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Short-Chain Fatty Acid Fermentation Products of Plant Fiber Affect Glucose Metabolism of Isolated Rat Hepatocytes (41958)

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Abstract. Short-chain fatty acids (SCFA) produced during fermentation of plant fibers and absorbed from the colon may affect hepatic glucose metabolism. We examined the effects of different fatty acids on rates of glucose production and glycolysis in isolated rat hepatocytes. Acetate, butyrate, and long-chain fatty acids significantly increased glucose production from lactate. However, propionate and valerate significantly decreased glucose production from lactate. Whereas 5 m*M* butyrate increased the incorporation of [^{14}C]lactate into [^{14}C]glucose by 80%, 5 m*M* propionate produced a 67% decrease. Glycolysis was significantly decreased by acetate, butyrate, and long-chain fatty acids. However, propionate and valerate significantly increased glycolysis. Thus propionate, which inhibits hepatic acetate metabolism, acts to increase glucose use and decrease glucose production. Plant fibers may influence hepatic glucose metabolism via their SCFA metabolites.

Plant fibers influence the digestion, absorption, and metabolism of dietary carbohydrates (1). Supplementing the diet with selected fibers lowers postprandial plasma glucose concentrations (1, 2) whereas high-fiber diets lower insulin requirements of adult diabetic patients (3, 4). The mechanisms responsible for fiber-induced improvements of glucose metabolism are not well delineated but may include hepatic or extrahepatic effects (1). As most plant fibers are fermented by bacterial action in the colon to short-chain fatty acids (SCFA) and other products (1, 5) and these SCFA are absorbed (5), we examined the effects of SCFA on rates of glucose production and glycolysis in isolated rat hepatocytes. These studies may have further clinical application because propionate can induce hypoglycemia in humans under certain circumstances (6).

Materials and Methods. Suspensions of liver cells were prepared by a modification (7) of the method of Berry and Friend (8). Male Sprague–Dawley rats, 250–500 g, were fasted for 24 hr. They were anesthetized by an intraperitoneal injection of sodium thiopental (Pentathal). The portal vein was cannulated and perfused for 10 min in a non-recirculating system under a 95% O_2–5% CO_2 atmosphere with calcium-free Krebs–Ringer bicarbonate buffer, 8 ml/min, using an Ambec perfusion apparatus (Ambec, Inc., Aurora, Colo). After 10 min collagenase (Worthington Type I), 0.4 mg/ml, was added to the perfusate and the liver was then placed in a beaker containing calcium-free Krebs–Ringer bicarbonate buffer plus 1.5% gelatin. The tissue was torn with dissecting scissors under a 95% O_2–5% CO_2 atmosphere for 5 min. The tissue suspension was poured through nylon (350-μm mesh) and the filtrate centrifuged at 50*g* for 30 sec. The cells were washed twice and resuspended in Krebs buffer containing 1.5% gelatin to give a final concentration of 10–15 $\times$ 10^6 cells/ml. The final cell suspension routinely contained 95–98% intact cells as determined by trypan blue exclusion.

The cell suspension was gassed with 95% O_2–5% CO_2 and 0.8 ml of the suspension added to 10-ml Erlenmeyer flasks containing substrate and fatty acid. The total reaction volume was 1 ml. The flasks were immediately stoppered and incubated for 30 min at 37°C in a shaking water bath. The reaction was stopped by the addition of 0.2 ml 10% trichloracetic acid (TCA) or 2 ml of 8% perchloric acid for gluconeogenesis or glycolysis experiments, respectively.

In the gluconeogenesis experiments lactate (Sigma Chemical Co., St. Louis, Mo.) was used as substrate. Glucose production rates from propionate or valerate alone were subtracted from values obtained with lactate and these fatty acids. The TCA supernatants were collected by centrifugation at 1000*g* for 20

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min. Glucose in the supernatant was assayed using an enzymatic determination with glucose oxidase (Sigma kit 510). Glucose production was linear over 30 min.

