3-Hydroxyisobutyric Aciduria: An Inborn Error of Valine Metabolism

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ABSTRACT. 3-Hydroxyisobutyric aciduria, a disorder of valine metabolism, has been found in a boy in whom the clinical picture was that of a typical organic acidemia with repeated episodes of ketoacidosis requiring admission to hospital and parenteral fluid therapy, along with impressive failure to thrive and chronic lactic acidemia. The excretion of 3-hydroxyisobutyric acid ranged from 170 to 390 mmol/mol of creatinine. The administration of valine increased this to 18 700 mmol/mol of creatinine and reproduced the clinical picture of ketoacidosis. Concentrations of free carnitine were low, and esterified carnitine was elevated. Treatment with carnitine and a diet restricted in protein appeared to be beneficial. (*Pediatr Res* 30: 322-326, 1991)

Inborn errors of amino acid metabolism in which organic acid intermediates accumulate often present with life-threatening attacks of ketoacidosis. Lactic acidemia occurs in disorders of carbohydrate metabolism and abnormalities of electron transport as well as in the oxidation of pyruvate. It is the purpose of this report to describe a patient with an inborn error of valine metabolism in which the typical pictures of organic acidemia and lactic acidemia were associated with failure to thrive. 3-Hydroxyisobutyric acid was found in large quantities in the urine and increased after the administration of valine.

CASE REPORT

S. B. was a 6-y-old Caucasian boy who was referred to the University of California San Diego Medical Center because of recurrent episodes of vomiting, acidosis, and dehydration.

He weighed 1.83 kg at delivery by elective cesarean section after a 37-wk gestation. He was the second child of healthy nonconsanguineous parents. An older sibling and a younger sibling were well. Dysmorphic features were noted at birth. His length was 45 cm, and his head circumference was 27 cm. The anterior fontanel appeared large, and the testes were undescended. A karyotype was normal. He was difficult to feed from the start and required gavage feedings for the 1st wk and was

kept in the hospital for 2 wk. He was first admitted to hospital for vomiting and failure to thrive at 4 mo of age. A systolic ejection murmur was heard best in the aortic area, and an ECG indicated hypertrophy. At 3 y of age, he was admitted after a grand mal seizure and was found to have a serum concentration of bicarbonate of 8 mmol/L (8 mEq/L). The pH was 7.26. He responded to parenteral fluid and electrolyte therapy. He continued to have episodes of vomiting, lethargy, and acidosis requiring admission to hospital and parenteral fluid therapy. During these episodes, his breath smelled of acetone and the urine tested positive for large amounts of ketones. Kussmaul respirations were often observed. On at least one occasion, a blood sugar of 2.50 mmol/L (45 mg/dL) was recorded. At 6 y of age, during such an episode, he was found to have a serum concentration of lactate of 6.77 mmol/L (61 mg/dL) and a pyruvate of 0.33 mmol/L (2.9 mg/dL). The patient preferentially avoided protein-containing foods, disliking meat, milk, and eggs.

Early development was delayed; he sat at 9 mo, walked at 18 mo and said only three words at 3 y. Developmental progress was satisfactory, and, ultimately, he was doing grade-level work in school appropriate for age, although he repeated the 3rd grade. At 3½ y of age, he underwent a surgical repair of hypospadias and repair of a by then unilateral cryptorchidism. At 10 y of age, his intelligence quotient was 100.

Physical examination at 6 y of age revealed the multiple minor dysmorphic features. His face was small and triangular, and the philtrum was long. His ears looked small and low; the left measured 4.6 cm, less than the 3rd percentile for age, and the right was 4.8 cm. There were bilateral simian creases and clinodactyly of the 5th fingers. There was mild bilateral syndactyly of the 2nd and 3rd toes. His weight was 18.4 kg less than the 5th percentile and was at the 50th percentile for a boy 3 y of age. His height was 119 cm, at the 5th percentile, and his head circumference was 51.5 cm, at the 35th percentile. Neurologic examination was normal. There was a very soft systolic murmur at the left sternal border.

An EEG revealed a persistent vertex spike discharge and occasional generalized bursts of spike and wave activity consistent with a seizure disorder. The ECG was consistent with ventricular hypertrophy by voltage criteria, but an echocardiogram was completely normal. Muscle biopsy revealed a preponderance (68%) of type I over type II (32%) fibers, a reversal of the usual ratio, and electron microscopy revealed increased amounts of glycogen.

