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ANTI-OBESITY EFFECT OF PORK-LIVER PROTEIN HYDROLYSATE IN DIABETES AND OBESITY MODEL RATS

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Introduction

Obesity is defined as the accumulation of excess adipose tissue resulting from various metabolic disorders. It is a strong risk factor of hyperlipidemia, heart disease and type II diabetes mellitus, and it is associated with low capacity of insulin to regulate glucose and lipid metabolisms. Recently, much attention has been focused on several food materials such as soy protein or buckwheat protein in survey of materials to prevent obesity. Liver has been traditionally used as a food ingredient to supply several nutrients. However, limited information is available on the physiological functions of liver extract.

Objectives

The aim of this study was to investigate the efficacy of pork-liver protein hydrolysate (PLH) on body fat accumulation in Otsuka Long-Evans Tokushima Fatty (OLETF) rat that is an animal model of non-insulin-dependent diabetes mellitus and obesity.

Materials & Methods

Preparation of pork-liver protein hydrolysate(PLH)

Pork liver was cut into very small pieces and dissolved in water at 280g/ L. The pork liver solution was hydrolyzed by a proteinase at pH 7.0 and 45 ℃ for 4 h. A proteinase was added to the protein solution at the 10g/ L. After the enzyme reaction, the enzyme was inactivated by heating at 95 ℃ for 1 h and then centrifuged at 3,000 x g for 10 min. The supernatant was ultrafiltrated through a membrane which separated molecular weights below 1,000. The permeable solution was spray-dried as a PLH.

Animals and Diets

Male 4 wk old OLETF rats were obtained from Tokushima Research Institute (Otsuka Pharmaceutical Company, Tokushima, Japan) and housed individually. The

animals were fed a standard laboratory chow plus tap water ad libitum until 19 wk old and assigned to experimental groups with the same average body weight. Then, two groups of 7 rats were fed experimental diet with or without PLH, and adapted to the experimental conditions and diets from 20 to 34 wk old. Composition of the experimental diets is shown in Table 1. The rats were individually pair-fed the PLH diet and the casein diet. Food intake and body weight were measured daily. Blood samples were collected from the tail vein at 24, 28 and 32 wk old rat. After the experimental period (34 wk old), rats were killed by decapitation under light anesthesia with diethyl ether. The liver, adipose tissues such as perirenal and epididymal fat and gastrocnemius muscle were excised and immediately weighed. They were then frozen using liquid nitrogen and kept at -80 ℃ until use.

Analysis of plasma parameters

Plasma concentrations of triglyceride and free fatty acid (FFA) were measured using enzymatic kits obtained from Wako Pure Chemical Industries (Osaka, Japan). Plasma leptin and insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) kit according to the manufacture's directions (Shibayagi, Gumma, Japan and Morinaga, Yokohama, Japan, respectively).

Assays of enzyme activities

Portions of the liver from individual rats were homogenized in an ice-cooled 10 mM Tris-HCl buffer (pH 7.4) containing 0.25M sucrose and 1 mM EDTA. Cytosolic and mitochondrial fractions were prepared as described previously. The supernatant was used for the assay of glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS). G6PDH and FAS activities were assayed spectrophotometrically as described by Kelly et al. (1, 2). Activity of carnitine palmitoyltransferase I (CPT) in the mitochondrial fractions was measured using L-carnitine, palmitoyl CoA, and 2-nitrobenzonic acid according to the method of Markwell et al. (3). The protein concentration was determined according to the method of Lowry et al. (4), by using bovine serum albumin as a standard.

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 & Discussion

1. Dietary PLH suppressed the increase in body weight

Fig. 1 shows body weight of rats. During the feeding period from 20 to 34 wk old, the rats fed casein and PLH gained 191 g and 136 g, respectively. Supplementation of PLH significantly suppressed the increase in body weight compared with casein diet in 28 to 34 wk old rats. During the experimental period for 14 wks, casein and PLH diet groups consumed 2328 ± 0.1 g casein and 2319 ± 0.1 g PLH, respectively. Thus, PLH diet seems to possess the function to suppress the increase in body weight.

2. Dietary PLH significantly lowerd leptin concentration in 24, 28 and 32 wk old rats

Concentrations of plasma biochemical parameters in 24, 28 and 32 wk old rats were shown in Fig. 2. During these periods, plasma concentration of leptin was significantly lower in the PLH group compared to the casein group. Consumption of PLH tended to reduce plasma FFA and triglyceride ($P < 0.10$). There is no significant difference in plasma insulin level between the two groups. These results indicate that the PLH diet influences lipid metabolism. There are few possibilities that PLH diet enhances lipolysis of triglyceride in adipose tissue and leads to lower adipose tissue weight.

