ELSEVIER

Effects of propionate on rat hepatocyte metabolism

Sébastien Petitet, Christine Morand, Catherine Besson, Christian Rémésy, and Christian Demigné

Laboratoire des Maladies Metaboliques et Micronutriments, Centre de Recherche en Nutrition Humaine, Ceyrat, France

The aim of the present study was to investigate the effects of propionate or even-chain fatty acids (butyrate, octanoate, and oleate) on glucogenic or ketogenic substrate metabolism as well as ureagenesis. Therefore, rat hepatocytes were incubated in complex media containing near-physiological concentrations of glucose, lactate, alanine, and glutamine. On hepatocytes from fed rats, propionate impaired lactate metabolism, whose threshold for net utilization was shifted from 2.5 mM to more than 4 mM, whereas even-chain fatty acids presented the opposite effect. Furthermore, propionate, in contrast to even-chain fatty acids, effectively lowered lactate utilization in hepatocytes from starved rats. Determination of the cellular concentration of glycolysis effectors (citrate, fructose 2,6-bisphosphate, and xylulose 5-phosphate) indicates that this pathway might be accelerated by propionate and depressed by even-chain fatty acids. Cellular pyruvate was markedly increased by propionate and depressed by even-chain fatty acids. Thus, the sparing effect of propionate on lactate seems mainly a consequence of sustaining high pyruvate concentrations in hepatocytes. Ammonia increased lactate utilization, and propionate partially thwarted the effect of ammonia on lactate flux. Propionate did not influence alanine utilization, but favored the production of alanine in an amino acid-free medium. Ketogenesis, very active in hepatocytes from starved rats, was significantly decreased in the presence of propionate, whatever the fatty acid precursor (butyrate, octanoate, or oleate) together with β-hydroxybutyrate/acetoacetate ratio. Because propionate did not affect oleate utilization, it could switch fatty acids from ketogenesis to other pathways like reesterification. Finally, propionate exerts important effects on cellular metabolism, and it helps to reduce the catabolism of some substrates such as lactate or oleate. (J. Nutr. Biochem. 9:652–658, 1998) *© Elsevier Science Inc. 1998*

Keywords: propionate; short-chain fatty acids; glucose; fatty acids; isolated hepatocytes; ketone bodies

Introduction

Intestinal breakdown of dietary fibers by the microflora leads to production of substantial amounts of short-chain fatty acids (SCFAs), mostly acetate, propionate, and butyrate, which are almost completely absorbed along the digestive tract.[1] Whereas butyrate is widely metabolized by enterocytes, propionate and acetate are poorly consumed in the intestinal mucosa and may therefore reach the liver via the portal vein. The hepatic uptake of acetate depends on its availability, but efferent blood is almost completely devoid of propionate whatever the nutritional status.[2] In fact, propionate availability for hepatocytes is maximal during the late absorption and postabsorptive periods. The hepatic metabolism of propionate contributes to the synthesis of four-carbon dicarboxylates such as malate and oxaloacate, which may enter the gluconeogenic pathway.[2] Thus, it is conceivable that propionate, when available, may interfere with the utilization of various substrates in this pathway, when active in liver cells.

Although it has been reported that propionate-supplemented diets reduce both postprandial hyperglycemia and hyperinsulinemia in normal humans,[3] it seems that the effects of propionate on hepatic glucose output and peripheral glucose utilization are not physiologically significant either in normal or diabetic rats.[4,5] Propionate and butyrate, however, affect lactate utilization by liver cells.[2,6] Furthermore, propionate may affect lipid metabolism. Although its

Address correspondence and reprint requests to Dr. S. Petitet, Laboratoire des Maladies Métaboliques et Micronutriments, Centre de Recherche en Nutrition Humaine, INRA de Clermont-Ferrand/Theix, 63122 Ceyrat, France.
Received February 27, 1998; accepted August 4, 1998.

655 Avenue of the Americas, New York, NY 10010
0955-2863/98/$19.00
PII S0955-2863(98)00073-4

effects on cholesterogenesis remain questionable in vivo,[7,8] propionate is an effective inhibitor of fatty-acid and cholesterol synthesis in vitro.[9–11] Moreover, propionate has a high affinity for carnitine, a component of the acyl-carnitine tranferase system (ACT), which permits transfer of long-chain fatty acids from the cytosol to the mitochondrial matrix.

The aim of the present study was to investigate modulation of fuel selection by isolated hepatocytes. Therefore, we examined a possible sparing effect of propionate on lactate utilization by hepatocytes isolated from fed or starved rats, incubated in media containing almost physiological concentrations of glucogenic substrates. Moreover, experiments have been carried out to further document effects of propionate on nitrogen and long-chain fatty acid metabolism.

