Increased Urinary Metabolite Excretion during Fasting in Disorders of Propionate Metabolism

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ABSTRACT. Odd-chain fatty acids are recognized precursors of propionate in man, but their clinical significance in disorders of propionate metabolism has not been well studied. Urinary excretion of methylmalonate, methylcitrate, propionylglycine, and 3-hydroxypropionate was measured in five children with methylmalonic acidemia and three with propionic acidemia during frequent or continuous feeding and after 10–18 h of fasting. There was a significant ($p < 0.01$) increase in the mean total measured metabolite excretion during fasting (fed 38.1 $\mu\text{mol/kg/h}$, fasting 54.6 in methylmalonic acidemia, fed 1.45, fasting 2.98 in propionic acidemia). Percentage rises in each subject were similar for all measured metabolites. These increases in metabolite excretion are most easily explained by mobilization and oxidation of odd-chain fatty acids in the fasting state. Prolonged fasting should be avoided in children with disorders of propionate metabolism. (*Pediatr Res* 27: 413–416, 1990)

Abbreviations

MMA, methylmalonic acidemia
PA, propionic acidemia

MMA and PA have long been recognized as disorders of valine, isoleucine, methionine, and threonine metabolism (1). However, the therapeutic response to dietary restriction of these amino acids is often less than optimal, suggesting that production of propionate from other sources may be significant in these conditions (1, 2). Gut bacterial propionate production appears to make a substantial contribution to metabolic imbalance in MMA as evidenced by the reduction in propionate metabolite excretion in urine after inhibition of gut bacterial activity with neomycin or metronidazole (3, 4).

A further source of propionate that is well defined but has not previously received attention is that arising from fat metabolism. Both the side-chain of cholesterol and odd-chain fatty acids are recognized precursors of propionate (5). Cholesterol turnover in man is, however, only about 2 $\mu\text{mol/kg/h}$ (6, 7), which is well below measured rates of propionate production in MMA and PA (8) and is unlikely to be quantitatively significant. However, odd-chain fatty acids accumulate in many tissues in disorders of propionate metabolism (9–11). Propionate is an obligate intermediate in odd-chain fatty acid oxidation, and catabolism of fatty acids is greatly increased in the fasting state. To gain a measure of the relative importance of odd-chain fatty acid me-

tabolism in MMA and PA, we have examined urinary metabolite excretion in the fed and fasted states.

MATERIALS AND METHODS

Five children with MMA and three with PA were studied. The clinical details appear in Table 1. The subjects were diagnosed on the basis of clinical presentation, urinary metabolite excretion pattern and *in vitro* enzyme assays. Methylmalonyl-CoA mutase activity ranged from <1–6% incorporation of [¹⁴C]propionate into protein compared with [³H]glycine in MMA subjects (normal 21–43) and propionyl-CoA carboxylase activity from <0.005 to 0.02 nmol/min/mg protein in PA subjects (normal 0.33–0.58). None of the MMA subjects were responsive to vitamin B₁₂. All studies were undertaken with the informed consent of the subjects' parents and all complied with the World Medical Association Declaration of Helsinki recommendations for conduct of clinical research.

Both fed and fasting studies were undertaken when the subjects had been clinically well and had remained on a constant treatment regime for the preceding month. Treatment consisted of restriction of dietary protein intake to provide 17–67 mg/kg/24 h of valine and, in most cases, supplementation of the diet with essential amino acids free of valine, isoleucine, methionine, and threonine. Four subjects (two with PA, subjects 2, 5, 6, and 8) were fed by continuous nasogastric infusion for at least 20 h/day. No subjects were receiving carnitine supplements.

Urinary propionate metabolites were measured in timed urine samples collected during feeding and fasting. Urinary metabolite excretion varies substantially within individuals; excretion was therefore measured over a number of collection periods lasting between 1 and 4 h. A total of six to 10 collections was made in the fed state and four collections in the fasted state. The bladder was emptied at the start of each collection period. Fed state collections were made within 4 h after a meal or during continuous nasogastric feeding on at least 4 different d. Fasting collections were made during the interval between 10 and 18 h after cessation of all oral intake in children under age 4 y, and between 14 and 21 h in subjects over age of 4 y. The collections were made after longer fasting periods in older patients because of the increased metabolic tolerance to fasting with age (12). The four fasting collections were made during two periods of fasting separated by at least 1 wk (two collections during each fast). There was no clear trend in urinary metabolite excretion patterns in the different samples collected within the feeding or fasting states; all values from each individual for each state were therefore meaned for further analysis.

