Effects of dietary pectin on the hepatic activities of hydroxymethyl glutaryl CoA reductase and acyl CoA cholesterol acyltransferase in cholesterol supplemented mice

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Plasma lipoprotein cholesterol and hepatic activities of hydroxymethyl glutaryl CoA reductase (HMGR) and acyl CoA cholesterol acyltransferase (ACAT) in mice fed a semi-synthetic diet supplemented with 5% low methoxyl pectin with or without addition of 0.5% cholesterol for 4 weeks were examined. While plasma concentrations of total cholesterol and triglycerides were significantly reduced, the hepatic levels of total and esterified cholesterol were markedly increased, and triglyceride levels remained unaffected when cellulose in the diet was substituted for by pectin in the cholesterol-enriched diet. The administration of 0.5% cholesterol to control animals resulted in a 50% reduction in the hepatic HMGR activity. This activity was restored to its control level when cellulose was substituted by pectin in the diet. The hepatic ACAT activity, on the other hand, was significantly increased when fed a diet containing 0.5% cholesterol. This effect remained unchanged when pectin was administered. The hypolipidemic action of pectin does not appear to be mediated through inhibition of cholesterol synthesis. It is possible that the decreased plasma level of cholesterol is the reflection of its accumulation in the liver.

Keywords: pectin; HMG CoA reductase; ACAT; total cholesterol; cholesterol ester; triglycerides; HDL

Introduction

Pectin is a water-soluble dietary fiber found in fruits, such as apples and bananas, and succulent vegetables, such as carrots and squash. Our recent study indicated a cholesterol-lowering effect of pectin,[1] which confirms observations in animals[2-4] and in human subjects.[5-7] The underlying mechanism for the hypocholesterolemic action of pectin is not clearly understood. It has been hypothesized that the production and absorption of short-chain fatty acids, the fermentation products of soluble fiber such as pectin in colon, may reduce the hepatic synthesis of cholesterol.[8] The hypocholesterolemic action of pectin has also been suggested to be due to the sequestration of bile acids in the intestine and increasing cholesterol turnover.[9,10] In addition, pectin is believed to interfere with lipid and cholesterol absorption by causing intestinal morphofunctional changes.[11,12]

In an attempt to explore whether pectin may be involved in modulating cholesterol synthesis and metabolism, the present study was undertaken to investigate the effects of dietary pectin on hepatic activities of two important enzymes. These were hydroxymethylglutaryl CoA reductase (HMGR), which is involved in cholesterol biosynthesis, and acyl CoA cholesterol acyltransferase (ACAT), an essential enzyme for transport of cholesterol from the liver. This study was carried out in mice receiving a semi-synthetic diet enriched with 0.5% cholesterol.

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Table 1 Diet composition (%)

Ingredient	Diet A	Diet B	Diet C
Casein[1]	20	20	20
Olive oil[2]	10	10	10
Cholesterol[3]	0	0.5	0.5
Vitamin mix[4]	2	2	2
Mineral mix[5]	3.5	3.5	3.5
Cellulose powder[6]	5	5	0
Corn starch[7]	59.5	59.0	59.0
Pectin powder[8]	0	0	5

[1]Casein from Teklad Test Diets (Madison, WI, USA).
[2]Olive oil (No. 102501) from ICN Biochemicals Divisions (Cleveland, OH, USA).
[3]Cholesterol (No. 101380) from ICN Biochemicals Divisions.
[4]Vitamin Mix A.O.A.C. (No. 40055), from Teklad Test Diets, composition (g/Kg of diet): vitamin A and D powder, 0.08 (vitamin A acetate, 40,000 U and Vitamin D_2, 4000 U); dry vitamin E acetate, 0.4 (200 U); menadione (vitamin K_3), 0.010; choline dihydrogen citrate (41% choline), 9.7561; p-aminobenzoic acid 0.2; inositol, 0.2; D-calcium pantothenate, 0.08; riboflavin, 0.016; thiamin-HCl, 0.01; pyridoxine HCl, 0.01; folic acid, 0.004; biotin, 0.008; vitamin B_{12} (0.1% trituration in mannitol), 0.00006; dextrose, anhydrous, 9.0931.
[5]Mineral Mix, Bernhart-Tomarelli (No. 170750), from Teklad Test Diets, composition 1(g/Kg): $CaCo_3$, 21; $CaHPo_4$, 735.0; Mg0, 25; $K_{2HP}O_4$, 68.0, NaCl, 30.6; Na_2HPO_4, 21.4; $Cu_2C_6H_4O_7.2\ 1/2H_20$ 0.46; ferric citrate (16.7% Fe), 5.58; manganese citrate (13.9%) Mn, 8.35; KI, 0.0072; zinc citrate, 1.33; citric acid, 2.2727.
[6]Cellulose from Teklad Test Diets.
[7]Cornstarch from ICN Biochemicals Divisions.
[8]Pectin Citrus (No. 102587) from ICN Biochemical Divisions.