Methods and materials

Animals and diets

Male Wistar rats weighing 200–220 g derived from the colony of laboratory animals of I.N.R.A (National Institute of Agronomic Research, Clermont-Ferrand/Theix) were fed ad libitum a standard diet (55% carbohydrates, 30% lipids, 15% proteins); food was removed 15 hr before experiments on starved animals. Rodents were on a 12 hr light/12 hr dark cycle. Rats were anesthetized by intraperitoneal injection with sodium pentobarbital (40 mg/kg body weight). Liver perfusion was performed at 9.30 A.M. (i.e, 1.5 hr after light was on) for the isolation of hepatocytes involving collagenases as previously described by Berry and Friend.[12]

Incubation of hepatocytes

Cells were resuspended in Krebs' buffer (pH 7.4) flushed with a gas mixture of oxygen (95%) plus carbon dioxide (5%) and supplemented with bovine serum albumin 2%, glucose 7 mM, and glutamine 0.5 mM. Density of cells in the medium was adjusted to 40 mg cell/mL for intracellular metabolites assays and to 20 mg cell/mL for other measurements. Staining with trypan blue was performed to evaluate initial cell quality (90–95%). Aliquots (2.5 mL) of cellular suspension were then transfered into 10-mL glass vials sealed under a O_2:CO_2 (95:5 vol/vol) atmosphere and containing appropriate effectors and substrates. Hepatocytes were then incubated during 45 min at 37°C with appropriate agitation, and were gased every 15 min. Hepatocyte metabolism was stopped by centrifugation (1 min; 14,000 rpm). The supernatant was then rapidly removed and cooled down to 4°C, whereas cells were frozen in liquid nitrogen until analysis.

Assay of metabolites

All chemicals were purchased from Sigma (Sigma-Aldrich, L'Isle-d'Abeau, France). Supernatants were used either directly, after deproteinization with perchloric acid (0.6 M; 2 vol/vol supernatant), or after deproteinization and neutralization with potassium carbonate (0.75 M; 1 vol/3.5 vol acid supernatant). Xylulose 5-phosphate (X5P) concentration was measured in cells after deproteinization with perchloric acid (0.6 M; 500 μL) and neutralization with potassium carbonate (1.6 M; 1 vol/4.5 vol). Although assays of X5P by fluorimetric method have been already reported,[13] the procedure we have developed is an adaptation of those reported by Casazza[14] and Racker[15] in order to have a spectrophotometric determination. Elimination of ketopentose phosphate was then required and obtained with addition of NaOH (10 M; 1 vol/9 vol; 10 min; 25°C) and further neutralization by addition of HCl (10 M; 1 vol/9 vol). Then the supernatant was diluted in 2.5 vol of concentrated buffer (glycylglycine 250 mM pH 7.7; $MgCl_2$ 0.3 M; thiamine pyrophosphate 5 g/L; erythrose 4-phosphate 0.7 mM; NADH 0.18 mM; triose-phosphate isomerase 7 u/sample; glycerol-phosphate dehydrogenase 0.7 u/sample). Absorbance was measured before and after addition of transketolase (0.5 u/sample), used to initiate the reaction. This final solution was then incubated at 25°C for a 5-min-long period before absorbance measurement. The X5P concentration was then calculated using standard solutions. Oleate and octanoate concentrations were directly measured in the supernatant using a commercial kit (Nefa C*, Wako Chemicals GmbH, Neuss, Germany). Fructose 2,6-bisphosphate ($F2,6P_2$) was determined in alkaline extracts as described by van Schaftigen.[16] Lactate,[17] alanine,[18] urea,[19] and β-hydroxybutyrate[20] (β-OH-but) were determined enzymatically in acid extracts. Ammonia,[21] citrate,[22] pyruvate,[23] and acetoacetate[24] (Acac) were measured in deproteinized and neutralized extracts.

Statistics

Values are given as the means ± SEM of four isolation procedures. Significance of difference ($P < 0.05$) between mean values was determined by analysis of variance (ANOVA).

Results

A first set of experiments has been carried out to determine the concentration of lactate required to reach the equilibrium between incubation medium and liver cells. Hepatocytes isolated from fed rats were incubated in a basal medium with increasing lactate concentrations (0–5 mM). Furthermore, pyruvate was simultaneously added to maintain a constant lactate/pyruvate ratio (close to 8). It appears that, independently of the addition of short-chain fatty acids and within a given range of lactate concentrations, a linear relationship exists between lactate flux and its initial concentration (*Figure 1*). When hepatocytes were incubated in a SCFA-free medium, a near equilibrium between utilization and production was obtained in the presence of lactate (2 mM). When a physiological concentration of lactate (2.5 mM) was used, the effect of propionate and even-chain fatty acids was particularly significant (*Figure 2*). Lactate was removed at a slow rate (−0.05 μmol/min/g cell) from the external medium by cells incubated in control conditions, whereas it was released (+0.44 μmol/min/g cell) when propionate was present. Conversely, its net utilization was markedly increased (up to 1.2 μmol/min/g cell) by the addition of either butyrate, octanoate, or oleate.

