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Propionate May Mediate the Hypocholesterolemic Effects of Certain Soluble Plant Fibers in Cholesterol-Fed Rats (41791)

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Abstract. The effects of propionate on serum and liver lipid concentrations were studied in cholesterol-fed rats. Both serum and liver cholesterol levels were significantly lower in rats fed the cholesterol-propionate diet than in rats fed the cholesterol diet without propionate. Liver triglyceride levels were also significantly lower in the propionate-treated group. Serum triglyceride concentrations were not influenced by the propionate feeding. Propionate intake was not associated with histologic changes in liver tissue. This study indicates that 0.5% sodium propionate-supplemented diets slightly but significantly reduced cholesterol accumulation in both serum and liver of cholesterol-fed rats. Thus propionate, a metabolic product of fiber fermentation, may mediate some of the hypocholesterolemic effects of certain soluble plant fibers.

Supplementing the diet with certain soluble plant fibers lowers serum cholesterol concentrations in humans and animals by ill-defined mechanisms (1). The hypocholesterolemic effect of plant fiber may be related to the fiber-induced alterations of intestinal absorption, intestinal or pancreatic hormone secretion, lipoprotein metabolism, bile acid metabolism, or metabolic effects in the colon (1). Certain soluble plant fibers such as pectin are almost completely fermented by colonic bacteria to short chain fatty acids such as acetate, propionate, and butyrate and other products in human and rat (2). These short chain fatty acids are virtually completely absorbed (3). Since propionate can significantly inhibit cholesterol synthetic rates in isolated rat hepatocytes (4), this study was designed to examine the effects of propionate-supplemented diets on lipid metabolism of rats.

Materials and Methods. Male Sprague–Dawley rats (Harland Industries, Cumberland, Ind.), 250–300 g, were individually housed in stainless-steel cages with raised mesh floors. They were randomly divided into four dietary groups of 10 rats each. Their diets were essentially identical in carbohydrate, protein, and fat content (Table I). The control diet contained neither cholesterol nor sodium propionate. The control-propionate diet contained 0.5% sodium propionate without cholesterol. The cholesterol diet contained 0.3% cholesterol without sodium propionate. The cholesterol-propionate diet contained 0.3% cholesterol and 0.5% sodium propionate. Food and water were allowed *ad libitum*. Food intake and body weight were recorded weekly. After 2 weeks, rats were fasted for 24 hr, anesthetized with pentobarbital, and, then, 0.5 ml blood was withdrawn by cardiac puncture. Serum glucose was measured by the glucose oxidase method (5). After 3 weeks the rats were anesthetized with pentobarbital and blood samples were withdrawn by cardiac puncture; livers were excised and blotted to remove excess blood. Plasma and portions of the left lobe of liver were stored at −20°C for lipid determinations (6).

To evaluate liver function and morphology, we measured serum concentrations of γ glutamyl transpeptidase (7) and examined sections of the left lobe of liver by light microscopy. Samples of liver were placed in 10% formaldehyde–phosphate buffer for fixation. Specimens were embedded in paraffin, sectioned, and stained by hematoxylin and eosin.

Data were analyzed using randomized one-way analysis of variance and Duncan's multiple-range test (8).

Results. Weight gains, final body weights, and food intakes were similar among the rats fed the four experimental diets (Table II). Fasting serum glucose concentrations were not affected by propionate supplements. Although serum γ glutamyl transpeptidase varied widely within groups, average values were not significantly different between groups.