Each urine sample was tested for the presence of ketones by dipstick and was then frozen at –20°C until analyzed. The major propionyl-CoA metabolites, methylmalonate, methylcitrate (2S,3R and 2S,3S stereoisomers), propionylglycine and 3-hydroxypropionate were extracted from urine together with other organic acids and minor propionyl-CoA metabolites using DEAE Sephadex (Pharmacia Fine Chemicals, Piscataway, NJ) and the

Received August 29, 1989; accepted November 29, 1989.
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dry extracted acids converted to their ethoxime and trimethylsilyl derivatives. The organic acid derivatives were separated using capillary gas-liquid chromatography on fused silica columns (25m CP-Sil 5 CB; Chrompack, London, UK) using a temperature programme from 70–250°C at 6°C/min with a 2- μ L sample and a 70:1 split injection. Carrier gas was helium, 1.4 mL/min. Components, including methylcitrate diastereoisomers, were identified from gas chromatograph retention data and, where necessary, capillary gas chromatography/mass spectrometry, and quantified by reference to *n*-tetracosane internal standard using relative response factors obtained with authentic standards (5). Metabolite excretion was expressed as mmol/mol creatinine and as an absolute excretion rate (μ mol/kg/h) for both individual metabolites and for the sum of all measured metabolites. Although urinary metabolite excretion values are conventionally expressed as mmol/mol creatinine, expression as μ mol/kg/h gives a value that can be compared with previously described rates of propionate production *in vivo* in MMA and PA. Urinary concentrations of free propionate were also measured by gas chromatography in selected samples from fed and fasting studies (13). Neither propionate nor the minor metabolites contributed significantly to total metabolite excretion in any subject. These metabolites are normally virtually or completely absent in MMA and PA subjects who are well.

Fed and fasted values were compared statistically using the Wilcoxon signed rank sum test. Statistical comparisons were made in relation to changes in activity of the precursor pathways to propionate rather than in relation to the enzymic disposal routes. The physiologic responses of the precursor pathways would be expected to be similar in each of the various disorders of propionate metabolism studied. However, enzyme activity, although heterogenous within the overall study group, would not be expected to change between the fed and fasted states. Results from all subjects were therefore combined for statistical comparisons.

RESULTS

All urine samples collected in the fed state were negative for ketones, whereas those collected in the fasting state were uniformly positive (usually ++ or ++++) and showed vastly increased concentrations of both β -hydroxybutyrate and acetoacetate on gas chromatographic analysis. These findings suggest that fasting urine collections were made during periods when subjects were dependent primarily on fat oxidation as a source of energy.

The urinary excretion of virtually all propionate metabolites increased during fasting in each individual (Table 2). Increases in methylcitrate (2S,3S and 3S,3R), 3-hydroxypropionate, and total metabolite excretion were statistically significant. The percentage increase with fasting (not shown) was similar for each metabolite, suggesting that the increased metabolite excretion resulted from increased propionate production in the fasting state. The urinary metabolite increase was not accompanied by clinical evidence of metabolic decompensation in any subject.

Table 1. *Clinical details of subjects*

Subject	Diagnosis	Age (y)	Clinical score*
1	MMA	6.5	5
2	MMA	0.6	7
3	MMA	3.6	1
4	MMA	5.5	4
5	MMA	1.4	5
6	PA	2.4	8
7	PA	22	0
8	PA	1.9	1

* Graded on a scale of 0 (mild) to 9 (severe) as described previously (12).

DISCUSSION

Excretion of propionate metabolites was increased substantially in all subjects during fasting. The magnitude and generalized nature of this increase suggest that there is a significant rise in propionate production in the fasting state in children with inborn errors of propionate metabolism. While the urinary metabolite changes during fasting did not manifest clinically, it is likely that the apparent increase in propionate production during fasting could precipitate a metabolic crisis in children with MMA and PA who are metabolically unstable.

