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Effects of Dietary Pectin, Tangerine Pulp Meal, Propionate, Lactate or Fumarate on Serum and Liver Cholesterol Levels, and Dietary Pectin on Cholesterol Absorption in Rats*

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ABSTRACT

The effect of dietary pectin, tangerine pulp meal, propionate, lactate or fumarate on cholesterol (C) and triacylglycerol(TG) levels in the serum and liver, and the effect of pectin on dietary C absorption were studied in a series of three experiments. Mature female Sprague Dawley rats were fed a control diet or diets containing 5% pectin, 5% tangerine pulp meal, 3% propionate, 3% lactate 3% fumarate, or 10% pectin. Serum total C levels were lower($p<0.05$) in rats fed the diet containing 5% pectin than in control rats after a 4-week feeding period(93.8 vs 119.2mg/100mL). Serum HDL, LDL+VLDL C levels were not different among diet groups. Liver total C level was also lower($p<0.05$) in rats fed the diet containing 5% pectin than in control rats, but liver TG level was not influenced by diet. Dietary propionate, lactate or fumarate did not reduce serum C, indicating that propionate is not a regulator of serum C. However, dietary pectin(10%) increased fecal excretion of dietary C(or its metabolites) more than 70% over a control value. Our data indicate that dietary pectin reduces serum and liver C levels by increased fecal secretion of dietary C, but not by its fermentation product propionate or other gluconeogenic substrates. (*Korean J Nutrition* 31(5) : 914~920, 1998)

KEY WORDS : rats · cholesterol · pectin · propionate · fumarate · lactate.

Introduction

Cholesterol has long been known as a risk factor for cardiovascular diseases, and many investigators have attempted to reduce cholesterol in the body by dietary manipulations. Soluble fiber(such as that in pectin, guar gum, psyllium, oat bran and barley) has been shown to decrease plasma cholesterol levels in experimental animals[1] and also in humans.[2] Among soluble fiber sources, pectin invariably has had a more pronounced effect on lowering the serum cholesterol level in animals[3,4] and humans[5].

The presence of soluble fiber in the gastrointestinal tract is known to increase viscosity and as a result to interfere with micelle formation and lipid absorption[6]. This effect of soluble fiber was achieved by only moderate increases in viscosity[7]. In addition, certain fiber sources were suggested to bind or adsorb bile acids and neutral sterols, enhancing their removal from enterohepatic circulation[8].

Propionate, a fermentation product of soluble fiber has been reported to reduce blood cholesterol level, perhaps by inhibiting endogenous cholesterol synthesis in the liver[9,10]. These findings suggest that the hypocholesterolemic effect of dietary soluble fiber is mediated through the fermentation product propionate. However, other studies[11,12] found no hy-

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pocholesterolemic effect of propionate.

The present study was carried out to evaluate the effect of dietary pectin, tangerine pulp meal, propionate, fumarate and lactate on serum and liver cholesterol or triacylglycerol levels in rats. (These organic acids are good substrates for gluconeogenesis and are often used as growth promotants for young pigs.) Particularly, our study was done to explore tangerine pulp meal, which is a byproduct of tangerine juice manufacture and becomes a polluting waste, as a possible cholesterol-lowering food additive.

Materials and Methods

1. Animals and diets

Adult female Sprague Dawley rats(Korea Institute of Chemistry, Tae-Jun, Korea) were housed individually in suspended wire cages in a room maintained at 20-25℃ with a 12-hr light(0700 to 1900) and dark(1900 to 0700) cycle. Twenty-one adult female rats(219g) were divided into three groups, and each group was fed a control diet or diets containing 5% pectin or 5% tangerine pulp meal in experiment 1. For the other two experiments, a group of 5 rats (130g for rats used in experiment 2 or 230g in experiment 3) was fed a control diet or diets containing 3% disodium propionate, 3% calcium lactate or 3% hemicalcium fumarate(experiment 2), and a control or a diet containing 10% pectin(experiment 3). (These acids were used because of their gluconeogenic nature.) The composition of the experimental diets is shown in Table 1.

Diets and water were provided for ad libitum consumption. A 3-day adjustment period was allowed before rats were fed experimental diets for four weeks in experiments 1 and 2. At the end of experiments 1 and 2, rats were fasted for 16 hours and killed by decapitation, and blood and liver samples were collected. Serum was obtained from the blood which was centrifuged(2,200×g) for 10 min. Liver samples were weighed and frozen at −20℃ until analyzed in experiment 1.