Serum cholesterol concentrations were sig-

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TABLE I. COMPOSITION OF DIETS (g/100 g)

	Control	Control-propionate	Cholesterol	Cholesterol-propionate
Sucrose	46.5	46.5	46.5	46.5
Starch	17.2	16.7	16.9	16.4
Casein	15.0	15.0	15.0	15.0
DL-Methionine	0.3	0.3	0.3	0.3
Cotton seed oil	6	6	6	6
Vitamin mixture[a]	1	1	1	1
Salt mixture[b]	4	4	4	4
Cellulose[c]	10	10	10	10
Cholesterol	—	—	0.3	0.3
Sodium propionate	—	0.5	—	0.5

[a] Vitamin diet fortification mixture, ICN Nutritional Biochemicals, Cleveland, Ohio.
[b] USP XVII salt mixture supplemented with zinc sulfate 0.08% ICN Nutritional Biochemicals, Cleveland, Ohio.
[c] Alphacel, ICN Nutritional Biochemicals, Cleveland, Ohio.

nificantly lower in rats fed the cholesterol-propionate diet than in rats fed the cholesterol diet without propionate. Serum cholesterol concentrations were similar between the rats fed the control-propionate diet and control diet. Serum high-density lipoprotein (HDL) cholesterol concentrations tended to be lower in cholesterol-fed rats than in rats fed control diets, but these differences were not statistically significant. Propionate feeding did not affect serum HDL-cholesterol concentrations. Serum triglyceride concentrations tended to be higher in cholesterol-fed rats than in rats fed the control diet, but these differences were not statistically significant. Propionate feeding did not affect serum triglyceride concentrations.

Total lipid concentrations of liver were not different among the rats fed the four different diets. Liver cholesterol concentrations were significantly higher in cholesterol-fed rats than in rats fed control diets. Liver cholesterol concentrations were significantly lower in rats fed the cholesterol-propionate diet than in rats fed the cholesterol diet without propionate. Liver cholesterol concentrations were similar between the rats fed the control diet and the control diet with propionate. Liver triglyceride concentrations were significantly higher in cholesterol-fed rats than in rats fed control

TABLE II. EFFECT OF PROPIONATE ON BODY WEIGHT, FOOD INTAKE, AND PLASMA AND LIVER LIPID LEVELS OF CHOLESTEROL-FED RATS[a]

	Control	Control-propionate	Cholesterol	Cholesterol-propionate
Body weight (g)				
Initial	268 ± 4	268 ± 4	270 ± 6	268 ± 3
Final	336 ± 4	329 ± 3	335 ± 6	333 ± 4
Food intake (g/day)	19.7 ± 0.2	19.4 ± 0.3	19.8 ± 0.4	19.9 ± 0.3
Serum γ-glutamyl transpeptidase (u/liter)	−0.43 ± 0.86	−0.14 ± 0.34	−0.38 ± 0.82	−0.71 ± 0.57
Serum fasting glucose (mg/dl)	116 ± 5	114 ± 4	118 ± 3	117 ± 5
Serum cholesterol (mg/dl)	102 ± 3*	102 ± 5*	101 ± 5*	87 ± 3†
Serum HDL cholesterol[b] (mg/dl)	58 ± 7	55 ± 7	39 ± 5	44 ± 5
Serum triglyceride (mg/dl)	80 ± 12	78 ± 9	116 ± 13	116 ± 9
Liver total lipid (mg/g)	23.3 ± 4.6	24.7 ± 5.4	29.4 ± 5.4	25.2 ± 3.6
Liver cholesterol (mg/g)	1.96 ± 0.05*	2.08 ± 0.06*	4.31 ± 0.28†	3.56 ± 0.22‡
Liver triglyceride (mg/g)	4.55 ± 0.96*	4.92 ± 0.96*	9.17 ± 1.46†	5.87 ± 0.80*

[a] Values are means ± SE for 8–10 rats. Means in the same line with different superscript are significantly different ($P < 0.05$).
[b] High-density lipoprotein cholesterol.

diets. However, rats fed the cholesterol-propionate diet had significantly lower liver triglyceride concentrations than rats fed the cholesterol diet without propionate.

Histological sections of liver showed normal hepatic lobular architecture. There were no significant differences histologically among the various dietary groups. No evidence of cirrhosis, hepatocyte necrosis, cholestasis, or significant inflammation was identified in any section.