Methods and materials

Animals and diets

Male adult ICR albino mice (University of Alberta, Canada), weighing 30–35 g (60–100 days old) were used throughout. The animals were individually housed in plastic cages. The room was temperature controlled (25° ± 2° C), and it was on a 12-hr light-dark cycle.

The animals were randomly divided into three groups of eight each. All groups received an experimental semi-synthetic diet (*Table 1*). Group 1 was fed a diet containing 5% cellulose powder (diet A) as a source of dietary fiber. Groups 2 and 3 were fed a diet containing 0.5% cholesterol with 5% cellulose powder (diet B) and 5% pectin powder (diet C), respectively. Group 1 was considered to be normocholesterolemic, and the remaining two groups to be hypercholesterolemic animals. All animals had free access to water and their respective diets for 4 weeks; body weights were recorded once a week. At the end of 4 weeks, the mice were fasted overnight and blood was collected through cardiac puncture in heparinized tubes while the animals were anesthesized with chloroform. Plasma was separated by centrifugation at 600*g* for 10 min. The livers were quickly removed, excised, weighed, and homogenized. The separated plasma and homogenized liver samples were frozen (−40° C) until analyzed.

Lipid analysis

Plasma total cholesterol, free cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides were determined using kit methods (Boehringer Mannheim, Indianapolis, IN and Sigma Chemical Co., St. Louis, MO USA). The difference between total and free cholesterol gave an estimation of esterified cholesterol levels. Liver tissue was subjected to lipid extraction by the method of Folch et al.[13] Aliquots of total lipid extracts were taken to determine cholesterol (total, free, and esterified) and triglycerides by the methods described above.

HMGR and ACAT assays

Microsomes were prepared from the liver tissues of animals fed various dietary regimes following the method described elsewhere.[14] HMGR activity was measured essentially as described by Garg and Sabine.[15] Microsomal protein (100–125 μg) was pre-incubated for 5 min at 37° C in 50 μl of 0.25 M sucrose/5 mmol/L potassium phosphate/l mmol/L EDTA buffer, pH 6.8, containing 0.01 M dithioerythritol. Then 30 μL of 0.02 M EDTA/KOH, pH 6.8, containing 5 μmol of glucose 6-phosphate, 0.5 μmol of $NADP^+$, and 1 unit of glucose-6-phosphate dehydrogenase was added. After another 5 min, 20 μL of 1 mmol/L-DL-hydroxy [3-^{14}C]-methylglutaryl-CoA (1100 dpm/nmol) was added and the incubations were continued for another 30 min. The reaction was stopped with 25 μL of 4 M-HCl containing 4 μmol of [5-^{3}H] mevalonic acid (500 dpm/μmol) as an internal standard. The mixture was further incubated for at least 15 min at 37° C to permit the mevalonic acid to form its lactone derivative, and then centrifuged for 5 min at 2000*g* to sediment denatured proteins. A 100-μL portion of this protein-free supernatant was applied directly to activated silica-gel G thin layer chromatographic plates and developed in a solvent system comprised of benzene/acetone (1:1, vol/vol) and air dried. The region (R_F 0.5–0.8) containing the mevalonolactone was scraped off directly into a scintillation vial and counted for radioactivity with 10 mL of scintillation fluid. Enzyme activity was expressed as pmol of mevalonic acid synthesized/min per mg of microsomal protein.

ACAT was assayed essentially by the method of Spector et at.[16] The reaction mixture contained in a final volume of 0.5 mL: 100–200 μg of microsomal protein, 0.1 M potassium phosphate buffer, pH 7.2, and 1 mmol/L dithiothreitol. The reaction was started by the addition of 10 nmol of [1-^{14}C]oleoyl-CoA (0.05 μCi). Incubations were carried out for 5 min at 37° C with shaking, and the reaction was terminated with 2 mL of chloroform/methanol (2:1 vol/vol) containing 0.005% (wt/vol) butylated hydroxytoluene as an antioxidant.