For measuring the incorporation of [U-^{14}C]lactate into glucose we used 10 m*M* lactate as substrate. After 30 min, the incubation was terminated by the addition of 0.5 ml of 5% $ZnSO_4$; 0.5 ml of 0.15 *M* Ba $(OH)_2$, and 2 ml of water were then added and the precipitate removed by centrifugation as described by Claus *et al.* (9). We separated [^{14}C]glucose from the supernatant by the procedure of Exton and Park (10). An aliquot of the resin-treated supernatant was added to 10 ml of Aquasol and analyzed for [^{14}C]glucose in a Packard liquid scintillation counter.

In experiments measuring glycolysis, 10 m*M* glucose was used as the substrate. After chilling for 5 min in ice, the perchloric acid supernatants were collected by centrifugation at 1500*g* for 10 min. Lactate was determined in the supernatant using lactate dehydrogenase and measuring the production of NADH (Sigma kit 826-UV). Lactate production was linear over 30 min.

Results. *Effects of SCFA on hepatic glucose production rates.* SCFA with even numbers of carbon atoms, acetate and butyrate, were not substrates for glucose production but did increase glucose production from lactate. Maximal rates of glucose production were observed with acetate and butyrate at concentrations of 5 m*M*; at these concentrations glucose production from lactate was 48 and 68% higher with acetate and butyrate, respectively, than values without these SCFA (Table I).

In sharp contrast SCFA with odd numbers of carbon atoms, propionate and valerate, decreased glucose production rates. Although propionate and valerate were converted to glucose to a limited extent, their net effects were to decrease glucose production. Maximal rates of glucose production were 2.2 and 1.4 μmole/30 min/g hepatocyte from propionate and valerate, respectively, when lactate was not present; these values were subtracted from values in the presence of lactate to compute net gluconeogenic rates. Glucose production from lactate was maximally decreased with propionate concentrations of 2 m*M* and valerate concentrations of 10 m*M*. Net glucose production from lactate was decreased by 28% with 5 m*M* propionate and 38% with 5 m*M* valerate (Table I).

The conversion of [^{14}C]lactate to [^{14}C]-glucose with various SCFA is summarized in Fig. 1. Acetate, 5 m*M*, increased glucose production by 55%. Butyrate increased glucose production at concentrations of 0.1 m*M* and produced an 80% increase in lactate to glucose conversion at 5 m*M*. In sharp contrast, propionate produced a marked decrease in glucose production. Propionate, 5 m*M*, produced a 67% decrease whereas 10 m*M* propionate decreased lactate to glucose conversion by 76%.

TABLE I. EFFECTS OF FATTY ACIDS (FA) ON GLUCOSE PRODUCTION RATES IN ISOLATED RAT HEPATOCYTES

	Substrate			
			Lactate	
	FA concn (m*M*)	FA	No FA	With FA
Acetate	5	0	8.7 ± 0.4	12.9 ± 1.1 (6)[a]
Propionate	5	2.2 ± 0.3	8.9 ± 0.4	6.4 ± 0.6 (9)[b]
Butyrate	5	0	8.5 ± 0.5	14.3 ± 1.2 (6)[a]
Valerate	5	1.4 ± 0.3	8.9 ± 1.2	5.6 ± 1.3 (4)[c]
Nonanoate	1	0	9.1 ± 1.1	13.7 ± 2.3 (3)
Oleate	1	0	8.6 ± 0.4	15.1 ± 1.3 (8)[b]

Note. Values are means ± SEM expressed as μmoles glucose produced/30 min/g hepatacyte (wet wt) with number of experiments in parenthesis. Glucose production rates without lactate or FA were 0.8 ± 0.1 μmole/30 min/g (N = 23). Glucose production rates from propionate or valerate alone were subtracted from values obtained with lactate. Significant differences are indicated. Lactate, 10 m*M*, was used as substrate.

[a–c] P vs value with no FA: [a], <0.005; [b], <0.001; [c], < 0.025.