Amino acid catabolism would not appear responsible for the increase in metabolite excretion on theoretical grounds. In in-born metabolic errors where the deficient enzyme is closely related to the degradation of an essential amino acid, such as phenylketonuria and maple syrup urine disease, the levels of the precursor amino acid (phenylalanine or leucine) rise during fasting rather than falling as they do in normal individuals (15, 16). This rise reflects the net catabolism of whole body protein that occurs during fasting that results in the net release of amino acids into the plasma (17). Normal subjects have an obligate requirement for oxidation of amino acids that, during fasting, exceeds the net rate of entry of amino acids into the plasma from protein so that plasma amino acid concentrations fall (18). However, when the immediate metabolism of an amino acid is impaired by an enzyme defect, the net rate of entry of that amino acid into the plasma from protein will exceed its removal rate resulting in an increase in the plasma concentration. However, a more distal defect in amino acid catabolism, such as MMA or PA, will not impair the obligate requirement for amino acid oxidation, as is evidenced by the failure of precursor amino acid concentrations to rise significantly in these conditions. Hence, the production of propionate from precursor amino acids will be governed by the rate of oxidation of these amino acids. It has been demonstrated that the oxidation of valine (19), threonine (20), and leucine (21) is decreased in the fasting state even during dietary restriction of these amino acids, and so it would be expected that the production of propionate from precursor amino acids would also decrease during fasting.

Increased oxidation of odd-chain fatty acids would, however, be a much more plausible explanation for the increased metabolite excretion. The usual physiologic response to fasting is to increase the oxidation of fatty acids (22). In normal children the production of free fatty acids is about 600 μ mol/kg/h during fasting (23, 24), about 30% of which is oxidized (25). Turnover of individual fatty acids is closely related to their representation in total fatty acid composition (26). The oxidation rate of odd-chain fatty acids during fasting may therefore be estimated from their representation in total fatty acids. In normal subjects odd-chain fatty acids represent less than 1% of tissue total fatty acids (9). However, concentrations of odd-chain fatty acids are increased in a wide variety of tissues in MMA and PA (9–11), and may represent 15% of total fatty acids in plasma (11). The increases vary widely in different tissues and individuals. If, however, odd-chain fatty acids were presumed to represent 8% of total fatty acids oxidized in MMA and PA, then odd-chain fatty acid oxidation would be expected to be about 15 μ mol/kg/h during fasting. Propionyl-CoA is an obligate intermediate in the complete oxidation of odd-chain fatty acids (22), and so odd-chain fatty acids may be presumed to contribute about 15 μ mol/kg/h to propionate production in the fasting state. This figure is similar to the increase in urinary metabolite excretion noted in the current study in MMA subjects. A smaller increase in urinary metabolite excretion was noted in PA subjects, presumably because the metabolites measured in urine are much less representative of total metabolite excretion than in MMA (5).

It has recently been demonstrated that only about 50% of total propionate production can be attributed to amino acid catabolism in MMA in the fasting state (2, 8) (Thompson GN, Walter JH, Leonard JV, Saudubray J-M, Halliday D, unpublished observations). The reduction in urinary metabolite excretion after

Table 2. Urinary metabolite excretion during feeding and fasting*

Subject	Methylmalonate		Propionylglycine	
	Fed	Fasted	Fed	Fasted
a. mmol/mol creatinine				
1	14716	18621	ND	ND
2	14766	25855	ND	ND
3	623	1091	ND	ND
4	5986	7188	ND	ND
5	7647	8761	ND	ND
6	ND	ND	239	ND
7	ND	ND	ND	33
8	ND	ND	ND	ND
Mean	8747	12303		
<i>p</i>		NS		
b. μ mol/kg/h				
1	60.1	73.6	ND	ND
2	53.0	92.6	ND	ND
3	3.6	5.3	ND	ND
4	47.6	56.6	ND	ND
5	23.3	38.7	ND	ND
6	ND	ND	0.2	ND
7	ND	ND	ND	0.2
8	ND	ND	ND	ND
Mean	37.5	53.4		
<i>p</i>		NS		
Subject	Methylcitrate			
	2S,3S		2S,3R	
Subject	Fed	Fasted	Fed	Fasted
a. mmol/mol creatinine				
1	22	26	30	33
2	65	118	80	112
3	16	36	11	23
4	37	51	28	37
5	75	128	91	117
6	234	234	193	179
7	173	221	89	121
8	44	36	51	54
Mean	83	106	72	84
<i>p</i>		<0.05		<0.01
b. μ mol/kg/h				
1	0.09	0.10	0.12	0.10
2	0.23	0.44	0.29	0.41
3	0.09	0.17	0.07	0.11
4	0.29	0.40	0.22	0.29
5	0.24	0.57	0.28	0.53
6	0.32	0.56	0.27	0.43
7	1.20	1.82	0.62	1.00
8	0.07	0.11	0.09	0.18
Mean	0.32	0.52	0.24	0.38
<i>p</i>		<0.01		<0.01
Subject	3-Hydroxypropionate		Total metabolites	
	Fed	Fasted	Fed	Fasted
a. mmol/mol creatinine				
1	4	8	14772	18687
2	22	143	14932	26228
3	24	55	675	1204
4	8	13	6059	7288
5	316	525	8128	9531
6	312	870	978	1283
7	145	247	408	622
8	113	316	208	406
Mean	118	272	5770	8156
<i>p</i>		<0.01		<0.01