Lipids from these samples were recovered in the chloroform phase by the extraction method of Folch et al.[13] Cholesteryl ester fraction, produced as a result of ACAT action, was separated by thin layer chromatography on silica-gel G plates, using a solvent system comprised of light petroleum (boiling point: 60–80° C), diethyl ether, and acetic acid (80:20:1, by vol) as described previously.[14] The zones across the chromatographic plates corresponding to cholesterol esters were scraped off directly into scintillation vials containing 5 mL of scintillation fluid. From the known specific activity of the [1-^{14}C] oleoyl CoA substrate, ACAT activity was expressed as pmol of a cholesterol oleate formed/min per mg of microsomal protein.

Statistical analysis

Statistically significant differences among means were evaluated by one-way analysis of variance. The level of significance was set at $P < 0.05$.

Results

Mice fed a semi-synthetic diet with no cholesterol or with 0.5% cholesterol in the presence or absence of 5%

Table 2 Effect of dietary pectin (5%) on hepatic microsomal activities of acyl CoA cholesterol acyltransferase (ACAT) and hydroxy-methyl glutaryl CoA reductase (HMGR) in mice fed diets supplemented with 0.5% cholesterol for four weeks

Diet		Body wt gain	Liver wt	ACAT	HMGR
Chol (%)	Pectin (%)	(g)	(g)	pmol/mg microsomal protein	
—	—	10.1 ± 0.03[a]	2.1 ± 0.04[a]	58 ± 6.0[a]	1600 ± 108[a]
0.5	—	10.6 ± 0.02[a]	1.9 ± 0.3[a]	81 ± 5.6[b]	800 ± 56[b]
0.5	5.0	11.0 ± 0.03[a]	1.9 ± 0.03[a]	86 ± 6.1[b]	1300 ± 97[a]

Values are means ± SEM, n = 8 mice. Means with different superscript letters are significantly different ($P < 0.05$).

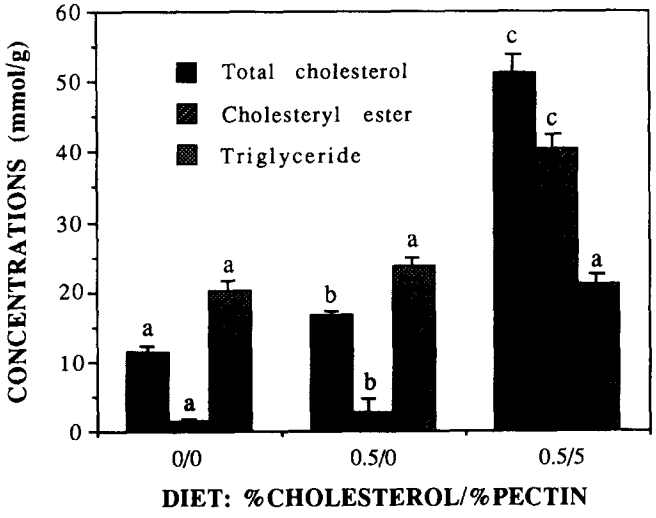

Figure 1 Effect of dietary pectin (5%) on liver cholesterol and triglyceride levels in mice receiving diets supplemented with 0.5% cholesterol for 4 weeks. In bars for each parameter, values (± SEM) not sharing a common superscript letter are significantly different at $P < 0.05$.

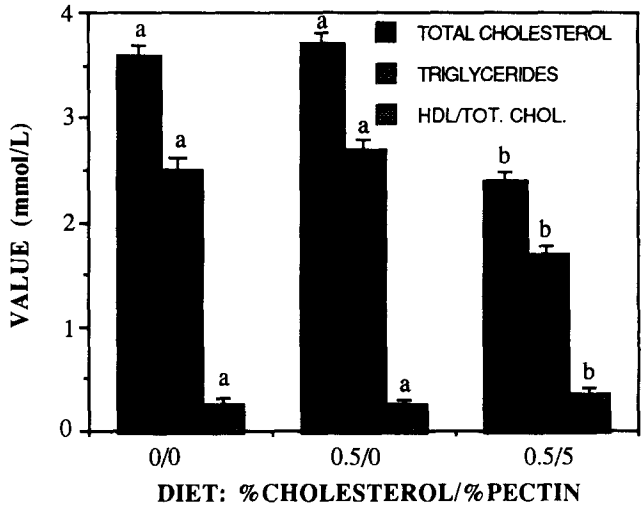

Figure 2 Effect of dietary pectin on plasma cholesterol and triglyceride levels in mice fed diets supplemented with 0.5% cholesterol for 4 weeks. In bars for each parameter, values (± SEM) not sharing a common superscript letter are significantly different at $P < 0.05$.