3. Dietary PLH lowerd abdominal fat pad weight, but did not affect muscle weight

The weight (g) and relative weight (%) of abdominal fat pad in the PLH group was significantly lower than those of the casein group (Table 2). The weight of gastrocnemius was unaffected by the dietary treatment. The ratio of the fat pad and gastrocnemius muscle weights in the PLH group was significantly lower than that in the casein group. It was concluded that dietary PLH lower abdominal fat pad weight but not other tissues such as muscle.

4. Dietary PLH lowerd the activities of hepatic G6PDH and FAS

The activities of hepatic G6PDH and FAS in the OLETF rats were significantly lower in the PLH group compared with the casein group (Table 3). This result indicates that consumption of PLH diet markedly suppressed hepatic activities of lipogenesis enzymes in the liver. The activity of CPT was also significantly lower in the PLH group compared with the casein group (Table 3). However, this slight reduction of hepatic CPT activity does not seem to influence. From these results, the reduction of hepatic lipogenesis by PLH diet seems to bring about anti-obesity of rats and the decrease of plasma markers such as leptin.

Conclusions

We first discovered PLH diet suppress the development of obesity in OLETF rats. The effect of PLH diet on anti-obesity appears to be, at least in part, due to lowed hepatic lipogenesis enzyme activities, especially G6PDH and FAS.

References

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Tables and Figures

Table 1 Composition of experimental diets

Ingredients	**Casein**	**PLH**
	g/ kg	
Casein[1]	230	66
PLH[2]	0	200
Corn starch	270	234
Sucrose	300	300
Cellulose powder	50	50
Soybean oil	100	100
Mineral mixture[3]	35	35
Vitamin mixture[3]	10	10
DL-Methionine	3	3
Choline bitartate	2	2

[1] Protein component of casein is 87.0% (w/w) (N X 6.25).

[2] Protein component of PLH is 77.1% (w/w) (N X 6.25).

[3] The mineral mixture and vitamin mixture are prepared according to the AIN-76 mixture.

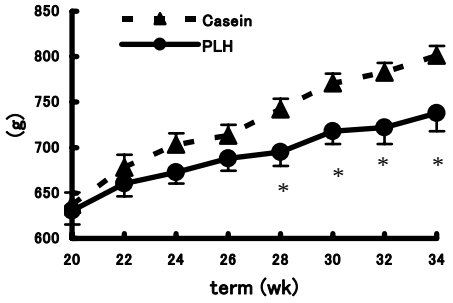

Fig. 1 Effect of dietary PLH on body weight

Table 2 Effect of dietary PLH on the weights of adipose tissue and muscle

	Casein	PLH
Fat pad (perirenal+epididymal)		
wt[a] (g)	114 ± 3	92 ± 5**
relative wt (g/ kg body wt)	139 ± 2	123 ± 3**
Gastrocnemius muscle		
wt[b] (g)	5.5 ± 0.1	5.3 ± 0.2
relative wt (g/ kg body wt)	6.7 ± 0.1	7.1 ± 0.2
Fat/ muscle (a/b)	19.7 ± 0.5	16.4 ± 0.9**

**p<0.01

Table 3 Effect of dietary PLH on the activities of hepatic lipogenesis enzymes

	Casein	PLH
G6P dehydrogenase (nmol/ min mg pritein)	95.0 ± 4.2	18.9 ± 5.2*
Fatty acid synthase (nmol/ min mg pritein)	15.6 ± 0.8	8.8 ± 1.2*
Carnithine palmitoyltransferase (nmol/ min mg pritein)	3.97 ± 0.20	3.26 ± 0.25*

*p<0.05

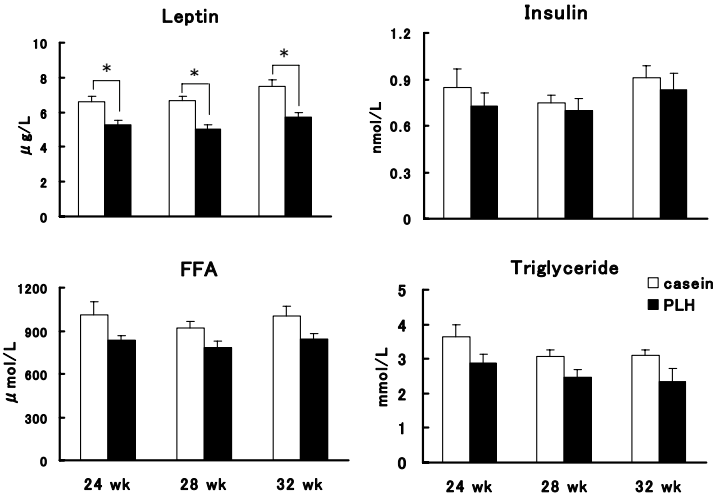

Fig. 2 Effect of dietary PLH on plasma parameters