Table 1 represents cellular concentrations of key metabolites either regulating or reflecting glucose metabolism in hepatocytes. Experimental conditions were similar to those described for *Figure 2*. Concentrations of pyruvate were significantly increased in the presence of propionate (0.59 ± 0.06 vs. 0.40 ± 0.05 μmol/g cell in control conditions), whereas it was decreased (−70%) by the other fatty acids. The cellular content of $F2,6P_2$ was not affected by propionate (12.0 ± 1.4 vs. 14.0 ± 1.3 μmol/g cell in controls) but it was significantly decreased with even-chain fatty acids, especially with octanoate (−73%). The cellular content of X5P was not significantly modified by fatty acids (whether even-chain fatty acids or propionate).

Further experiments have been performed to determine

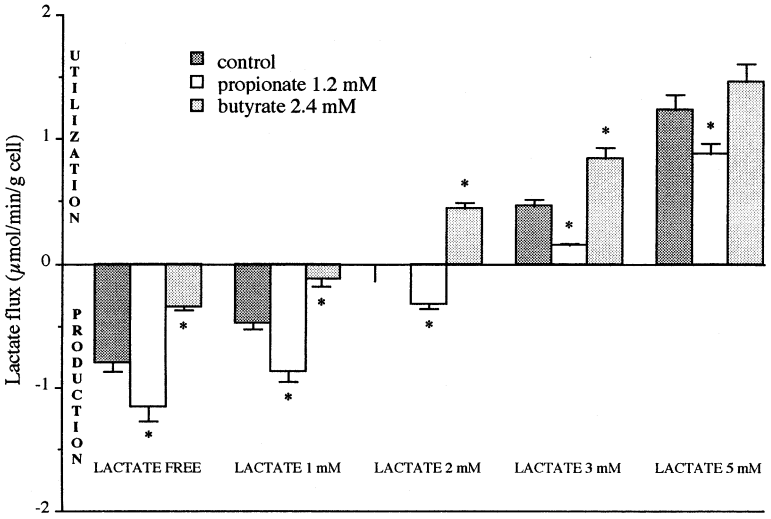

Figure 1 Influence of propionate or butyrate on lactate metabolism by hepatocytes from fed rats. Each value is the mean ± SEM, $n = 4$. *Significantly different from control conditions ($P < 0.05$).

the effects of propionate on ureagenesis and alanine flux. The effects of ammonia (2.5 mM) have been studied on hepatocytes isolated from fed rats and incubated in a medium lacking amino acids. In these conditions, ammonia led to a large increase of lactate utilization and shifted alanine utilization toward a slight output (*Figure 3a*). Moreover, it is noteworthy that ammonia thwarted the effect of propionate on lactate flux. Propionate did not significantly affect ammonia utilization or urea production. Alanine utilization at 0.7 mM was also not influenced by propionate addition (results not shown).

As shown in *Figure 4*, in contrast to fed state, there was a concentration-dependent uptake of lactate by hepatocytes from starved rats, whatever its initial concentration. Furthermore, this utilization of lactate was higher than in fed rats. The flux of lactate (initial concentration 2.5 mM) was significantly decreased (−57%) by propionate and increased by butyrate (+45%) or oleate (+57%). Therefore, it appears that propionate had a lowering effect on lactate utilization even in hepatocytes isolated from starved rats.

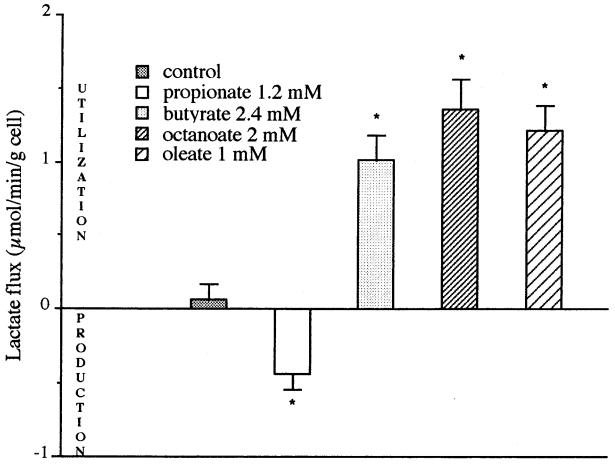

Figure 2 Influence of fatty acids on lactate flux in isolated hepatocytes incubated with lactate 2.5 mM. Each value is the mean ± SEM, $n = 4$. *Significantly different from control conditions ($P < 0.05$).

To assess the effects of propionate on substrate selection in the liver of starved animals, further experiments on fatty acid utilization were performed. As shown on *Figure 5*, oleate consumption (0.5 μmol/min/g cell) was not affected by propionate at either physiological (0.6 or 1.2 mM) or relatively high (2.4 mM) concentrations (results not shown). The production of ketone bodies, a major metabolic pathway during starvation, was determined, butyrate, octanoate, and oleate being used as ketone body precursors. The total production of acetoacetate and β-hydroxybutyrate (*Table 2*) was very high (up to 3 μmol/min/g cell). Oleate and octanoate promoted ketogenesis chiefly as β-hydroxybutyrate (81%), whereas acetoacetate was the major end-product (72%) when butyrate was the precursor. Octanoate at 2 mM was the most effective precursor, whereas oleate at 1 mM was less effective. Propionate at 1.2 mM had a potent anti-ketogenic effect, and it led to a decrease of approximately 0.5 μmol/min/g cell, whatever the precursor. Nevertheless, the impact of propionate seems different according to the chain-length of fatty acids. High values of β-hydroxybutyrate/acetoacetate ratio in control conditions (up to 4) were markedly depressed with propionate (down to 2).

