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PAPAIN-DIGESTED PORK REDUCES SERUM CHOLESTEROL LEVELS IN RATS

Kohji URASHIMA[1], Yasuki TAGUCHI[1], Fumiki MORIMATSU[1], Yuji FURUKAWA[2] and Ryoji YAMADA[1]

[1] Research and Development Center, Nippon Meat Packer, Inc., Tsukuba, Ibaraki 300-2646; and
[2]Department of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Miyagi 981-0914, Japan

Background:
Dietary protein is one of the important determinants of the serum cholesterol level. It has been reported that animal protein induces a higher serum cholesterol level than dose vegetable protein. Especially, soy protein isolate (SPI) is well known to induce a lower cholesterol level than dose casein, a representative of animal proteins. However, only a few studies have been carried out to examine for the effects of pork meat protein on the serum cholesterol level.

Objective:
The objective of this study was to examine the effects of pork meat protein and its enzymatically digested products on the serum cholesterol concentration.

Materials and methods:
Preparation of pork meat protein. Pork meat (PM) protein was prepared by defatting minced *Muscuius longissimus dorgi* with n-hexane. PM suspended in four volumes (v/w) of water was digested with papain (0.25 % of PM) at pH 6.0 and 50℃ for 24 hours and heated for 60 min at 90℃ to inactivate the enzyme. The digested pork meat protein was separated into soluble and insoluble fractions by centrifugation at 5,000 x *g* for 20 min. The sediment, the insoluble fraction (ISF), was washed several times and lyophilized. The filtrate was subjected to ultrafiltration with a membrane which separates molecular weights lower than 150,000 from those higher than 150,000. The permeable fraction was lyophilized (low molecular weight fraction ; LMF). The yields of ISF and LMF were 50.3 % and 46.1 %, respectively.
Animals and diets. Six-week-old male Sprague-Dawley rats (Funabashi Farm Inc., Chiba, Japan) were used in the experiments. After the rats had been fed on a commercial diet (type F2, Funabashi Farm Inc., Chiba, Japan), they were divided randomly into five groups. Each group consisted of seven rats with body weights approximately 80 g. Each group was given *ad libitum* a different diet containing 1% cholesterol and one of different proteins (20 %), PM, ISF, LMF, or SPI, for 21 days. At the experiment, the rats were sacrificed by withdrawing blood from the abdominal aorta under ether anesthesia after overnight (20:00-08:00) fasting . The liver was excised immediately.
Lipid analysis. Total and free cholesterol concentrations of the serum samples were analyzed by enzymatic assay with kits purchased from Wako Pure Chemical Industries. Liver lipids were extracted by the method of Folch et al. [1] and the cholesterol concentration was measured [2]. Serum lipoproteins were separated by ultracentrifugation [3]. The cholesterol concentration of each lipoprotein was analyzed by an enzymatic method [4].
Measurement of ^{14}C excretion after oral administration of [^{14}C] -cholesterol. To study the effects of pork meat protein in the digestive tract, the rats were fed PM or LMF and [4-^{14}C] -cholesterol was administrated. After oral administration, feces were collected for 2 days and radio activity of ^{14}C in the feces was measured with a scintillation counter.

Results and discussion:
Serum and liver cholesterol concentrations and the cholesterol percentage of each lipoprotein fraction of the serum are shown in Table 1. LMF exerted a hypocholesteromic effect more markedly than did PM. The cholesterol level of the rats fed on LMF was significantly lower than those fed on SPI. With the LMF group, cholesterol concentrations of chylomicron, VLDL and LDL decreased, whereas that of HDL increased. Thus, LMF of papain-digested pork meat proteins suppressed serum and liver cholesterol concentrations more markedly than did PM.
Generally, two hypotheses may explain the hypocholesteromic effects of concerned proteins: [1] amino-acid composition of the protein [5,6] and [2] behavior of the protein in the digestive tract [7], *i.e.*, digestibility of the protein and physicochemical properties of the digested products.
In this case, the former was denied, because no significant difference in amino-acid composition was observed between LMF and PM. However, the rats fed on LMF excreted larger quantities of ^{14}C cholesterol than did those fed on PM (Fig. 1), suggesting that LMF prevented absorption of cholesterol from the digestive track and reduced the serum cholesterol level.