Table 2.—Continued

Subject	3-Hydroxypropionate		Total metabolites	
	Fed	Fasted	Fed	Fasted
b. $\mu\text{mol/kg/h}$				
1	0.02	0.03	60.4	73.8
2	0.07	0.60	53.6	94.1
3	0.14	0.27	3.9	5.8
4	0.07	0.10	48.2	57.3
5	0.95	2.31	24.8	42.1
6	0.43	1.92	1.3	2.9
7	0.96	2.05	2.8	5.1
8	0.15	0.69	0.3	1.0
Mean	0.35	1.00	24.4	35.3
<i>p</i>	<0.01		<0.01	

* Urinary metabolite excretion data in each subject represent the means of between six and 10 collections in the fed state and four collections in the fasted state (see text). The mean values and the level of significance (Wilcoxon signed rank sum test) were calculated by including all subjects for the excretion of methylcitrate, 3-hydroxypropionate, and total metabolites and MMA subjects alone for methylmalonate excretion. ND, not detected. Detection limits were about 0.35 mmol/mol Cr for all metabolites.

inhibition of gut bacterial propionate production with metronidazole suggests that about 20 $\mu\text{mol/kg/h}$, or 25%, of propionate production may be attributable to gut bacteria (3, 4, 27). This leaves an unaccounted portion of about 20 $\mu\text{mol/kg/h}$, or 25% of propionate production during fasting. Theoretical considerations referred to above, and the results of the current study, suggest that this unaccounted portion arises primarily from odd-chain fatty acid oxidation.

It is clear that the avoidance of prolonged fasting will better prevent adverse increases in urinary metabolite excretion in MMA and PA. Although this study would support measures aimed at reducing fatty acid oxidation, it does not necessarily follow that reduction of dietary fat intake would also be of benefit, because odd-chain fatty acids are likely to be poorly represented in dietary fats.

MMA and PA should be considered not only as disorders of the catabolism of selected amino acids, but also as disorders involving fatty acid metabolism and gut bacteria. Treatment should include avoidance of prolonged fasting, and continuous or frequent intake of carbohydrate should be beneficial during acute or chronic periods of metabolic instability. Carbohydrate may be best administered by continuous nasogastric infusion or as cornstarch in a manner analogous to that used in glycogenoses.

Acknowledgments. The authors are grateful to Prof. J.-M. Saudubray and Dr. J. V. Leonard for allowing access to their patients who participated in this study, and to Mrs. S. Bartlett for skilled technical assistance.

