8-P25

EFFECT OF DIETARY PROTEIN HYDROLYZATE IN LIVESTOCK PRODUCTS ON THE PLASMA AND LIVER LIPID COMPONENTS IN RATS.

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Background:
Increased caloric intake and decreased energy consumption induce obesity which causes diabetes, hepatic disease, hyperlipidemia, hypercholesterolemia and other cardiovascular diseases. Although improvement of lipid metabolism clearly prevents these diseases, it is difficult to change the dietary habits and implement a moderate exercise program. Therefore, common foods that improve the lipid metabolism would be useful. Many studies have examined the effect of various proteins on lipid metabolism and the mechanisms have been discussed. In particular, soy protein which is commonly used for healthy food in Japan was recognized as a highly hypolipidemic food material. On the other hand, few studies have investigated the hypolipidemic effects of livestock products. Beef is a nutritious protein source, but the high fat level and fatty acid composition are undesirable. Recently, it was found that the muscle components, such as carnitine and creatine, promote energy metabolism. Therefore, beef components may be useful for diseases derived from abnormal lipid metabolism.

Objectives:
This investigation was carried out to assess the usefulness of livestock products for diseases associated with abnormal lipid metabolism. The effect of cattle muscle and red blood cell hydrolyzates which were reported to be hypolipidemic materials[1,2] for lipid metabolism was studied in rats fed a high cholesterol diet.

Methods:
Materials: Cattle round muscle, cattle heart muscle and red blood cells were hydrolyzed by protease (Alcalase 2.4L, Novo Co. Ltd.) and the water soluble fraction was extracted. The cells were spray-dried to obtain feed materials as a powder form. Soy protein was purchased by Fuji oil Co. Ltd.. Casein was purchased by Meiji Milk Products Co. Ltd..
Animal: Male Wistar strain rats (Japan SLC Inc., Hamamatsu, Japan) weighing about 90g were used. Room temperature was maintained at 22±2℃ with a 12hr cycle of light (08:00-20:00) and dark. All the rats were individually housed and provided with food and water *ad libitum*. The rats were fed commercial stock diet (CE-2, Japan, CLEA Co. Ltd., Tokyo) for 3 days to allow them to adapt to the new environment, after which they were fed an experimental diet containing 0.5% cholesterol for 10 days. Rats were grouped into 4 experimental groups; group supplied with 20% casein diet (I), group supplied with I + 5% soy protein diet (II), group supplied with I + 5% cattle round muscle hydrolyzate diet (III), group supplied with I + 5% cattle heart hydrolyzate diet (IV) and group supplied with I + 5% cattle red blood cell hydrolyzate diet (V). After experimental diet feedings, the rats were fasted for 24hr (09:00-09:00), and then killed within a short period. The livers were immediately excised to analyze liver lipids. Blood was collected by heparinized cardiac puncture to analyze of plasma lipids.
Analytical procedure: Plasma and liver cholesterol levels were measured by an enzymatic colorimetric method (Monotest Cholesterol, CHOD-PAP method, Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo). The plasma concentrations of high-density lipoprotein (HDL) cholesterol were assayed with a commercially available kit (HDL cholestase Nissui Co. Ltd., Tokyo), and plasma and liver triglyceride levels were also assayed with a commercially available kit (Triglycolour III, Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo). Plasma phospholipid was assayed with a commercially available kit (Phospholipid-Test; Wako Pure Chemical Ind., Ltd., Osaka) by a method described by Zilversmit and Davis.[3] Liver lipids were extracted by the method of Folch et al.[4] Liver phospholipid levels were calculated from total liver lipids minus the liver cholesterol and triglyceride.
Statistical analysis: All analytical data were evaluated and compared to those of group I by the Student's t-test[5].

