Propionate Inhibits Hepatocyte Lipid Synthesis (43113)

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Abstract. **Oat bran lowers serum cholesterol in animals and humans. Propionate, a short-chain fatty acid produced by colonic bacterial fermentation of soluble fiber, is a potential mediator of this action. We tested the effect of propionate on hepatocyte lipid synthesis in rats using [1-^{14}C]acetate, $^{3}H_2O$, and [2-^{14}C]mevalonate as precursors. Propionate produced a statistically significant inhibition of cholesterol biosynthesis from [1-^{14}C]-acetate at a concentration of 1.0 m*M* and from $^{3}H_2O$ and [2-^{14}C]mevolonate at concentrations of 2.5 m*M*. Propionate also produced a significant inhibition of fatty acid biosynthesis at concentrations of 2.5 m*M* using [1-^{14}C]acetate as a precursor. The demonstration of propionate-mediated inhibition of cholesterol and fatty acid biosynthesis at these concentrations suggests that propionate may inhibit cholesterol and fatty acid biosynthesis *in vivo* and may mediate in part the hypolipidemic effects of soluble dietary fiber. Further studies are needed to clarify this action of propionate and to establish the exact mechanisms by which the inhibition occurs.**

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Serum cholesterol concentrations are lowered by a variety of products rich in soluble fiber, including oat bran, pectin, guar, and beans through mechanisms that are not well defined (1–3). There is, however, much evidence that the microbial breakdown of carbohydrates in the large bowel yields short-chain fatty acids, including acetic, propionic, and butyric acids (4). Propionate has been reported to be a potential mediator of the hypocholesterolemic effect of certain dietary fibers (5). Since portal vein concentrations of propionate can range up to 0.8 m*M* in rats fed a variety of diets high in soluble fiber (6), these levels can be considered physiologic. Whereas propionate has been demonstrated to inhibit *de novo* biosynthesis of both cholesterol and fatty acids, the concentrations of propionate used were within the range of 18 m*M* (6). In this study, we found statistically significant inhibition of cholesterol biosynthesis over a range of propionate concentrations of 1.0 to 2.5 m*M* and higher. These observations suggest that propionate may inhibit cholesterol biosynthesis *in vivo* and in part mediate the hypocholesterolemic effects of soluble fiber.

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Materials and Methods

Suspensions of liver cells were prepared from male Sprague-Dawley rats weighing 250–500 g. All rats were fed Purina Laboratory Rodent Chow 5001 *ad libitum* until sacrificed. Isolated hepatocytes were prepared according to the method described by Berry and Friend (7), with some modifications (8), and were 95–98% viable using trypan blue dye exclusion testing (9). The isolated hepatocytes were then suspended in Krebs buffer containing 1.5% gelatin to give a final concentration of 10–15 $\times$ 10^6 cells/ml.

Synthesis of cholesterol and fatty acid was assessed by measuring the incorporation of [^{14}C]acetate (5 m*M*), [^{14}C]mevalonic acid (1 m*M*), and tritiated water (2 mCi) into cholesterol or fatty acid according to previously published procedures (9, 10). The radioactive label was added to 0.8 ml of cell suspension and the appropriate concentrations of propionate (0.1–25 m*M*) to give a final volume of 1 ml. The 10-ml flasks containing the cell suspension, substrate, and fatty acid were perfused briefly with an O_2:CO_2 (95:5) mixture, after which the flasks were capped tightly and incubated at 37°C in a shaking water bath (100 oscillations/min) for 60 min.

Incubations were terminated by adding 0.5 ml of 10 *N* potassium hydroxide followed by shaking for 30 min at 37°C. The contents of the flasks were transferred quantitatively to screw-cap culture tubes. The mixture was saponified at 85°C for 1 hr. The sterol fraction was then extracted with petroleum ether and precipitated

with 0.5% digitonin in 50% ethanol (11). It was presumed that this fraction contained mainly cholesterol because it is the major mammalian sterol. After removal of the sterol fraction, the alkaline-alcohol mixture was titrated with a few drops of 12 *N* HCl until acidic and then fatty acids were extracted with petroleum ether. The incorporation of the label into sterols and fatty acids was given as nanomoles of precursor incorporated per gram of fresh tissue. The incorporation of titrium into cholesterol and fatty acids was determined as described by Brunengraber *et al.* (12).

