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Cholesterol-lowering activity of linear- and gel-type sodium polyacrylate as dietary fiber models

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Effects of linear- and gel-type (cross-linked) sodium polyacrylate and partially desalted sodium polyacrylates on serum cholesterol levels were investigated in rats fed cholesterol-free diets. Gel-type sodium polyacrylate had the lowest serum cholesterol concentration among the experimental groups. In the case of partially desalted gel-type sodium polyacrylate, the serum cholesterol-lowering effect was not found. It was thought that the water-holding capacity caused the cholesterol-lowering activity. The linear-type sodium polyacrylate and partially desalted ones reduced the serum cholesterol levels significantly ($P < 0.05$). The effects of the degree of polymerization of linear-type sodium polyacrylates on serum cholesterol levels were observed, but the differences among them were not significant ($P < 0.05$). In the case of linear-type sodium polyacrylate, their viscosity was more effective on cholesterol lowering than water-holding capacity was. Diets of 1% sodium polyacrylates did not approximately affect the body weight gain and visceral organ weight. (J. Nutr. Biochem. 8:351–354, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Numerous studies have demonstrated that certain fibers have a variety of effects on gastrointestinal function, stool weight, fecal constituents, bacterial population, the enterohepatic circulation, and bile. There are also effects on the cholesterol-lowering in animals of soluble and insoluble dietary fibers.[1–11] In human studies, consumption of certain sources of dietary fiber lead to beneficial treatment of hypercholesterolemia and possibly in preventing coronary heart disease.[12–15]

Although large number of studies have been reported on cholesterol-lowering activity of dietary fibers, it is not likely to be sufficient to explain completely what kind of critical chemical and/or physical properties of dietary fibers are responsible for the effect, because of the complex structure of dietary fibers. If we use different kinds of tailor-made synthetic polymers as model compounds of dietary fibers, we could get information more easily about the relationship between chemical or physical properties of the polymers and their physiological effects.

From this point of view, we chose linear-type sodium polyacrylate (LPNa) as a model of soluble dietary fiber, which is being used as a food additive in Japan, and gel-type sodium polyacrylate (GPNa) as a model of insoluble ones. A few studies have been reported concerning the effects of LPNa on physiological function in animals. A diet containing 0.1 to 0.5% LPNa was fed to swine and its anti-ulcer effect was reported by Yamaguchi et al.[16] Tsuji and coworkers investigated the effect of synthetic polymers such as LPNa, polyethylene glycol, and polyvinyl alcohol on serum cholesterol in rats fed a diet containing cholesterol but did not find any serum cholesterol-lowering activity.[17]

In the present study, the effects of sodium polyacrylate (LPNa and GPNa) and partially desalted sodium polyacrylate (LPNa-D and GPNa-D) on serum cholesterol level were investigated in rats fed cholesterol-free diets.

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Table 1 Composition of experimental diets (in percent)

Ingredients	Control (cellulose)	LPNa	LPNa-D	GPNa	GPNa-D
Polymers	5	1	1	1	1
Casein	20	20	20	20	20
Mineral mix[1]	5	5	5	5	5
Vitamin mix[2]	2	2	2	2	2
Corn oil	5	5	5	5	5
Corn starch	63	67	67	67	67

[1]Mineral mixture (%): $CaHPO_4 \cdot 2H_2O$, 14.56; KH_2PO_4; NaH_2PO_4, 9.35: NaCl, 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; $MgSO_4$, 7.17; $ZnCO_3$, 0.11; $MnSO_4 \cdot 4H_2O$, 0.12; $CuSO_4 \cdot 5H_2O$, 0.03; KI, 0.01. (Mineral mixture was modified from McCulum salt.)
[2]Vitamin mixture (in 100 g): vitamin A acetate, 17.2 mg; vitamin D_3, 250 μg; vitamin $B_1 \cdot HCl$, 120 mg; vitamin B_2, 400 mg; vitamin $B_6 \cdot HCl$, 80 mg; vitamin B_{12}, 0.05 mg; vitamin C, 3000 mg; vitamin E, 500 mg; vitamin K_3, 520 mg; biotin, 2 mg; folic acid, 20 mg; Ca pantothenate, 500 mg; p-aminobenzoic acid, 500 mg; nicotinic acid, 600 mg; choline chloride, 20 g. (The vitamin supplement was custom-mixed by Oriental Yeast Co., Ltd.)

Methods and materials

Polymers

LPNa was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and purified by reprecipitation from water/ethanol and freeze-drying from distilled water. LPNa with different degrees of polymerization (7200, 15000 to 20000, and 22000 to 66000) were used (abbreviated as l-, m- and h-LPNa, respectively). GPNa (Sunwet-IM 5000) was commercially supplied by Sanyo Chemical Industry, Ltd. and used after washing with distilled water and freeze-drying. Partially desalted sodium polyacrylates (l-, m- and h-GPNa-D and GPNa-D) were prepared by dialysis of l-, m- and h-LPNa and GPNa using a cellulose membrane.