Discussion

In various monogastric species, SCFAs provide 5–10% of total energy supply.[25] In fact, the energetic impact of SCFAs depends on the tissues. Previous studies have shown that acetate is taken up when its portal concentration is high (>0.5 mM), whereas propionate and butyrate are completely removed from portal blood.[2] Because butyrate is extensively metabolized in the colon mucosa,[26] it appears that only propionate is likely to influence liver metabolism. In herbivorous species, propionate is mostly directed to gluconeogenesis, whereas this pathway is poorly active in fed monogastrics. Thus, propionate may be channeled to alternative pathways in fed monogastrics.

Lactate plays a key role in glucose cycling, and its hepatic uptake is modulated by both nutritional and metabolic factors (stimulation with a lipids-rich diet or starvation). In hepatocytes isolated from fed rats, the utilization of

Table 1 Effect of short-chain, medium-chain, or long-chain fatty acids on the intracellular concentrations of metabolites involved in glucose metabolism regulation by hepatocytes from fed rats

Conditions	Pyruvate (μmol/g cell)	Citrate (μmol/g cell)	$F2,6P_2$ (nmol/g cell)	X5P (nmol/g cell)
Control	0.40 ± 0.05	0.95 ± 0.07	14.0 ± 1.3	38.6 ± 4.2
Propionate (1 mM)	0.59 ± 0.06*	0.88 ± 0.07	12.0 ± 1.4	45.1 ± 3.5
Butyrate (2.5 mM)	0.25 ± 0.03*	1.90 ± 0.22*	8.5 ± 0.2*	27.6 ± 2.8*
Octanoate (2 mM)	0.12 ± 0.02*	2.10 ± 0.15*	3.3 ± 0.5*	32.2 ± 2.2*
Oleate (1 mM)	0.18 ± 0.08*	2.03 ± 0.17*	6.4 ± 0.6*	33.2 ± 3.7*

*Significantly different from control conditions ($P < 0.05$).

lactate is closely dependent on its extracellular concentration as it is released when extracellular contents are below values ranging from 2 to 2.5 mM.[27] Fatty acids such as acetate, propionate, and butyrate are liable to affect the threshold and the flux of lactate utilization. The inhibitory effect of propionate on lactate utilization is clearly shown, whatever the conditions.

Butyrate exerts an effect similar to that caused by either octanoate or oleate. Glycolysis or pyruvate mitochondrial pathways may be affected by fatty acids, and measurement of the cellular concentrations of both metabolites and effectors of glycolysis (*Table 2*) affords a better understanding of these processes.[6,28] Citrate was not affected by propionate, although it was significantly increased by even-chain fatty acids. This is in agreement with the increase of acetyl-CoA availability caused by fatty acids and influencing the cellular contents of citrate. This last is a very effective inhibitor of PFK-2, enhancing the direct inhibition of PFK-1. $F2,6P_2$, one of the most specific effectors of glycolysis, is generated in the cytosolic compartment where it exerts its action.[29] Propionate had no effect on this effector, which was, in contrast, markedly depressed by even-chain fatty acids.

For the control of glycolysis, mechanisms are probably complex: increase of citrate or, as recently reported in the literature,[30] involvement of X5P, a metabolite of the pentose-phosphate pathway. Its increase would lead to the activation of a specific protein phosphatase 2A, which in