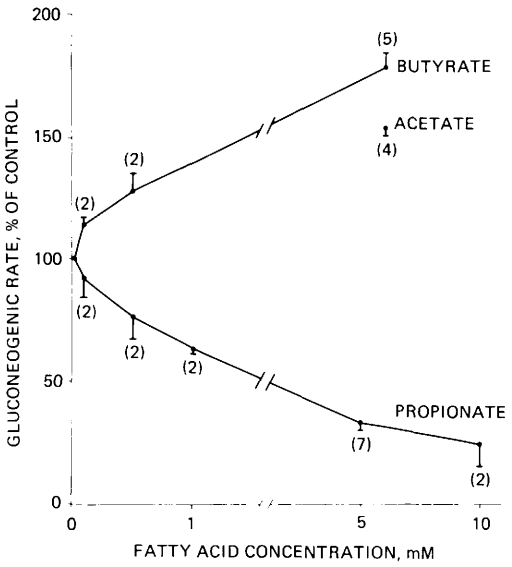

FIG. 1. Effects of butyrate, acetate, and propionate on glucose production from [^{14}C]lactate in isolated rat hepatocytes. Values are expressed as percentage of values in the absence of fatty acids with SEM indicated by bars. Number of experiments are indicated in parentheses.

Effect of SCFA on hepatic rates of glycolysis. When the rates of conversion of glucose to lactate were measured, acetate and butyrate decreased this process while propionate and valerate increased it (Table II). Acetate and butyrate were not accompanied by measurable increases in lactate production in the absence of glucose but lactate production rates from glucose were significantly decreased by acetate (12%) and butyrate (15%). Maximal rates of lactate production were 6.0 and 4.3 μmole/g/30 min from propionate and valerate, respectively, in the absence of glucose; these values were subtracted from values in the presence of glucose to compute net glycolytic rates. Furthermore, 5 m*M* concentrations of propionate and valerate significantly increased net rates of glucose conversion to lactate by 25 and 15%, respectively.

Effect of nonanoate and oleate on hepatic gluconeogenesis and glycolysis rates. Because of the consistent differences between SCFA with even vs odd numbers of carbon atoms, we compared the effects of nonanoate, a 9-carbon fatty acid, and oleate, an 18-carbon fatty acid, on glucose production and glycolysis. The effects of nonanoate and oleate were similar and were consistent with the well-documented effects of long-chain fatty acids on hepatic glucose metabolism (11). Nonanoate at 1 m*M* concentrations significantly increased glucose production rates from lactate by 51% (Table I). Oleate also had a pronounced effect on glucose production with maximal effects observed at 1 m*M* concentration. The net effects of nonanoate and oleate on glucose production rates were similar in direction to the effects of acetate and butyrate but opposite in direction to the effects of propionate and valerate.

Both nonanoate and oleate decreased rates of glycolysis; maximal decreases were observed at oleate concentrations between 0.5 and 1 m*M*. Lactate production rates from

TABLE II. EFFECTS OF FATTY ACIDS (FA) ON GLYCOLYTIC RATES IN ISOLATED RAT HEPATOCYTES

		Substrate		
			Glucose	
	FA concn (m*M*)	FA	No FA	With FA
Acetate	5	0	13.0 ± 1.0	11.5 ± 1.2 (8)[a]
Propionate	5	6.4 ± 0.4	13.6 ± 1.0	16.9 ± 1.1 (9)[b]
Butyrate	5	0	13.0 ± 1.0	11.1 ± 1.2 (8)[a]
Valerate	5	4.3 ± 0.6	11.8 ± 1.8	13.6 ± 1.7 (3)
Nonanoate	5	0	10.0 ± 1.7	7.7 ± 1.7 (3)
Oleate	1	0	12.3 ± 1.1	10.3 ± 1.1 (7)[c]

Note. Values are means ± SEM expressed as μmoles lactate produced/30 min/g hepatocyte (wet wt) with number of experiments in parenthesis. Lactate production rates without glucose or FA were 1.4 ± 0.2 μmole/g/30 min (N = 16). Significant differences are indicated. Glucose, 10 mM, was used as substrate.