Animals

Male Sprague-Dawley rats were obtained from a commercial supplier. They were housed individually in cages with wire mesh bottoms in a room kept at 24 ± 1°C and with a 12 hr light/dark cycle. Vivarium was lit between 21:30 and 09:30. The animals had access to tap water. Body weight and food intake were determined every day.

Diets and feeding protocol

The composition of the experimental diets is given in *Table 1*. The animals were randomly allocated to test groups of six animals each. One group received the diet with 5% cellulose as a control group. The other groups were the basal diets to which 1% (wt/wt) polymers had been added. The animals were given ad libitum access to food for 4 weeks.

Blood and tissue samples

At the end of the experimental diet period, food was removed at 10:00, 24 hr before killing. Water was provided ad libitum after food removal. The animals were anesthetized with pentobarbital. The peritoneal cavity was opened rapidly, and blood was collected from trunk blood into a centrifuge tube and kept on ice until serum was separated by centrifugation (3000 rpm, 0°C, 15 min.). Serum was stored at −20°C until later biochemical determinations. After blood sampling, kidneys, liver, spleen, and testis were removed. Adherent fat and mesentery were detached and the organs were washed and blotted dry with paper tissue before weighing.

Table 2 Effect of sodium polyacrylates on body weight gain (g)[1]

Week	Control	m-LPNa	m-LPNa-D	GPNa	GPNa-D
1st	7.8 ± 0.3[a]	6.1 ± 0.3[b]	6.4 ± 0.3[b]	5.5 ± 0.5[b]	5.5 ± 0.2[b]
2nd–4th	7.6 ± 0.3	7.5 ± 0.3	7.8 ± 0.2	7.4 ± 0.1	7.6 ± 0.2
1st–4th	7.7 ± 0.3	7.1 ± 0.3	7.4 ± 0.2	7.0 ± 0.1	7.1 ± 0.2

[1]Values are means ± SEM (n = 6). Values of body weight gain within a row with different superscript letters are significantly different from each other ($P < 0.05$).

Cholesterol analysis

Concentration of serum total cholesterol was determined with a Wako Cholesterol Diagnostic Kit (T-Cho I C II, Wako Pure Chemical Industries, Ltd.). The assay measures quinoneimine dye produced enzymatically by peroxidase from hydrogen peroxide that is generated enzymatically by cholesterol oxidase from free cholesterol. The sample is treated initially with cholesterol esterase to convert all cholesterol esters to free cholesterol.

Statistics

All statistical analyses were performed by one-way analysis of variance (ANOVA) using SPSS/PC + (SPSS Japan, Inc.). Means were considered to be significantly different for $P < 0.05$ as determined by the Duncan test for multiple comparisons.

Results and discussion

Growth and visceral organs

Data on body weight gain of rats fed diets of 1% of the polymers (GPNa, LPNa, GPNa-D and LPNa-D) are summarized in *Table 2*. At the end of the first week, the weight gain in the rats was significantly lower than that in those fed the control diet ($P < 0.05$). No significant differences in the body weight gain was found over the experimental period from the second to fourth week and the first to fourth week. It was obvious that the rats became familiar with the polymers after 1 week of feeding.

Table 3 shows the relative weight of visceral organs to body weight. Except the spleen, there was no difference between the relative weight of visceral organs to body weight. The relative weight of spleen to body weight in rats fed m-LPNa was slightly smaller ($P < 0.05$) than that in those fed the control diet. In the case of relative weight of kidneys, liver, and testis, there was no difference between

Table 3 Effect of the polymers on weight ratio[1] of visceral organs to body weight (in percent)

Groups	Kidneys	Liver	Spleen	Testis
Control	0.83 ± 0.05	3.22 ± 0.16	0.30 ± 0.02[a]	1.11 ± 0.05
m-LPNa	0.78 ± 0.02	3.08 ± 0.07	0.23 ± 0.01[b]	1.17 ± 0.05
m-LPNa-D	0.84 ± 0.04	3.17 ± 0.11	0.29 ± 0.02[a]	1.11 ± 0.05
GPNa	0.79 ± 0.02	3.64 ± 0.44	0.28 ± 0.02[a]	1.25 ± 0.13
GPNa-D	0.78 ± 0.03	3.50 ± 0.17	0.29 ± 0.02[a]	1.03 ± 0.03

[1]Values are means ± SEM (n = 6). Values within a column with different superscript letters are significantly different from each other ($P < 0.05$).