In experiment 3, which was done to determine the effect of dietary pectin on dietary cholesterol absorption, rats were fed a control diet or a diet containing 10% pectin for three days and fasted for 16 hours prior to being fed 3 g of their respective diet labeled with ^{14}C-cholesterol. The diets were made into slurry by adding 0.2μCi[4-^{14}C]cholesterol (Amersham International Plc, Buckinghamshire, England) which was premixed with 20μl corn oil, and also by adding appropriate amounts of water. These slurry

Table 1. Composition of experimental diets(%)

Ingredient	Control[1]	Exp 1(Exp 3)		Exp 2
		Pectin[2]	Tanger[3]	Organic acid[4]
Casein	20.0	20.0	20.0	20
L-Methionine	0.3	0.3	0.3	0.3
Lard	10.0	10.0	10.0	10.0
Sucrose	10.0	10.0	10.0	10.0
Cholesterol	0.5	0.5	0.5	-
Vitamin mix[5]	1.0	1.0	1.0	1.0
Mineral mix[5]	3.5	3.5	3.5	3.5
Choline chloride	0.5	0.5	0.5	0.5
Pectin		5.0(10)		
Tangerine pulp meal[6]			5.0	
Organic acid[4]				3.0
Corn starch	54.2	49.2(44.2)	49.2	51.7

a : Control diet was used for experiments 1, 2 and 3, but in experiment 2, 0% cholesterol and 54.7% corn starch were used instead of 0.5% cholesterol and 54.2% corn starch, respectively

2 : Diet used for exp 3 contained 10% pectin and 44.2% corn starch

3 : Waste product from a local tangerine juice manufacturer, which contained 30% pectin on the dry matter basis(thus the diet contained 1.5% pectin)

4 : Organic acid was sodium propionate, hemicalcium fumarate or calcium lactate, purchased from Sigma Chemical Co. (St. Louis, MO, USA)

5 : AIN 76

diets were fed through a stomach tube, and feces were collected daily for two days after the labeled diet was fed.

2. Analysis of cholesterol and triacylglycerol in the serum

Total cholesterol, high-density lipoprotein(HDL) cholesterol and triacylglycerol concentrations in the serum were determined using commercial assay kits (International Reagent Corp., Tokyo, Japan for the former, and WAKO Pure Chemical Ind., Osaka, Japan for the lattertwo) according to the manufacturer's instruction. Low-density lipoprotein(LDL)+very low-density lipoprotein(VLDL) cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

3. Analysis of cholesterol and triacylglycerol in the liver

Liver samples from rats used in experiment 1 were prepared by modification of the method described in De Hoff, et al.[13] to determine cholesterol and triacylglycerol contents. Liver tissue(1g) was homogenized in 6 mL of chloroform/methanol mixture(2/1 by volume) and 2mL of distilled water using a tissue homogenizer. Chloroform fraction was located under the methanol+water fraction when centrifuged. A 0.5-mL aliquot was taken from the chloroform fraction (assuming that total volume was 4mL) and dried under flowing nitrogen gas. To the dried sample, a 1 : 1 Triton X-100/chloroform solution (50μL, roughly six times the weight of the dried residue) was added. The mixture was diluted to 0.5 ml with chloroform and thoroughly mixed. Then a 50-μL sample was transferred to a test tube and the chloroform was evaporated under nitrogen. Total cholesterol concentrations were determined as described for determination of the serum cholesterol.

The method used for extracting liver cholesterol was checked by measuring radioactivity recovered in chloroform fraction after a known amount of ^{14}C-cholesterol was added to a 1-g liver sample. The radioactivity in the chloroform fraction(1mL) was determined using a liquid scintillation counter(Wallac Dy, Turku, Finland) after 15 mL of Biosafe II (Research Products International Corp. IL, USA) was added and the dpm value was calculated using an internal standard(14C-toluene). All of the radioactivity added to the liver sample was recovered in 4-mL chloroform fraction.

To determine liver triacylglycerol concentrations, a 20-μL aliquot of the chloroform fraction remaining from the total cholesterol assay was taken and dried under nitrogen. The residue was dissolved in 100-μL methanol, and triacylglycerol was determined as described above for the serum triacylglycerol assay.

4. Determination of dietary cholesterol absorption

Fecal excretion of radioactivity from diets labeled with ^{14}C-cholesterol was used to determine absorption of cholesterol and its metabolites from the gastrointestinal tract. Feces were collected every 24 hours for two days after feeding the labeled diet, dried and weighed. Dried feces were ground in glass scintillation vials with a small pestle. The radioactivity in fecal samples was determined as described above in the cholesterol recovery test for liver sample preparation.