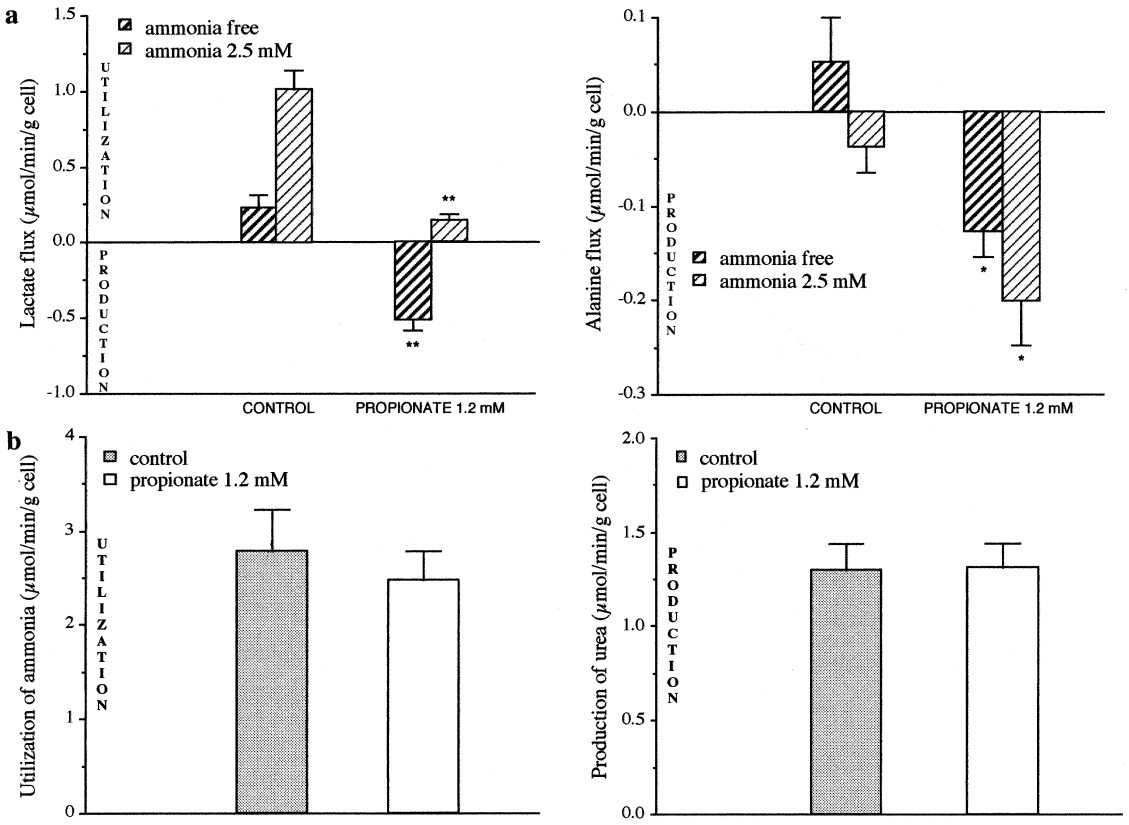

Figure 3 Effect of propionate on lactate and alanine fluxes with or without ammonia (a), and comparison of ammonia utilization and ureogenesis (b) with or without propionate. Each value is the mean ± SEM, $n = 4$. Both * and ** are significantly different from control conditions ($P < 0.05$, $P < 0.01$).

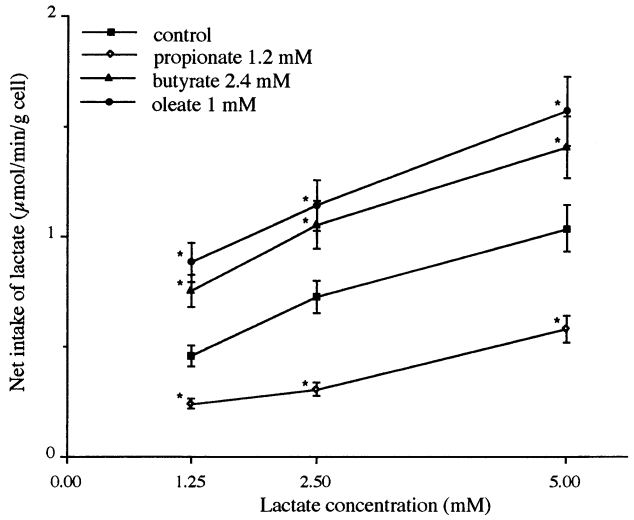

Figure 4 Influence of propionate, butyrate, and oleate on lactate utlization by isolated hepatocytes from starved rats. Each value is the mean ± SEM, $n = 4$. *Significantly different from control conditions ($P < 0.05$).

turn activates the PFK-2 by catalyzing the dephosphorylation of the enzymatic complex, hence an increase of $F2,6P_2$ concentrations. Nevertheless, measurement of X5P concentrations, in contrast to those of other effectors, were not conclusive.

The most effective fatty acid on X5P concentration was apparently butyrate, which also slightly affected cellular contents of $F2,6P_2$. Simultaneous decreases of X5P and $F2,6P_2$ have already been reported[31] in presence of acetate, propionate, or butyrate, but SCFAs in this case were at a high concentration (5–10 mM) and the liver was previously perfused with glucose (40 mM). Thus, only a slight stimulation of glycolysis by propionate could be observed, as shown by variations of citrate, pyruvate, or $F2,6P_2$ and the

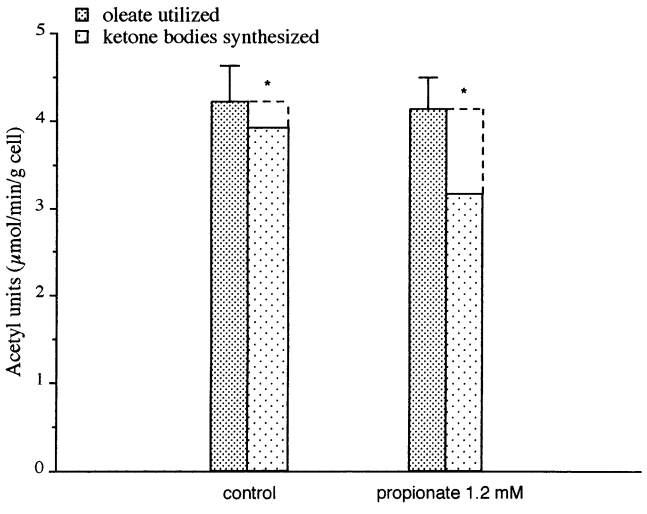

Figure 5 Effect of propionate on oleate utilization and on ketone body synthesis: Both metabolic fluxes are expressed in μmol acetyl units/min/g cell. Each value is the mean ± SEM, $n = 4$. *Significantly different from control conditions ($P < 0.05$).