Table 4 The effect of the polymers on serum cholesterol level

Ingredients	Serum cholesterol[1] (mmol/L)
Control	2.94 ± 0.23[a]
l-LPNa	2.37 ± 0.33[b]
m-LPNa	2.25 ± 0.24[b]
h-LPNa	1.97 ± 0.09[bc]
l-LPNa-D	1.94 ± 0.08[bc]
m-LPNa-D	1.97 ± 0.09[bc]
h-LPNa-D	2.16 ± 0.12[b]
GPNa	1.58 ± 0.12[c]
GPNa-D	2.54 ± 0.23[a]

[1]Values are means ± SEM (n = 6). Values with different superscript letters such as the combination of a and b or a and bc, are significantly different from each other ($P < 0.05$) but not in case of the superscript containing the same letter such as b and b or b and bc.

rats fed the polymers and the control diet ($P < 0.05$). From the above results, it was concluded that diets of 1% sodium polyacrylate did not approximately affect the body weight gain and the visceral organ weight.

Cholesterol-lowering effect

We chose LPNa as a model of soluble dietary fiber and GPNa as that of an insoluble one. The effects of LPNa, GPNa, LPNa-D, and GPNa-D on serum cholesterol levels in rats fed cholesterol-free diets were investigated. To know the effects of dietary fibers on cholesterol metabolism, we used cholesterol-free diets according to the method of Arituka and coworkers.[18]

The effect of the synthetic high polymers on serum cholesterol levels were summarized in *Table 4*. The cholesterol levels for l-, m- and h-LPNa were significantly lower than that of the control ($P < 0.05$). Tsuji and coworkers investigated the effect of LPNa on serum cholesterol in rats fed a diet containing cholesterol, but did not find any cholesterol-lowering activity.[17] As we used cholesterol-free diets in this work, our result for LPNa could not be compared with that of Tsuji's directly.

The serum cholesterol levels of LPNa decreased with the increasing the degree of polymerization, but the differences between l-, m- and h-LPNa were not statistically significant ($P < 0.05$). The effect of the degree of polymerization on serum cholesterol is likely because of the increase in viscosity. The intake of LPNa makes the contents in the intestinal tract viscous. It is thought that the viscous condition may prevent the diffusion of cholesterol from the intestinal lumen to the absorptive surface of the enterocytes.

Although the serum cholesterol concentrations of the partially desalted LPNa were significantly lower than that of the control ($P < 0.05$), but the differences between l-, m-, and h-LPNa-D were not significant ($P < 0.05$). The values of l- and m-LPNa-D are lower than those for l- and m-LPNa, respectively. Partial desalting of LPNa leads to a reduction in solubility in an aqueous solution because of the conversion of the functional group from -COONa to -COOH. In the case of l- and m-LPNa-D, the conversion made the contents in the intestinal tract more viscous and reduced the serum cholesterol concentration.

On the other hand, the serum cholesterol concentrations for h-LPNa-D were larger than that for h-LPNa although those for l- and m-LPNa-D are lower than those for l- and m-LPNa, respectively. The differences among them were not statistically significant ($P < 0.05$). These conflicting findings may result from the lack of difference in viscosity of both polymers (h-LPNa and h-LPNa-D). It is possible that desalting h-LPNa does not lead to an increase in viscosity because h-LPNa is originally highly viscous. The conversion of the functional groups from -COONa to -COOH may also affect the serum cholesterol concentration for desalted LPNa. These may be the reasons why the conversion from l- and m-LPNa to l- and m-LPNa-D leads to lower cholesterol levels, but desalting h-LPNa leads to an increase in cholesterol. However, the authors recognize that the above mentioned discussion is limited by the fact that the difference between the serum cholesterol levels for LPNa and LPNa-D was not significant ($P < 0.05$).

The lowest value of serum cholesterol levels was obtained in the rats fed GPNa. It was significantly lower than those of the control and the other polymers ($P < 0.05$). On the contrary, the serum cholesterol-lowering effect was not found in the case of GPNa-D.

It has been reported that, generally, insoluble nonviscous fibers such as cellulose or wheat bran are relatively ineffective in lowering serum cholesterol levels.[19] The cholesterol lowering activity of GPNa, which belongs to insoluble nonviscous type dietary fiber, was larger than that for the control (cellulose). The cholesterol level of GPNa-D was close to that of the control. The difference in the three dimensional structure of GPNa and GPNa-D might affect serum cholesterol levels. GPNa swells well in water or in an aqueous solution. The water-holding capacity for GPNa is extremely high, 413 mL/g polymer. GPNa-D has a shrink-structure in an aqueous solution, because the loss of sodium cation reduces the repulsion of carboxy anions ($-COO^-$). The water-holding capacity for GPNa-D is 2 mL/g polymer.

From the above facts, it was thought that the water-holding capacity of GPNa play an important role on serum cholesterol levels. GPNa has quite a high water-holding capacity. It seems that the block of GPNa, swollen by its absorption of a large amount of water, prevents the diffusion of cholesterol from the intestinal lumen to the absorptive surface of the enterocytes. The water-holding capacity of GPNa-D (2 mL/g) was one 200th of that of GPNa. This was the reason why GPNa-D had no cholesterol-lowering ability.

In the case of LPNa, the amount of bonded water is about 0.5 mL/g and the water absorption (water-holding) is negligible. It was thought that the viscosity of LPNa was more effective on cholesterol lowering than water-holding capacity was.

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