Conclusion:

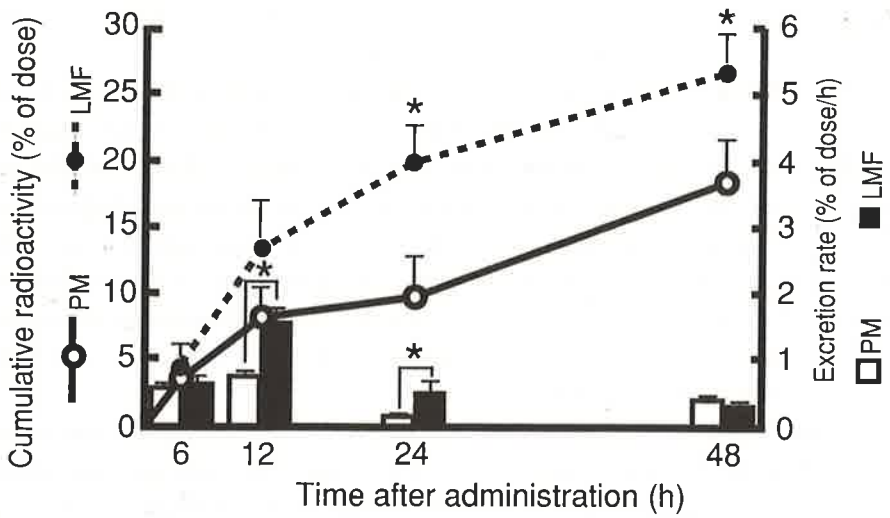
PM has a hypocholesteromic activity as does SPI, which is well known to reduce the serum cholesterol level. The activity of PM was enhanced by papain digestion. The increase in the activity may be related to LMF's peculiar behavior in the digestive tract: LMF may suppress more strongly the absorption of cholesterol.

Table 1. Cholesterol concentrations of the serum, liver and lipoprotein of rats fed different nitrogen sources for 21 days

	Dietary group			
	PM(7)	ISF(7)	LMF(7)	SPI(7)
Serum				
Total cholesterol (mg/dl)	277 ± 21^{a}	238 ± 13^{a}	117 ± 10^{b}	228 ± 12^{a}
Free cholesterol	31.3 ± 2.4^{a}	26.9 ± 2.5^{a}	15.6 ± 1.3^{b}	28.4 ± 1.9^{a}
Lipoprotein				
Chylomicron (% of cholesterol)	43.9 ± 1.1^{a}	43.3 ± 1.6^{a}	37.6 ± 1.0^{b}	43.5 ± 1.1^{a}
VLDL	28.0 ± 0.9^{a}	23.1 ± 0.8^{b}	13.2 ± 0.6^{c}	28.4 ± 0.8^{a}
LDL	12.3 ± 0.6^{a}	12.9 ± 0.6^{a}	7.5 ± 0.3^{c}	9.1 ± 0.4^{b}
HDL	13.8 ± 0.5^{c}	18.0 ± 0.6^{b}	39.4 ± 1.0^{a}	16.0 ± 0.5^{b}
Liver				
Total cholesterol (mg/g tissue)	68.4 ± 1.9^{a}	50.7 ± 1.6^{b}	24.1 ± 1.8^{c}	47.3 ± 1.8^{b}

Each value is indicated as ± S.E. Values with the same superscript in the same horizontal row are not significantly different at $p<0.01$. The number of rats examined are indicated in parentheses.

Figure 1. Cumulative ^{14}C excretion and ^{14}C excretion rate in feces after oral administration of [4-^{14}C] cholesterol to rats.



Pertinent literatures:

1) Folch, J.,Lee, M., Stanley,G.H.S. (1957): *J. Biol. Chem.*,**226**, 497-509.

2) Cho, B. H. S. (1983): *Clin. Chem.*,**29**, 166-168.

3) Havel, R. J., Eder, H. A., Bragdon, J. H. (1955): *J. Clin. Invest.*,**34**, 1345-1353

4) Richmond, W. (1973): *Clin. Chem.*,**19**, 1350-1356

5) S'anchez, A., Rubano, D. A., Shavlik, G. M., Hubbard, R., Horning, M. C. (1988): *Arch. Latinoam. Nutr.*,**38**, 229-238.

6) Tanaka, k., Sugano, M. (1989): *J. Nutr. Sci. Vitaminol.*, **35**, 323-332.

7) Terpstra, A. H. M., Sanchez-Muniz, F. J. (1981): *Atherosclerosis*, **43**, 381-391.