The hematocrit was 36, and the leukocyte count was 4600/mm³ with 52% polymorphonuclear forms and 48% lymphocytes. The concentration of sodium was 140, potassium was 4, chloride was 107, and bicarbonate was 20 mmol/L (20 mEq/L). The concentration of lactate was 2.02 mmol/L (18.2 mg/dL), and that of pyruvate was 0.14 mmol/L (1.2 mg/dL). The concentration of alanine was 730 µmol/L (6.5 mg/dL). There was no β-alanine on amino acid analysis of blood or urine.

The excretion of lactate in the urine ranged from 1580 to 2380 mmol/mol of creatinine (normal, 0-25). A number of studies

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were carried out to investigate the mild lactic acidemia. Fasting for as long as 26 h was without acidosis or hypoglycemia. Carbohydrate challenge tests were administered in a dose of 1 g/kg as a 20% solution 6 to 12 h postprandially. Blood was obtained at 0 and 45 min. After glucose, the concentration of lactate rose from 2.0 to 2.66 mmol/L (18 to 24 mg/dL) without a change in pyruvate. After fructose, the lactate rose from 1.54 to 4.42 mmol/L (13.9 to 39.8 mg/dL) and pyruvate rose from 0.08 to 0.19 mmol/L (0.7 to 1.7 mg/dL). After alanine, the lactate rose from 1.06 to 3.08 mmol/L (9.5 to 27.7 mg/dL) and pyruvate rose from 0.006 to 0.17 mmol/L (0.5 to 1.5 mg/dL). After galactose, lactate rose from 2.53 to 4.53 mmol/L (22.8 to 40.8 mg/dL) and pyruvate rose from 0.11 to 0.16 mmol/L (1.0 to 1.4 mg/dL). After glycerol, lactate rose from 2.09 to 3.37 mmol/L (18.8 to 30.4 mg/dL) and pyruvate rose from 0.14 to 0.18 mmol/L (1.2 to 1.6 mg/dL). On the basis of these data, he was given a diet low in simple sugars without obvious effect on his course or diminution in episodes of acute acidosis. Assay of cultured fibroblasts by Dr. Brian Robinson in Toronto revealed normal activity for pyruvate dehydrogenase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase, as well as the whole cell oxidation of [$1\text{-}^{14}\text{C}$]pyruvate.

MATERIALS AND METHODS

Loading tests with isoleucine and valine were carried out 3 h postprandially in dosages of 100 mg/kg. The patient was 9½ y of age and weighed 17.42 kg. Blood was obtained at 0, 1, 2, 3, and 4 h for glucose, 3-hydroxybutyrate, and amino acids. Urine was collected in three 8-h samples. The patient was admitted to the General Clinical Research Center for these challenge tests after a number of months without intercurrent infection or episodes of acidosis. He was receiving at that time his normal diet, which was still fructose free but contained 2.5 g/kg of protein and 103 kcal/kg.

Concentrations of amino acids were determined by column chromatography on an automatic amino acid analyzer (1). Organic acids in urine were determined by liquid partition chromatography followed by gas chromatography-mass spectrometry (2) and by batchwise liquid partition chromatography on silicic acid followed by gas chromatography-mass spectrometry with automated identification and quantification of the trimethylsilyl derivatives (3). 3-Hydroxyisobutyric acid was quantified by using the unique fragment ion at m/z 177, and 3-hydroxybutyric acid was quantified by using the unique fragment ion at m/z 199 (4). Concentrations of carnitine in blood and urine were determined by the method of McGarry and Foster (5), and 3-hydroxybutyrate was quantified by the method of Maughan (6).

RESULTS

Organic acid analysis revealed the presence of substantial amounts of 3-hydroxyisobutyric acid in the urine. The amounts ranged from 170 to 390 mmol/mol of creatinine (normal, 2–33). The concentration of free carnitine in the plasma was 17.0 $\mu\text{mol/L}$, and that of the esterified carnitine was 11.2 $\mu\text{mol/L}$. The urinary free carnitine was 2.3 mmol/mol, and the esterified fraction was 64.7 mmol/mol of creatinine.