5. Statistical analysis

Data were analyzed by the analysis of variance or the Student t-test.[14] The main source of variation for all variables was dietary treatments in the analysis of variance. When the F-value in the analysis of variance was significant, the Newman-Keuls test was used to compare individual means[15].

Results

Serum total cholesterol levels were significantly($p<0.05$) lower in rats fed the pectin diet(81.9mg/100mL) than in control rats(119.2mg)(Table 2). Dietary tangerine pulp meal (containing 30% pectin and 15% crude fiber on a dry matter basis) tended to decrease the serum cholesterol level, as compared to a control value(93.8 vs 119.2mg/100mL serum), but the difference was not significant($p=0.05$) due to large individual variations. HDL-cholesterol levels or the ratios of HDL-cholesterol to total cholesterol, and triacylglycerol levels in the serum were not affected by diet(Table 2).

Liver total cholesterol levels(14.2mg/g liver) in control rats were higher($p<0.05$) than in rats fed the pectin diet(7.94mg/g)(Table 2). Dietary tanger-

Table 2. Effect of diets containing 5% pectin or tangerine pulp meal on cholesterol and triacylglycerol levels in the serum and liver of rats - (Experiment 1)[1]

Item	Control	Pectin	Tangerine
	mg/100mL serum		
Total cholesterol	119.2 ± 9.1[a]	81.7 ± 5.4[b]	93.8 ±12.0[ab]
HDL cholesterol	40.1 ± 4.2	36.7 ± 4.1	33.7 ± 3.6
LDL+VLDL cholesterol	79.1 ±11.9	45.0 ± 6.7	60.1 ±11.2
Triacylglycerol	91.0 ±18.4	82.8 ± 7.4	75.6 ±15.0
HDL/total cholesterol	0.36±0.13	0.46±0.14	0.39±0.13
	mg/g liver		
Total cholesterol	14.2 ± 1.2[a]	7.9 ± 1.0[b]	12.6 ± 1.6[ab]
Triacylglycerol	24.7 ± 5.1	14.9 ± 2.7	17.6 ± 4.4

[1]Values are means SEM of 7 rats

[ab]Values in the same row with no common superscripts differ($p<0.05$) and those without superscripts do not differ($p>0.05$)

Table 3. Effect of feeding a diet containing 10% pectin on radioactivity excreted into feces after feeding of a slurry diet labeled with ^{14}C-cholesterol in rats(Experiment 3)[1]

Diet	Period after feeding(days)	
	1	2
	%[2]	
Control	3.1±2.1	9.8±2.8a
Pectin	5.4±3.0	16.5±6.3b

[1]Values are means SE of 5 rats

[2]Cummulative percentage of the infused dose.

[ab]Values in the same column with different superscripts differ($p<0.1$) and those without superscripts do not differ($p>0.05$) according to the Student t-test($p<0.1$)

ine pulp meal also tended to reduce the liver cholesterol level, but not significantly. Liver triacylglycerol levels were not influenced by dietary treatments.

Dietary propionate, fumarate or lactate did not influence serum cholesterol levels. Total serum cholesterol levels were 68.6±2.5, 64.5±0.9, 75.6±10.1 or 76.3±4.8mg/100mL serum(means±SEM of 5 rats) in rats fed the control diet or diets containing 3% disodium propionate, 3% calcium lactate or 3% hemicalcium fumarate, respectively. However, radioactivity in the feces excreted by rats fed the diet containing 10% pectin was almost twice that found in control rats over a 2-day period after feeding the diet labeled with 14C-cholesterol(Table 3).

Discussion

Many investigators have reported that dietary pectin has a hypocholesterolemic effect[1)5)]. Most of these studies were done with diets supplemented with cholesterol. However, when animals were fed diets supplemented with no cholesterol, the influence was not found[3)], with some exceptions especially when young rats were used in studies[4)] or when rats were fed high fat diets[16)]. Most studies done on humans have also shown the effect of dietary soluble fiber on lowering blood cholesterol level[2)], possibly because human diets normally contain significant amounts of cholesterol.

Differences between findings on the hypocholesterolemic effect of dietary pectin with and without cholesterol supplementation suggest that dietary soluble fibers are more effective in binding with and excreting exogenous cholesterol. This contention has been supported by many investigators[4)6)8)] who have reported that dietary soluble fibers increased fecal steroids. Turley, et al.[17)] indicated that a hydrophilic, gel-forming polymer decreased cholesterol absorption from the intestine and prevented accumulation of cholesterol in the liver as well as in the plasma.