All reagents and chemicals used were of analytical grade. Tritated water (0.38 mCi/mmol) was purchased from Amersham (Arlington Heights, IL). DL-[2-^{14}C] Mevalonic acid, sodium salt, (47 mCi/mmol) was purchased from Research Products International (Mount Prospect, IL). Sodium [1-^{14}C]acetate (2.1 mCi/mmol) and Aquasol scintillation fluid were purchased from New England Nuclear (Boston, MA). Collagenase was purchased from Worthington Biochemicals (Freehold, NJ).

Data are shown as the mean ± SE of the number of trials in parentheses. All variables were analyzed by two-way analysis of variance, adjusting propionate level means for individual rat effects. Those variables exhibiting significant differences ($p < 0.05$) among propionate levels were then further analyzed by a protected least significant difference (13).

Results

Effect of Propionate on Cholesterol Biosynthesis. The effect of propionate on hepatic cholesterol biosynthesis was tested using [1-^{14}C]acetate, ^3H_2O, and [2-^{14}C]mevalonate as precursors. Table I shows the influence of propionate on cholesterol biosynthesis from [1-^{14}C]acetate. Propionate produced a statistically significant inhibition of cholesterol biosynthesis at a concentration of 1.0 m*M*. Inhibitory activity increased with increasing propionate concentration.

Table II shows the influence of propionate on cholesterol biosynthesis from ^3H_2O, and Table III shows the effects of propionate on cholesterol biosynthesis from [2-^{14}C]mevalonate. At a concentration of 2.5 m*M* propionate significantly inhibited cholesterol biosynthesis from ^3H_2O by 33% and from mevalonate by 20%.

Effect of Propionate on Fatty Acid Biosynthesis. The effect of propionate on fatty acid biosynthesis was tested using [1-^{14}C]acetate, ^3H_2O, and [2-^{14}C]mevalonate as precursors. Table I shows the influence of propionate on fatty acid biosynthesis from [1-^{14}C]acetate. Propionate significantly inhibited fatty acid biosynthesis at concentrations of 2.5 m*M*, reducing fatty acid biosynthesis by 95% at the 10.0 m*M* concentration. Propionate had no apparent effect on fatty acid biosyn-

Table I. Influence of Propionate on Hepatocyte Lipid Synthesis from [1-^{14}C]Acetate[a]

Propionate concentration (m*M*)	Cholesterol biosynthesis	Fatty acid biosynthesis
	(nmol/g liver/hr)	
0 (control)	101.4 ± 12.1 (9)	384.7 ± 98.2
	% of Control	
0.1	97.3 ± 1.3 (6)	97.5 ± 3.5 (6)
0.25	97.3 ± 0.9 (9)	91.4 ± 3.7 (9)
0.5	96.1 ± 1.6 (9)	86.7 ± 6.4 (9)
1.0	84.4 ± 3.5 (5)[b]	63.8 ± 10.2 (5)
2.5	56.8 ± 6.8 (5)[b]	29.4 ± 9.2 (5)[b]
5.0	47.2 ± 4.5 (5)[b]	7.2 ± 1.0 (5)[b]
10.0	43.0 ± 5.5 (3)[b]	5.0 ± 0.0 (3)[b]
25.0	42.0 ± 2.1 (3)[b]	6.7 ± 1.2 (3)[b]

[a] Values are the mean ± SE (*n*).
[b] $P \leq 0.05$.

Table II. Influence of Propionate on Hepatocyte Lipid Synthesis from ^3H_2O[a]

Propionate concentration (m*M*)	Cholesterol biosynthesis	Fatty acid biosynthesis
	(nmol/g liver/hr)	
0 (control)	210 ± 80 (3)	1010 ± 190 (3)
	% of Control	
0.25	83.5 ± 7.5 (2)	
0.5	87.7 ± 0.9 (3)	99.0 ± 3.1 (3)
1.0	86.3 ± 4.1 (3)	97.3 ± 7.3 (3)
2.5	67.0 ± 4.9 (3)[b]	93.3 ± 9.1 (3)
5.0	61.0 ± 2.3 (3)[b]	94.5 ± 24.0 (2)
10.0	58.0 ± 4.9 (3)[b]	104.7 ± 18.8 (3)
25.0	38.5 ± 9.5 (2)[b]	104 ± 22.0 (2)

[a] Values are the mean ± SE (*n*).
[b] $P \leq 0.05$.

