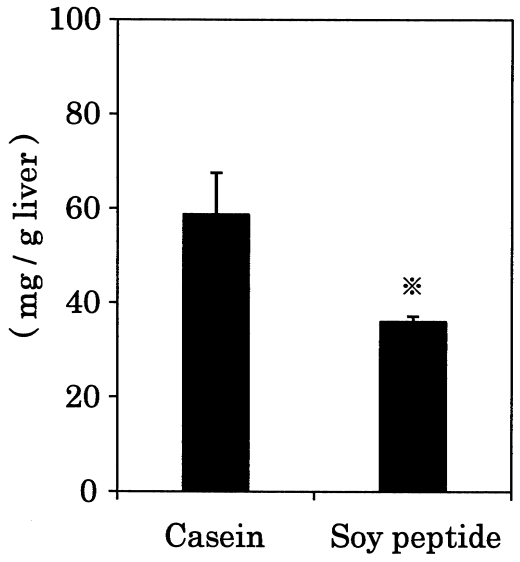

Fig. 1 Effect of soy peptide on hepatic triglyceride concentration in OLETF rats.

Young male OLETF rats were fed a semi-purified diet supplemented with soy peptide or casein for two weeks. Hepatic triglyceride concentration was measured as described in materials and methods.
※significantly different at $p < 0.05$.

3.3 Effect of soy peptide on energy metabolism.

Table 3 shows the effect of soy peptide on energy metabolism, obtained from respiratory gas analysis after 10 days of feeding. Interestingly, lipid oxidation was higher in the soy peptide group than in the casein group. In contrast, carbohydrate expenditure was tended to reduce in the soy peptide group. Protein oxidation decreased by 45 % in the soy peptide group compared with the casein group.

Table 3. Effect of soy peptide on energy metabolism in OLETF rats.

Parameters	Groups	
	Casein	Soy peptide
	(g / day / 100 g B. W.)	
Carbohydrate	7.03 ± 0.29[a]	6.56 ± 0.25[a]
Lipid	0.238 ± 0.128[a]	0.336 ± 0.090[a]
Protein	0.550 ± 0.045[a]	0.292 ± 0.035[b]

Young male OLETF rats were fed a semi-purified diet supplemented with soy peptide or casein. Energy expenditure was measured 24h after a 10-day feeding period. Data are mean±SE (n = 6). Values within a column that do not share a superscript letter are significantly different at $p < 0.05$.

4 Discussion

This study evaluated the effect of soy peptide on body weight, abdominal fat tissue, and energy metabolism in obese OLETF rats. OLETF rats are experimental animals that develop a syndrome of metabolic and hormonal disorders similar to many symptoms of human obesity[3, 16]. OLETF rats exhibit hyperphagia, owing to the lack of cholecystokinin receptors; as a result, they become obese and develop a fatty liver, diabetes, and hyperlipidemia.

When OLETF rats were fed diets with soy peptide or casein for two weeks, there was no different in the body weight of the two groups. Inoue et. al.[16] reported that soy protein hydrolysates significantly reduced hepatic TG concentration in genetically obese rats after four weeks of feeding. Concentrations of serum TG and apolipoprotein B_{100} were also reduced. This suggests that soy protein isolates have the ability to modify lipid metabolism. This study also showed that OLETF rats fed experimental soy peptide diets for two weeks, had reduced