flux through PFK-1.[6] Release of lactate in the presence of propionate seems a consequence of the increase of intracellular pyruvate. Sherry et al[32] have shown that some propionate could be converted to lactate through the Krebs' cycle and malic enzyme. Extensive utilization of propionate in the Krebs' cycle has priority on pyruvate utilization; thus, pyruvate generated through glycolysis is recovered as lactate.

Additionally, propionate may inhibit mitochondrial pyruvate utilization through the effects of its CoA-esters on pyruvate carboxylase. Stimulation of lactate utilization by even-chain fatty acids that yield acetyl-CoA is consistently observed. It is likely that the increase of acetyl-CoA and mitochondrial NADH inhibits the flux through pyruvate-dehydrogenase,[32] although acetyl-CoA also stimulates pyruvate-carboxylase.[33] Whatever the nutritional status, the present results suggest that propionate is effective in inhibiting lactate utilization even when this substrate is extensively metabolized. During the postabsorptive period, propionate may contribute in feeding the glucose precursor pool, which is important in reducing the intensity of glucose-lactate cycling or protein mobilization.

In the literature, data suggest that acetyl-CoA stimulates the mitochondrial synthesis of acetyl-glutamate, resulting in the activation of carbamoyl-phosphate synthase. Propionyl-CoA could interfere with the synthesis of acetyl-glutalmate, thus inhibiting ureagenesis.[34] Our experiments are not in keeping with this view, because propionate did not affect urea production. In contrast, results favor a slight activation of urea synthesis by even-chain fatty acids. It is noteworthy that ammonia may stimulate the utilization of lactate; the release of lactate caused by propionate is reduced by ammonia, but a net uptake by hepatocytes is not enhanced. This is of physiological interest because there is a permanent digestive absorption of ammonia simultaneously to SCFAs. Thus, ammonia stimulates lactate utilization and propionate thwarts its effects.

The rise in pyruvate induced by propionate promotes alanine synthesis, and recycling of ammonia from the digestive tract may participate in the synthesis of nonessential amino acids; therefore, high availability of propionate may contribute to nitrogen sparing. In this view, it has been shown on other experimental models that ammonia may channel propionate to amino-acid synthesis.[35] Glutamine may contribute to a large extent to nitrogen cycling, when synthesized instead of urea.[36]

Ketogenesis is an important metabolic pathway especially during starvation or in pathological situations such as NIDDM ketoacidosis.[37] The hepatic catabolism of free fatty acids into water-soluble 4-carbon molecules represents energetic fuel supply for peripheral tissues. Ketogenesis is chiefly regulated at the step of transfer of long-chain fatty acids into mitochondria, mediated by the ACT system.[38] Octanoate and butyrate, however, escape this step and are efficiently converted to ketone bodies.

In our model of starved-rat hepatocytes, it is noteworthy that propionate exerts a striking lowering effect on ketogenesis. Propionate also depressed the β-OH-but/Acac ratio. A possible mechanism of this effect may involve a reduced fatty-acid uptake by hepatocytes, although experimental data do not support this view. In vivo, administrating a

Table 2 Effect of propionate on ketone body synthesis from different fatty acids as precursors by isolated hepatocytes from starved rats

Conditions	Precursor	Production flux (μmol/min/g cell)			
		Acetoacetate	β-hydroxybutyrate	Total ketone bodies	β-OH but/acac
Control	butyrate 2.5 mM	1.66 ± 0.02	0.66 ± 0.03	2.31 ± 0.04	0.40 ± 0.01
	octanoate 2 mM	0.64 ± 0.05	2.69 ± 0.09	3.33 ± 0.05	4.27 ± 0.44
	oleate 1 mM	0.38 ± 0.02	1.62 ± 0.08	1.99 ± 0.07	4.31 ± 0.03
Propionate 1.2 mM	butyrate 2.5 mM	1.34 ± 0.01	0.41 ± 0.04	1.75 ± 0.03	0.31 ± 0.03
	octanoate 2 mM	1.01 ± 0.03	1.83 ± 0.06	2.84 ± 0.09	1.81 ± 0.01
	oleate 1 mM	0.61 ± 0.04	0.90 ± 0.11	1.51 ± 0.07	1.50 ± 0.25

mixture of SCFAs containing acetate may affect this process by lowering blood concentrations of free fatty acids owing to the antilipolytic effect of acetate.[39] Propionate could switch fatty acids from ketogenesis to other pathways, such as reesterification. Propionate may have an antiketogenic effect by increasing the mitochondrial availability of oxaloacetate, contributing to direct acetyl-CoA toward the citric acid cycle. Further mechanisms might be invoked: succinyl-CoA is an inhibitor of mitochondrial HMG-CoA synthase, which catalyzes the conversion of acetyl-CoA to acetoacetate.[40] The β-oxidation of fatty acids may be inhibited by popionyl-CoA.[41,42] Furthermore, the high affinity of propionyl-CoA for carnitine (leading to the formation of propionyl-carnitine exported out of liver cells) may contribute to the depletion of carnitine pool, hence lowering the flux of fatty acids from the cytosol to the matrix.[43,44]