Reasult and discussion:
Food consumption was significantly decreased in group IV and relative liver weight was significantly lower in group II. Plasma total cholesterol, LDL+VLDL-cholesterol concentration and plasma HDL-cholesterol / total cholesterol ratio were significantly improved in groups II and V. However, the plasma HDL-cholesterol concentration was significantly decreased in group

II and significantly increased in group V. In groups II, III and IV, the plasma phospholipid concentration was significantly decreased. Liver total lipid and phospholipid were significantly decreased in groups II, III and IV. In addition, liver triglycerol was significantly decreased in group III and liver total cholesterol was significantly decreased in group IV.

Soy protein feeding generally showed a higher hypolipidemic effect than casein feeding. Several mechanisms have been proposed, including effects on intestinal absorption of cholesterol, fecal bile acid excretion and hormonal change due to differences in amino acid compositon. In this study, the hypolipidemic effect of soy protein feeding was observed in the plasma and liver lipid components. On the other hand, the hypolipidemic effect of cattle muscle hydrolyzate feeding was detected in the liver lipid component alone and red blood cell hydrolyzate feeding was found in the plasma lipid component alone. It is difficult to completely explain the mechanisms of these effects, but several hypotheses could be based on differences in amino acid composition have been proposed. For example, the hydrophobic amino acid composition of red blood cells might cause increased fecal steroid losses. In rats fed cattle muscle hydrolyzate, their high arginine content might influence the hormonal regulation. Further study is necessary to clarify the mechanisms of these effects to determine the usefulness of livestock products as food material.

Conclusions:

Cattle muscle hydrolyzates may be useful as materials to prevent or improve hepatic diseases. On the other hand, red blood cell hydrolyzates may be useful to prevent or improve cardiovascular diseases. These hydrolyzates may reduce the risk factors of hepatic disease and cardiovascular disease in the future.

Pertinent literature:

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Table Effects of dietary protein supplementation on body and liver weight, food intake, serum and liver lipid components distribution in rats.

Group	C	S	CR	CH	R
Body weight gain (g/10days)	24.40±1.08	24.57±1.37	27.10±1.23	27.32±2.27	25.35±3.34
Liver weight (g/100g body weight)	4.22±0.08	3.96±0.07*	4.05±0.07	4.06±0.06	4.17±0.07
Food intake (Day 6)	14.23±0.40	13.00±0.46	13.55±0.45	12.21±0.41**	12.94±0.69
Serum cholesterol (mg/dl) (a)	142.27±6.37	115.21±4.01**	126.26±6.76	114.36±12.41	111.04±7.01**
HDL-cholesterol (mg/dl) (b)	32.23±1.29	30.75±1.85**	30.07±2.42	30.81±2.66	34.00±2.56**
LDL+VLDL-cholesterol (mg/dl)	110.04±6.15	84.46±3.02**	96.19±7.55	83.55±12.20	77.05±7.23**
(b)/(a)	0.23±0.01	0.27±0.01*	0.24±0.02	0.28±0.04	0.31±0.03*
Serum triacylglycerol (mg/dl)	81.13±9.43	64.15±5.49	87.11±9.44	68.24±9.05	68.87±3.92
Serum phospholipid (mg/dl)	120.71±5.64	100.43±3.14*	107.00±2.23*	96.29±8.85*	106.57±4.58
Total lipid (mg/g liver)	128.22±5.23	111.14±1.84*	110.86±1.30**	109.25±4.12*	115.94±5.55
Triacylglycerol (mg/g liver)	27.16±2.67	20.72±1.85	20.52±1.26*	23.84±2.38	24.45±3.52
Phospholipid (mg/g liver)	70.76±3.72	60.65±1.65*	60.06±2.60*	60.20±1.80*	62.81±3.24
Cholesterol (mg/g liver)	30.30±1.64	29.77±0.97	30.29±1.31	25.20±1.23*	28.45±1.92

C:20% casein
CR:20% casein + 5% cattle round hydrolyzate
CH:20% casein + 5% cattle heart hydrolyzate
S:20% casein + 5% soy protein
CH:20% casein + 5% cattle red blood cell hydrolyzate
Means±SEM.
Stastical significance compared with control (C) group (* : $p<0.05$, ** : $p<0.01$).