REFERENCES

- Rosenberg LE 1983 Disorders of propionate and methylmalonate metabolism. In: Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS (eds). *The Metabolic Basis of Inherited Disease*, 5th ed. McGraw-Hill, New York, pp 474–497
- Walter JH, Thompson GN, Leonard JV, Bartlett K, Halliday D 1989 Contribution of amino acid catabolism to propionate production in methylmalonic acidemia. *Lancet* 1:1298–1299
- Snyderman SE, Sansaricq C, Norton P, Phansalkar SV 1972 The use of neomycin in the treatment of methylmalonic acidemia. *Pediatrics* 50:925–927
- Bain MD, Jones M, Borriello SP, Reed PJ, Tracey BM, Chalmers RA, Stacey TE 1988 Contribution of gut bacterial metabolism to human metabolic disease. *Lancet* 1:1078–1079
- Chalmers RA, Lawson AM 1982 *Organic Acids in Man*. Chapman and Hall, London
- Samuel P, Lieberman S, Ahrens EH 1978 Comparison of cholesterol turnover by sterol balance and input-output analysis, and a shortened way to estimate total exchangeable mass of cholesterol by the combination of the two methods. *J Lipid Research* 19:94–102
- Samuel P, Perl W 1970 Long-term decay of serum cholesterol radioactivity: body cholesterol metabolism in normals and in patients with hyperlipoproteinemia and atherosclerosis. *J Clin Invest* 49:346–356
- Walter JH, Thompson GN, Jones M, Bartlett K, Halliday D, Leonard JV, Chalmers RA 1988 Metronidazole reduces *in vivo* propionate production and methylmalonate excretion in methylmalonic acidemia. Abstracts of the 26th SSIEM Annual Symposium, 1988. SSIEM, London, p 64
- Wendel U, Diekmann E, Laryea MD 1988 Odd-numbered long-chain fatty acid contents in erythrocyte membrane phospholipids in patients with an impaired propionate utilisation. *J Inher Metab Dis* 11(suppl 2):225–228
- Kishimoto Y, Williams M, Moser H, Hignite C, Biemann K 1973 Branched-chain and odd-numbered fatty acids and aldehydes in the nervous system of a patient with deranged vitamin B12 metabolism. *J Lipid Res* 14:69–76
- Wendel U, Laryea MD 1990 Increased production of odd-numbered long-chain fatty acids in patients with impaired propionate utilisation. In: Tanaka K, Coates PM (eds) *Clinical, Biochemical and Molecular Aspects of Fatty Acid Oxidation*. Alan R Liss New York (in press)
- Saudubray JM, Marsac C, Limal JM, Dumurgier E, Charpentier C, Ogier H, Coude FX 1981 Variation in plasma ketone bodies during a 24-hour fast in normal and in hypoglycemic children. *J Pediatr* 98:904–908
- Holdeman LV, Cato EP, Moore WEC 1977 *Anaerobe Laboratory Manual*, 4th ed. Polytechnic Institute and State University, Blacksburg, VA
- Walter JH, Michalski A, Wilson WM, Leonard JV, Barratt TM, Dillon MJ 1989 Chronic renal failure in methylmalonic acidemia. *Eur J Pediatr* 148:344–348
- Guttler F 1980 Hyperphenylalaninaemia: diagnosis and classification of the various types of phenylalanine hydroxylase deficiency in childhood. *Acta Paediatr Scand* 280 (suppl):7–80
- Haymond MW, Ben-Galim E, Strobel KE 1978 Glucose and alanine metabolism in children with maple syrup urine disease. *J Clin Invest* 62:398–405
- Matthews DE, Motil KJ, Rohrbach DK, Burke JF, Young VR, Bier DM 1980 Measurement of leucine metabolism in man from a primed continuous infusion of L-[1-¹⁴C]leucine. *Am J Physiol* 238:E473–479
- Owen OE, Reichard GA, Patel MS, Boden G 1979 Energy metabolism in fasting and feeding. *Adv Exp Med Biol* 111:119–188
- Meguid MM, Matthews DE, Bier DM, Meredith CN, Young VR 1986 Valine kinetics at graded valine intakes in young men. *Am J Clin Nutr* 43:781–786
- Zhao X, Wen ZM, Meredith CN, Matthews DE, Bier DM, Young VR 1986 Threonine kinetics at graded threonine intakes in young men. *Am J Clin Nutr* 43:795–802
- Meguid MM, Matthews DE, Bier DM, Meredith CN, Soeldner JS, Young VR 1986 Leucine kinetics at graded leucine intakes in young men. *Am J Clin Nutr* 43:770–780
- Newsholme EA, Leech AR 1983 *Biochemistry for the Medical Sciences*. Wiley & Sons, Chichester
- Lewis B, Wittman W, Krut LH, Hansen JDL, Brock JF 1966 Free fatty acid flux through plasma in protein malnutrition of infants. *Clin Sci* 30:371–375
- Boungneres PF, Karl IE, Hillman LS, Bier DM 1982 Lipid transport in the human newborn. Palmitate and glycerol turnover and the contribution of glycerol to neonatal hepatic glucose output. *J Clin Invest* 70:262–270
- Thompson GN, Leonard JV, Walter JH, Matthews EE, Halliday D 1988 Oxidation of [¹⁴C]palmitate and [¹⁴C]octanoate *in vivo* in children with defects in fatty acid oxidation. Abstracts of the 26th SSIEM Annual Symposium, 1988. SSIEM, London, p 100
- Hagenfeldt L 1975 Turnover of individual free fatty acids in man. *Fed Proc* 34:2246–2249
- Thompson GN, Walter JH, Bresson JL, Ford GC, Lyonnet SL, Chalmers RA, Saudubray JM, Leonard JV, Halliday D 1989 Sources of propionate production in inborn errors of propionate metabolism. *Metabolism* (in press)