[a-c] P vs value with no FA: [a], <0.001; [b], <0.005; [c], < 0.01.

glucose were 23% lower with 5 m*M* nonanoate and 15% lower with 1 m*M* oleate than values in the absence of these fatty acids (Table II). Thus the net effects of the longer chain fatty acids on glycolytic rates were similar in direction to the effects of acetate and butyrate but opposite in direction to the effects of propionate and valerate.

Discussion. Plant fiber intake alters the digestion, absorption and metabolism of carbohydrates by a variety of mechanisms. Certain fibers may alter the rate of gastric emptying (12), intestinal transit time (1), the rate of hydrolysis of carbohydrates (12), and the absorption rate of sugars (12). Fiber intake also influences the secretion of pancreatic and gut hormones (12) and may increase insulin receptor concentrations in certain tissues (12). The influence of SCFA fermentation products of fiber which are absorbed from the colon (5) on hepatic glucose metabolism, however, has not been thoroughly evaluated.

SCFA and longer-chain fatty acids have important hepatic effects on both glucose production and glycolysis. The distinctly different effects of propionate and valerate may be mediated by their unique metabolic products. Whereas acetate, butyrate, nonanoate and oleate are oxidized predominately to acetyl CoA (13, 14), propionate and valerate are converted sequentially to propionyl CoA, methylmalonyl CoA and succinyl CoA (6, 15). Oleate appears to stimulate hepatic gluconeogenic rates largely by activating pyruvate carboxylase via acetyl CoA generation (13, 14). Acetate, butyrate and nonanoate probably stimulate gluconeogenesis by this same mechanism (16, 17). In sharp contrast, propionate administration appears to lower hepatic acetyl CoA concentrations (18) and inhibits pyruvate carboxylase activity further via methylmalonyl CoA and succinyl CoA, specific inhibitors of this enzyme (11, 15).

Glycolytic rates are influenced importantly by the activity of phosphofructokinase (14, 19); citrate appears to be an important metabolic inhibitor of this enzyme (14, 19). Oleate metabolism is accompanied by increased tissue concentrations of citrate in liver (11, 13), muscle (19), and heart (20); decreased rates of glycolysis in these tissues in the presence of oleate have been attributed to citrate-induced inhibition of phosphofructokinase. In sharp contrast, propionate metabolism is accompanied by a reduction in hepatic citrate concentration (11) and valerate may induce similar changes since it is metabolized in a similar manner to propionate (6). The increase in glycolysis we observed with propionate and valerate may be related to decreased hepatic citrate concentrations.

The effects of increased plant fiber intake on portal vein concentrations of SCFA are not well delineated. Recently Illman and colleagues (21) measured SCFA concentrations from portal vein blood samples of rats fed standard rat chow, pectin or wheat bran diets. They reported propionate concentrations of 0.21 m*M* from chow-fed rats and values of 0.15 m*M* from pectin-fed rats. These concentrations are in the range that produced significant decreases of hepatic gluconeogenesis (Fig. 1). Illman *et al.* (21) noted portal vein blood acetate concentrations which were approximately fivefold higher than propionate concentrations while butyrate concentrations were roughly equivalent to propionate concentrations. Acetate, the predominate SCFA produced in the colon (5, 22) is not well metabolized in the liver when propionate is present (15, 23). Using a variety of combinations of SCFA, we documented that propionate overrode the effects of acetate on glucose metabolism in rat hepatocytes (J. W. Anderson, unpublished observations). Thus, even in the presence of several-fold higher concentrations of acetate, propionate acted to inhibit hepatic glucose production and stimulate hepatic glycolysis. Much further study is required to delineate the mechanisms of action of SCFA on glucose metabolism and the physiologic significance of our observations.