dietary pectin for 4 weeks did not show any statistically significant difference in the body and liver weights compared with those of the control animals receiving the same semi-synthetic diet with no added cholesterol and pectin (*Table 2*). In spite of similarities in body and liver weights among all three groups of animals, the plasma and liver exhibited marked changes in the lipid concentrations. In animals receiving the cholesterol-enriched diet for 4 weeks the hepatic concentrations of total and esterified cholesterol but not triglycerides were significantly increased indicating hypercholesterolemia (*Figure 1*). Addition of pectin powder to this diet at a 5% level resulted in a further and profound increase in hepatic levels of both total and esterified cholesterol. In contrast, the cholesterol supplemented diet did not alter the level of either cholesterol or triglycerides in plasma (*Figure 2*). Furthermore, animals receiving cholesterol plus pectin powder (5%) had significantly lower plasma total cholesterol levels than animals receiving cholesterol alone. The animals receiving a diet containing 0.5% cholesterol with pectin also had their levels of plasma triglyceride significantly reduced and HDL-cholesterol/total cholesterol ratio increased in relation to those animals that received the 0.5% cholesterol diet alone (*Figure 2*).

The activity of HMGR in the hepatic microsomes of mice receiving the cholesterol-enriched diet was decreased by 50% when compared with that of the animals receiving a cholesterol-free diet (*Table 2*). The dietary cholesterol-associated decrease in the enzyme activity was, however, counteracted when pectin powder (5%) was added to the diet. In contrast, the ACAT activity was significantly increased when cholesterol was added in a diet either alone or in combination with pectin (*Table 2*).

Discussion

Pectin is a polysaccharide composed of essentially linear polymers of D-galactopyranosyl-uronic acid units joined in a D,1-4-glycosidic linkage. It has the important property of forming spreadable gels, which occurs when the polymer chains interact over a portion of their length to form a three-dimensional network held together through hydrogen bonding, divalent cation crossbridging and/or hydrophilic interactions.[17]

Using mice, the present study clearly demonstrates that the substitution of cellulose with low methoxylated pectin in a cholesterol enriched semi-synthetic diet reduces both total cholesterol and triglyceride levels in

plasma. The presence of pectin in the cholesterol-rich diet does not appear to affect the plasma level of HDL-cholesterol. However, because of the reduction in total cholesterol concentration, the HDL-cholesterol to total cholesterol ratio appears to be significantly increased following pectin administration. These results are in agreement with others who made similar observations in experimental animals,[2-4] as well as in human subjects.[5-7]

While the addition of pectin to the cholesterol-enriched standard diet reduced the plasma level of cholesterol, it enhanced the accumulation of total cholesterol as well as its esterified form in the liver. How pectin results in an increased hepatic concentration of cholesterol cannot be ascertained. It is likely that the hypocholesterolemic action of dietary fiber is due to a shift of cholesterol from plasma to the hepatic pools. Such a redistribution phenomenon has previously been observed when animals were fed a diet enriched with polyunsaturated (linoleic acid) fatty acids.[14]

Addition of exogenous cholesterol to the diet caused its hepatic production to be inhibited, as evident by the reduction in the activity of HMGR, the rate limiting enzyme for cholesterol synthesis, to 50% of that of the cholesterol-free diet. This is expected because of the homeostasis control of cholesterol metabolism.[15,18] It is, however, noteworthy that when mice were fed the cholesterol-enriched diet supplemented with 5% pectin, the hepatic HMGR activity was increased to the level of the animals fed a diet not containing any cholesterol. It is possible that dietary pectin hinders the absorption of lipid and cholesterol.[11,12] This may decrease the body pool size of cholesterol that promotes cholesterol biosynthesis.

It has been suggested that propionate resulting from large bowel fermentation may be a component of pectin responsible for lowering plasma cholesterol through inhibiting cholesterol synthesis.[19,20] The results of the present study do not support this hypothesis. Other investigators also failed to observe any pectin-associated decrease in cholesterol synthesis.[21]

The hepatic activity of ACAT enzyme was significantly increased when animals were fed the diet enriched with cholesterol. This is in agreement with our previous observations.[15] This increase in ACAT activity remained unaltered by addition of pectin to the diet. This is not surprising considering that dietary pectin redistributes cholesterol from blood to the liver pools, which would put additional demands on liver to esterify the redistributed cholesterol either for transport or storage in the hepatic tissue itself.

In conclusion, the pectin-associated decrease in plasma cholesterol may be the reflection of its decreased transport from the liver. The underlying mechanism for the observed effects of pectin on hepatic accumulation of cholesterol is far from being understood. More studies are needed to establish the mechanism of the cholesterol and lipid lowering effects of soluble fibers.

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