Table III. Influence of Propionate on Hepatocyte Lipid Synthesis from [1-^{14}C]Mevalonate[a]

Propionate concentration (m*M*)	Cholesterol biosynthesis	Fatty acid biosynthesis
	(nmol/g liver/hr)	
0 (control)	295.15 ± 81.9 (6)	22.6 ± 3.0 (4)
	% of Control	
0.25	99.1 ± 2.5 (4)	96.4 ± 3.9 (4)
0.50	101.4 ± 2.6 (6)	106.7 ± 5.8 (4)
1.0	94.4 ± 2.5 (6)	106.0 ± 3.8 (4)
2.5	79.5 ± 4.0 (6)[b]	104.3 ± 7.1 (4)
5.0	68.3 ± 5.0 (6)[b]	100.9 ± 7.4 (4)
10.0	64.5 ± 3.8 (6)[b]	99.6 ± 3.2 (4)
25.0	59.8 ± 5.1 (3)[b]	104.9 ± 15.3 (4)

[a] Values are the mean ± SE (*n*).
[b] $P \leq 0.05$.

thesis from ^3H_2O and [2-^{14}C]mevalonate (Tables II and III).

Discussion

This study demonstrates propionate-mediated inhibition of cholesterol biosynthesis across a wide range of propionate concentrations. Propionate exerts a stepwise, dose-related inhibition of cholesterol synthesis in rat hepatocytes using [1-^{14}C]acetate, ^3H_2O, and [2-^{14}C] mevalonate as precursors. Whereas previous work demonstrate propionate-mediated inhibition of cholesterol and fatty acid biosynthesis at propionate concentrations of 18 m*M* in the perfused rat liver, this study demonstrates propionate-mediated inhibition of cholesterol and fatty acid biosynthesis in hepatocytes at propionate concentrations of 1.0–2.5 m*M* and higher.

Propionate inhibited the incorporation of [1-^{14}C] acetate into fatty acids at 2.5 m*M* concentrations, but it did not inhibit the incorporation of ^3H_2O and [1-^{14}C] mevalonate into fatty acids with concentrations as high as 25 m*M*. Several mechanisms may explain this selective inhibition. First, propionate may inhibit the synthesis of acetyl-CoA from acetate by inhibiting acetyl-CoA synthetase. Tomodo *et al.* (14) recently described a novel class of compounds that selectively inhibit acyl-CoA synthetases. Second, propionate may compete with acetate during lipid synthesis and dilute the radiolabel rather than inhibit biosynthesis (15). Third, propionate may inhibit fatty acid biosynthesis through decreased cytosolic citrate concentrations. Previous work by Blair *et al.* (16) demonstrated that 10 m*M* propionate reduced cytosolic citrate concentrations by 56%. Because cytosolic citrate is an important allosteric regulator of acetyl-CoA carboxylase, a regulatory enzyme in fatty acid biosynthesis, reductions in cytosolic citrate concentrations would help explain the effects of propionate on lipid biosynthesis. Additionally, decreased citrate concentrations results in less available acetyl-CoA for incorporation into overall lipid synthesis. The inhibition of fatty acid biosynthesis from [1-^{14}C]acetate complements previous work demonstrating the lipid-lowering properties of soluble fiber and propionate (1–3, 5).

Dietary products rich in soluble fiber, such as oats and beans (17), have been shown to lower serum cholesterol in hyperlipidemic animals and humans (2, 3); whereas, products rich in insoluble fiber, such as wheat and cellulose, do not (18). The hypolipidemic properties of soluble dietary fiber occur through a variety of mechanisms. Most soluble fibers increase fecal excretion of bile acids and alter the percentages of various primary and secondary bile acids excreted by the liver (19). These changes could result in a shift of systemic cholesterol into bile synthesis and thus reduce the total body cholesterol pool. Also, most soluble fibers decrease the absorption of lipids in the proximal intestine and increase their absorption in the midintestine, which may alter the size and composition of lipoproteins secreted by the intestine (20). Certain dietary fibers have a significant impact on pancreatic and gastrointestinal hormones. Dietary fibers tend to decrease serum insulin concentration and increase peripheral insulin sensitivity which may affect the regulation of hepatic lipid synthesis (21, 22). Propionate, a product of carbohydrate fermentation, has been shown to lower serum cholesterol when fed to rats (5). The demonstration of propionate-mediated inhibition of hepatocyte lipid synthesis suggests that propionate may modulate *in vivo* hepatic processes and in part modulate the activity of soluble dietary fiber. Further studies are needed to clarify these roles.

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