1. Anderson JW, Chen W-JL. Plant fiber. Carbohydrate and lipid metabolism. Amer J Clin Nutr **32**:346–363, 1979.
2. Jenkins DJA, Leeds AR, Gassull MS, et al. Unabsorbable carbohydrate and diabetes: Decreased postprandial hyperglycaemia. Lancet **2**:172–74, 1976.
3. Anderson JW, Ward K. High carbohydrate, high fiber diets for insulin-treated men with diabetes mellitus. Amer J Clin Nutr **32**:2312–2321, 1979.
4. Rivellese A, Giacco A, Genovese S, *et al.* Effect of

dietary fibre on glucose control and serum lipoproteins in diabetic patients. Lancet **1**:447–450, 1980.
5. Cummings JH. Short chain fatty acids in the human colon. Gut **22**:763–779, 1981.
6. Oberholzer VG, Levin B, Burgess EA, Young WF. Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. Arch Dis Child **42**:492–504, 1967.
7. Leichter SB, Anderson JW. Exogenous, but not endogenous, cyclic GMP reduces hepatic pyruvate kinase activity. Biochim Biophys Acta **659**:199–204, 1981.
8. Berry MN, Friend DS. High yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. J Cell Biol **43**:506–520, 1969.
9. Claus TH, Pilkis SJ, Park CR. Stimulation by glucagon of the incorporation of U^{14}-C-labeled substrates into glucose by isolated hepatocytes from fed rats. Biochim Biophys Acta **404**:110–123, 1975.
10. Exton JH, Park CR. Control of gluconeogenesis in liver. I. General features of gluconeogenesis in the perfused livers of rats. J Biol Chem **242**:2622–2636, 1967.
11. Blair JG, Cook DE, Lardy HA. Interaction of propionate and lactate in the perfused rat liver. Effects of glucagon and oleate. J Biol Chem **248**:3608–3614, 1973.
12. Anderson JW. Dietary fiber and diabetes. In: Vahouny GV, Kritchevsky D, eds. Dietary Fiber. New York, Plenum, 1982.
13. Brocks DG, Siess EA, Wieland OH. Distinctive roles of oleate and glucagon in gluconeogenesis. Eur J Biochem **113**:39–43, 1980.
14. Pilkis SJ, Park CR, Claus TH. Hormonal control of hepatic gluconeogenesis. Vitam Horm (NY) **36**:383–460, 1978.
15. Baird GD, Lomax MA, Symonds HW, Shaw DR. Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows *in vivo* in relation to lactation and nutrient supply. Biochem J **186**:47–57, 1980.
16. Ballard FJ. Supply and utilization of acetate in mammals. Amer J Clin Nutr **25**:773–779, 1972.
17. Chan TM, Freedland RA. Effects of glucagon on gluconeogenesis from lactate and propionate in the perfused rat liver. Proc Soc Exp Biol Med **151**:372–375, 1976.
18. Chan TM, Freedland RA. The effect of propionate on the metabolism of pyruvate and lactate in the perfused rat liver. Biochem J **127**:539–543, 1972.
19. Rennie MJ, Holloszy JO. Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. Biochem J **168**:161–170, 1977.
20. Bricknell OL, Opie LH. Effects of substrate on tissue metabolic changes in the isolated rat heart during under-perfusion and on release of lactate dehydrogenase and arrhythmias during reperfusion. Circ Res **43**:102–115, 1978.
21. Illman RJ, Trimble RP, Snoswell AM, Topping DL. Daily variations in the concentrations of volatile fatty acids in the splanchnic blood vessels of rats fed diets high in pectin and bran. Nutr Rep Int **26**:439–446, 1983.
22. Cummings JH, Southgate DAT, Branch WJ, *et al.* The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. Brit J Nutr **41**:477–485, 1979.
23. Smith RM. Interaction of acetate, propionate and butyrate in sheep liver mitochondria. Biochem J **124**:877–881, 1971.

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