Discussion. This study indicated that 0.5% sodium propionate-supplemented diets significantly lowered cholesterol concentrations in both plasma and liver of cholesterol-fed rats. These observations suggest that propionate may mediate, in part, the cholesterol-lowering effects of certain soluble plant fibers. Previous studies (1, 9) indicate that certain soluble fibers increase fecal bile acid excretion. The loss of these cholesterol-containing compounds may contribute to the hypocholesterolemic effects of certain soluble fibers. However, beans (10) and gum arabic (11) have significant hypocholesterolemic effects but do not increase fecal bile acid excretion. Thus the loss of fecal bile acids cannot explain the hypocholesterolemic effects of certain soluble fibers. Consequently, we examined the effect of propionate on cholesterol metabolism.

When pigs were fed high levels of propionate, Thacker and colleagues (12–14) noted reductions in serum cholesterol concentrations but increases in cholesterol concentrations of liver and backfat. Using 3–9% dietary propionate (6 to 18-fold higher concentrations than we used), serum cholesterol concentrations were lower in propionate-fed pigs than in control animals. While liver cholesterol concentrations were slightly higher, backfat cholesterol concentrations were significantly higher in propionate-fed pigs than in control animals (12–14). Thus Thacker and colleagues (13, 14) suggested that propionate reduced total serum cholesterol by shifting cholesterol from serum to tissue pools. In our studies in rats using much lower levels of dietary propionate, we observed that the propionate-supplemented diet significantly decreased liver cholesterol and triglyceride concentrations and slightly decreased liver total lipid concentrations of cholesterol-fed rats.

To exclude the possibility that propionate feeding might alter serum and liver cholesterol value through some toxic mechanism, we measured a sensitive enzyme of hepatic function (γ-glutamyl transpeptidase) and examined histological sections of liver. No evidence of hepatocellular dysfunction was detected.

The hypocholesterolemic effect of propionate may be related to altered hepatic cholesterol synthesis. The synthesis of cholesterol from acety-CoA is mediated by the cytoplasmic form of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (15). Bush and Milligan (16) utilized bovine liver incubations and reported that propionic acid at concentrations of 30 and 15 m*M* inhibited HMG-CoA synthase activity by 58 and 30%, respectively. The activity of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis in animal tissues (17) was also affected by propionic acid. Ide et al (18) reported that shorter chain fatty acid tended to decrease HMG-CoA reductase activities, whereas longer chain fatty acids tended to produce higher enzyme activities. Recently in isolated rat hepatocytes, we (18) observed that propionate inhibited cholesterol synthetic rate by 45% and fatty acid synthesis by 84%.

Short chain fatty acids were produced in the colon by bacteria fermentation of polysaccharide carbohydrates. The major source of these acids in the human colon is plant fiber. These short chain fatty acids including acetate (approximately 60% of the total short chain fatty acids), propionate (approximately 20%), and butyrate (approximately 16%) are virtually completely absorbed from colon (2, 3). The effects of increased plant fiber intake on portal vein concentrations of short chain fatty acids are not well delineated. From available data (2) we assume that greater production and absorption of short chain fatty acid from the human colon would increase portal vein short chain fatty acid concentrations. In rats, oat-bran-supplemented diets were accompanied by significantly higher portal vein concentrations of acetate, propionate, and butyrate than were cellulose-supplemented diets (J. W. Anderson, unpublished observations). Illman *et al.* (19) observed that plasma short chain fatty acids were significantly higher in both hepatic portal venous and arterial blood of rats fed pectin compared with wheat-bran-fed rats.

The metabolic effects of rapid intestinal absorption of dietary propionate and gradual colonic absorption of plant fiber derived propionate may not be the same. Previous studies (1, 5) found that certain soluble plant fiber such as oat bran and pectin lowered the serum and liver cholesterol in cholesterol-fed rats; the present study finds that dietary propionate lowers the serum and liver cholesterol in cholesterol-fed rats, we therefore suggest that one mechanism for the hypocholesterolemic effect of certain soluble plant fiber can be related to the absorbed propionate, a fermentation metabolite of soluble plant fiber, which inhibits hepatic cholesterol synthesis.

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