Finally, in addition to the endocrine factors, substrate availability exerts a direct control on hepatic metabolism. Propionate displays important effects on cellular metabolisms that are directed to the saving of energetic compounds.

References

1 Morand, C., Rémésy, C., Levrat, M-A., and Demigné, C. (1992). Replacement of digestible wheat starch by resistant cornstarch alters splanchnic metabolism in rats. *J. Nutr.* **122,** 345–354

2 Rémésy, C., Demigné, C., and Morand, C. (1995). Metabolism of short-chain fatty acids in the liver. In *Physiological and Clinical Aspects of Short-Chain Fatty Acids* (J.H., Cummings, J.L. Rombeau, and T. Sakata, eds.), pp. 171–190, Cambridge University Press, Cambridge, UK

3 Venter, C.S. and Vorster, H.G. (1989). Possible metabolic consequences of fermentation in the colon for humans. *Med. Hypotheses* **29,** 161–166

4 Boillot, J., Alamovitch, C., and Berger, A.M. (1995). Effect of dietary propionate on hepatic glucose production, whole-body glucose utilization, carbohydrate and lipid metabolism in normal rats. *Br. J. Nutr.* **73,** 241–251

5 Cameron-Smith, D., Collier, G.R., and O'Dea, K. (1994). Effect of propionate on in vivo carbohydrate metabolism in streptozotocin-induced diabetic rats. *Metabolism* **43,** 728–734

6 Morand, C., Besson, C., Demigné, C., and Rémésy, C. (1994). Importance of the modulation of glycolysis by fatty acids in isolated hepatocytes from fed rats. *Arch. Biochem. Biophys.* **309,** 254–260

7 Berggren, A.M., Nyman, M.G.L., and Lundquist, I. (1996). Influence of orally and rectally administered propionate on cholesterol and glucose metabolism in obese rats. *Br. J. Nutr.* **76,** 287–296

8 Levrat, M.-A., Favier, M.-L., Moundras, C., Rémésy, C., and Demigné, C. (1994). Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats. *J. Nutr.* **124,** 531–538

9 Demigné, C., Morand, C., Levrat, M.-A., Besson, C., Moundras, C., and Rémésy, C. (1995). Effect of propionate on fatty acid and cholesterol synthesis and acetate metabolism in isolated rat hepatocytes. *Br. J. Nutr.* **74,** 209–219

10 Lin, Y., Vonk, R.J., and Maarten, J.H.S. (1995). Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. *Br. J. Nutr.* **74,** 197–207

11 Nishina, P.M. and Freedland, R.A. (1990). Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *J. Nutr.* **120,** 667–673

12 Berry, M.N. and Friend, D.S. (1969). High-yield preparation of isolated rat liver parenchymal cells. *J. Cell. Biol.* **43,** 506–520

13 Kaufman, F.C., Brown, J.G., Passoneau, J.V., and Lowry, O.H. (1969). Effects of changes in brain metabolism on levels of pentose phosphate pathway intermediates. *J. Biol. Chem.* **244,** 3647–3653

14 Casazza, J.P. and Veech, R.L. (1986). The measurement of xylulose 5-phosphate, ribulose 5-phosphate and combined sedoheptulose 7-phosphate and ribose 5-phosphate in liver tissues. *Anal. Biochem.* **159,** 243–248

15 Racker, E. (1974). D-xylulose 5-phosphate. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 3, pp. 399–406, Academic Press, New York, NY, USA

16 van Schaftigen, E. (1988). D-fructose 2,6-bisphosphate: determination with lactate dehydrogenase and NAD. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 6, pp. 335–341, Academic Press, New York, NY, USA

17 Gutmann, I. and Wahlefeld, A.W. (1974). In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 3, pp. 1464–1468, Academic Press, New York, NY, USA

18 Williamson, D.H. (1974). L-alanine: determination with alanine dehydrogenase. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 4, pp. 1679–1685, Academic Press, New York, NY, USA

19 Gutmann, I. (1974). Determination of urea with glutamate dehydrogenase as indicator enzyme. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 4, pp. 1794–1797, Academic Press, New York, NY, USA

20 Williamson, D.H. and Mellamby, J. (1974). D-(−)-3-hydroxybutyrate. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 4, pp. 1836–1839, Academic Press, New York, NY, USA