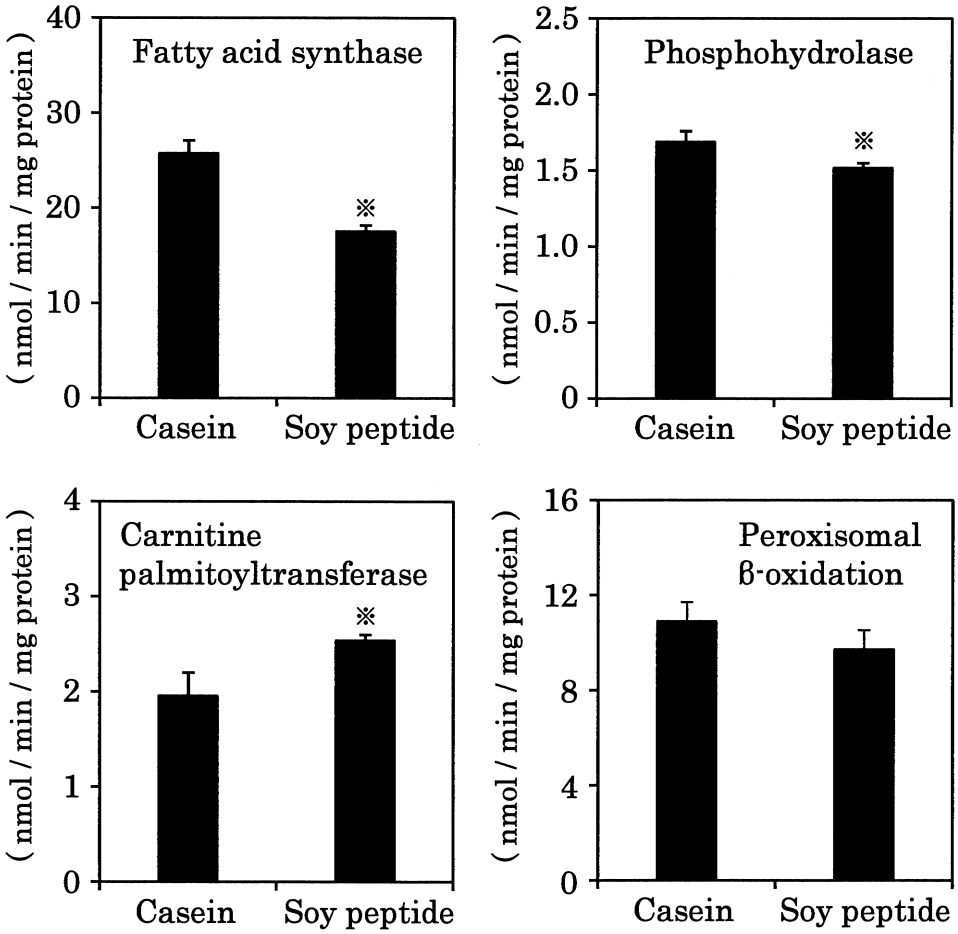

Fig. 2 Effect of soy peptide on hepatic enzyme activities related to lipid metabolism in OLETF rats.

Young male OLETF rats were fed a semi-purified diet supplemented with soy peptide or casein for two weeks. Hepatic enzyme activities were measured as described in materials and methods.

※significantly different at $p < 0.05$.

hepatic TG concentration and reduced abdominal fat tissue weight. Further long-term feeding experiments may explain whether soy peptide affects abdominal fat tissue weight and other physiological functions in the body.

To understand the underlying mechanism behind the lowering of hepatic TG by soy peptide, we examined key enzymes involved in lipid biosynthesis (FAS and PAP), and lipid degradation (CPT and peroxisomal enzyme). Soy peptide rats had lower FAS and PAP enzyme activities, and higher CPT activities. The data clearly suggest that soy peptide inhibited lipid synthesis and enhanced mitochondrial lipid degradation in the liver. In the previous study, we found that three peptides, Lys-Ala, Val-Lys, and Ser-Tyr, had TG lowering properties in HepG 2 cells[16].

To understand the effect of soy peptide on energy metabolism in obese rats, energy expenditure was studied after a 10-day feeding period. Interestingly, we found that soy peptide diet enhanced lipid oxidation rate, whereas carbohydrate and protein expenditure were reduced. Enhancement of lipid oxidation may ultimately be associated with preventing the accumulation of abdominal fat tissue and storage of body fat mass. Furthermore, the soy peptide diet suppressed protein oxidation in the body, suggesting that soy peptide has a protein-sparing action. Further studies are need to understand the physiological functions of dietary soy peptide in humans.

In conclusion, the present study indicates that dietary soy peptide decreased hepatic TG concentration and enhanced lipid expenditure in obese model rats.

5 References

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