By contrast, no difference was observed in the fecal cholesterol level between animals fed a control diet containing no soluble fiber and those fed a diet containing 12% guar gum[18)], perhaps due to an increased metabolism of cholesterol by intestinal bacteria in rats fed the guar gum diet. These differences in the effect of dietary soluble fibers on steroid excretion suggest that determination of fecal cholesterol excretion is not an appropriate method as a measure of cholesterol absorption, because it does not include a wide variety of cholesterol metabolites including microbial metabolites. In fact, dietary pec-

tin markedly increased fecal excretion of coprostanol, a microbial metabolite of cholesterol in rats, as compared to a control value[4,8], although other investigators[19] found an insignificant increase in fecal excretion of steroids and bile acids in men fed psyllium.

Based on our findings together with the reports that absorption of radio-labeled cholesterol through the intestine was diminished when rats were fed diets containing pectin[3], it is evident that excretion of exogenous cholesterol can be increased by dietary pectin or certain soluble fiber sources. The viscosity of soluble fibers might play an important role in binding with sterols and bile acids, preventing absorption in the intestinal tract[6,7]. In contrast, viscosity did not influence plasma or liver cholesterol levels when rats were fed diets supplemented with cholesterol and galactomannans of different origins[20] or diets supplemented with methylcellulose but with no cholesterol[21]. In those studies, however, there was a possibility that the intestinal viscosity of rats fed the diet containing 8% fenugreek gum(a source of galactomannans) or 8% methylcellulose of low viscosity was high enough to achieve a maximum effect, as shown by Gallaher, et al.[7] In the present study, rats were fed a diet containing 0.5% cholesterol, and thus dietary pectin was considered to remove significant amounts of the dietary cholesterol from absorption and consequently to depress the serum and liver cholesterol levels.

An alternative hypothesis that the hypocholesterolemic ability of dietary pectin or certain soluble fiber sources may in part be ascribed to their fermentation products, has been supported by the observation that blood cholesterol level was decreased by feeding propionic acid[9] or by infusing propionate solution into the rat cecum[10]. Soluble fiber or lactose that is not digested in the small intestine, enters the large intestine[22] where it is fermented by microflora, producing lactic acid and volatile fatty acids, especially increasing propionate fraction[23].

However, as shown in the present study, propionate may not be responsible for the hypocholesterolemic effects of pectin. Blood cholesterol concentrations were not affected in humans[12] and were increased in baboons by dietary propionate[11]. Serum cholesterol levels were even increased(15%) when propionate solution was infused into the pig cecum[12] or a mixture of acetate and propionate(3 : 1 by mole fraction) into human rectum[24], when compared to their control values. These reports support the hypothesis that propionate or volatile fatty acids produced in the large intestine from fermentation of dietary soluble fiber are an indicator of fermentability of soluble fiber but not a specific regulator of cholesterol metabolism, as suggested by Illman, et al.[25] Some of the non-specific hypocholesterolemic activity of volatile fatty acids produced in the large intestine may be attributable to their cathartic effect, i. e., decreased transit time possibly by the osmotic effect and acidic pH, resulting in decreased absorption of nutrients[26], perhaps including cholesterol and its metabolites.

Reviewing the literature accumulated to date, we believe that the hypocholesterolemic effect of dietary pectin or certain soluble fiber is mediated mainly by depressed absorption of cholesterol and its metabolites, but not through the fermentation product propionate because of the following reasons : 1) dietary soluble fiber usually(with a few exceptions) increases hepatic cholesterol synthesis(compensatory increases to a reduced cholesterol absorption), in contrast to the effect found with propionate, which is known to inhibit(though not always) cholesterol synthesis ; 2) non-fermentable, viscous soluble fiber(e.g., hydroxypropyl methylcellulose) has a hypocholesterolemic effect equivalent to that of fermentable, viscous soluble fiber(e.g., guar gum) ; 3) fermentable, non-viscous soluble fibers(e.g., acacia gum, lactulose) have relatively insignificant hypocholesterolemic effects(perhaps through the cathartic effect, if any) in comparison to the effect of viscous soluble fiber ; 4) a small fraction of the work done on propionate shows significant hypocholesterolemic effects when either fed in diets or infused into the large intestine ; and 5) the hypocholesterolemic effect of dietary soluble fiber is invariably more pronounced in animals fed diets containing high levels of cholesterol than in animals fed cholesterol-free diets.

Conclusions

The present study demonstrates the feasibility and

efficacy of pectin and possibly tangerine pulp meal (with an increased amount) as a cholesterol-lowering agent. Our findings also suggest that the positive effect of the soluble fiber pectin on lowering serum and liver cholesterol levels is mediated by decreased absorption of dietary cholesterol, its metabolites, or both, but not by the fermentation product propionate.

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