21 Kun, E. and Kearney, E.B. (1974). Ammonia. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 4, pp. 1802–1806, Academic press, New York, NY, USA

22 Möllering, H. (1989). Citrate. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 7, pp. 2–12, VCH, Weinheim, Germany

23 Czok, R. and Lamprecht, W. (1974). Pyruvate, phosphoenolpyruvate and D-glycerate-2-phosphate. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 3, pp. 1446–1451, Academic Press, Weinheim, Germany

24 Mellamby, J. and Williamson, D.H. (1974). Acetoacetate. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), vol. 4, pp. 1840–1843, Academic Press, New York, NY, USA

25 Livesey, G. (1990). Energy value of unavailable carbohydrate and diets: an inquiry and analys. *Am. J. Clin. Nutr.* **51,** 617–637

26 Ardawi, M.S. and Newsholme, E.A. (1985). Fuel utilization in colonocytes of the rat. *Biochem. J.* **231,** 713–719

27 Morand, C., Rémésy, C., and Demigné, C. (1993). Fatty acids are potent modulators of lactate utilization in isolated hepatocytes from fed rats. *Am. J. Physiol.* **264,** E816–E823

28 Hue, L., Maisin, S., and Rider, M.H. (1988). Palmitate inhibits liver

glycolysis: involvement of fructose 2,6-bisphosphate in the glucose/fatty acid cycle. *Biochem. J.* **251,** 541–545
29 Pilkis, S.J., El-Magharabi, M.R., and Claus, T.H. (1990). Fructose-2,6-bisphosphate in control of hepatic gluconeogenesis. *Diabetes Care* **13,** 582–599
30 Liu, Y.Q. and Uyeda, K. (1996). A mechanism of regulation of hepatic Fru 2,6-P2 concentration upon refeeding: involvement of xylulose 5-P and cyclic-AMPc. *Biochem. Biophys. Res. Commun.* **221,** 554–558
31 Liu, Y.Q. and Uyeda, K. (1996). A mechanism for fatty acid inhibition of glucose utilization in liver. *J. Biol. Chem.* **271,** 8824–8830
32 Sherry, A.D., Malloy, C.R., Roby, R.E., Rajapogal, A., and Jeffrey, F.M.H. (1988). Propionate metabolism in the rat heart by ^{13}C n.m.r. spectroscopy. *Biochem. J.* **254,** 593–598
33 Wieland, O.H. (1983). The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev. Physiol. Biochem. Pharmacol.* **96,** 124–169
34 Barritt, G.J., Zander, G.L., and Utter, M.F. (1976). The regulation of pyruvate carboxylase in mammalian species. In *Gluconeogenesis: Its Regulation* (R.W. Hanson and M.A. Mehlman, eds.), pp. 3–46, Wiley, New York, NY, USA
35 Coudé, F.X., Sweetman, L., and Nyhan, W.L. (1979). Inhibition by propionyl-coenzyme A of *N*-acetylglutamate synthesis in rat liver mitochondria. *J. Clin. Invest.* **64,** 1544–1551
36 Demigné, C., Yacoub, C., Morand, C., and Rémésy, C. (1991). Interactions between propionate and amino acid metabolism in isolated sheep hepatocytes. *Br. J. Nutr.* **65,** 301–317
37 Rémésy, C., Moundras, C., Morand, C., and Demigné, C. (1997). Glutamine or glutamate release by the liver constitutes a major mechanism for nitrogen salvage. *Am. J. Physiol.* **272,** G257–G264
38 Cahill, G.F. (1981). Ketosis. *Kidney Int.* **20,** 416–423
39 Sherratt, H.S.A. and Spurway, T.D. (1994). Regulation of fatty acid oxidation in cells. *Biochem. Soc. Trans.* **22,** 423–427
40 Akanji, A.O., Bruce, M.A., and Frayn, K.N. (1989). Effect of acetate infusion on energy expenditure and substrate oxidation rates in diabetic and non-diabetics subjects. *Am. J. Clin. Nutr.* **43,** 107–115
41 Lowe, D.M. and Tubbs, P.K. (1985). Succinylation and inactivation of 3-hydroxy-3-methylglutaryl-CoA synthase by succinyl-CoA and its possible relevance in the control of ketogenesis. *Biochem. J.* **232,** 37–42
42 Fedotcheva, N.I., Gessler, N.N., Anikeeva, S.P., Ignat'ev, D.A., Bykhovskii, V., and Konrashova, M.N. (1993). Metabolites of the propionate pathway as regulators of fatty and dicarboxylic oxidation in liver mitochondria. *Biokhimiia* **58,** 599–605
43 Shaw, L. and Engel, P.C. (1985). The suicide inactivation of ox liver short-chain acyl-CoA dehydrogenase by propionyl-CoA. *Biochem. J.* **230,** 723–731
44 Brass, E.P., Fennessey, P.V., and Miller, L.V. (1986). Inhibition of oxidative metabolism by propionic acid and its reversal by carnitine in isolated rat hepatocytes. *Biochem. J.* **236,** 131–136