Loading with isoleucine was uneventful (Table 1). The patient's blood sugar was maintained between 4.16 and 4.77 mmol/L (75 to 86 mg/dL), and the serum 3-hydroxybutyrate remained in the range of 300 to 460 $\mu\text{mol/L}$ (Fig. 1). Urinary organic acids were unchanged except for an increase in lactate to 1540 mmol/mol of creatinine (normal, 0–25) and in 3-hydroxybutyrate to 1640 mmol/mol of creatinine (normal, 0–3) (Fig. 2). Urinary excretion of free carnitine was 0.43, 0.54, and 1.03 μmol in each of the 8-h periods after the load; the esterified carnitine was 18.99, 14.75, and 25.95 μmol .

On the following day, a load of valine reproduced the syndrome of clinical illness (Table 1). Two hours after administra-

tion, the patient became flushed, lethargic, and diaphoretic, and he vomited. Ketonuria was prominent. The serum bicarbonate was 10 mmol/L. He improved clinically after the administration of parenteral fluids containing sodium bicarbonate, but the bicarbonate rose slowly to 18 mmol/L in 8 h and to 24 mmol/L 5 h later; he continued dry retching 2 more h after the onset of therapy. The serum concentration of glucose fell to 2.61 mmol/L (47 mg/dL) at 2 h (Table 1), at which time the serum concentration of valine had risen to 1528 $\mu\text{mol/L}$ (17.9 mg/dL). The serum concentration of 3-hydroxybutyrate rose to 2640 $\mu\text{mol/L}$. Urinary lactate rose to 30 000 mmol/mol of creatinine and urinary 3-hydroxybutyrate rose to 18 700 mmol/mol of creatinine. In addition, there were large amounts of dicarboxylic acids and 3-hydroxy acids in the urine, all concomitants of ketosis, as well as citric acid cycle intermediates.

On the basis of these data, treatment was initiated with a diet low in protein and 100 mg/kg of carnitine. The diet contained 0.75 g/kg of protein and 100 kcal/kg. This was associated with a marked reduction in the frequency and severity of attacks of acidosis. The patient's appetite did not permit an intake high in calories, and growth, though improved, remained slow. At 12 y of age, his weight of 21.9 kg was at the 50th percentile for a 6 y old and his height of 129 cm was in the 50th percentile for an 8 y old. His head circumference of 54 cm was in the 50th percentile for age.

The effects of valine and isoleucine on the urinary organic acids are shown in Figure 1. The administration of valine led to a major increase in the excretion of 3-hydroxyisobutyrate. During the first 8 h after the load, the patient excreted 1888 mmol/mol of creatinine and in the next 8 h the value was 2820, considerable elevations over the 8 h before the load when he excreted 130 mmol/mol of creatinine. In contrast, in a control child (J. A.), the baseline level of excretion was only 20 mmol/mol of creatinine and, after the same load of valine, it rose to 50 mmol/mol of creatinine. In another control child (Table 2), the excretion of 3-hydroxyisobutyrate in 8 h after valine was only 18 μmol (26 $\mu\text{mol/mol}$ of creatinine). Of the three control children given the same dose of valine, none became ill. Of two studied, neither increased the concentration of lactate, and, in all three, the blood sugar level was maintained (Table 1).

After valine, the excretion of 3-aminoisobutyrate by the patient increased in the second and third 8-h periods (Table 2). In contrast, after isoleucine, the amounts were too low to measure accurately. In contrast, this compound was undetectable after valine in the urine of two control individuals (J. A. and S. C.) and very slight in the third (Table 2).

In the patient, the valine load provided a total of 14.87 mmol. In the following 24 h, he excreted 35% of this administered load, most of it in the form of 3-hydroxyisobutyrate, with the rest in 3-aminoisobutyrate, acylcarnitine, and valine itself (Table 2). In the first 8-h period, 12.5% of the load was excreted. In the comparable period, the control individual (Table 2) excreted only 1.4% and this was virtually all carnitine ester; this is what would be expected because that individual (Z. M.) has been reported (7) to have organic acidemia, and he was receiving treatment with carnitine. The baseline excretion of carnitine ester of this boy was 397 mmol/mol of creatinine, and the value after valine was 315.

The clinically benign course after isoleucine was mirrored in the flat curves for glucose and lactate, whereas, after valine, the concentrations of glucose fell and those of lactate rose. The excretion of 3-hydroxyisobutyrate after isoleucine remained in the baseline range from 129 to 560 mmol/mol of creatinine (Fig. 1). The normal range in this laboratory is from 2 to 33 mmol/mol of creatinine. The excretion of 3-aminoisobutyrate did not increase after isoleucine.

Ketosis, as reflected in 3-hydroxybutyrate excretion, was directly proportional ($r = 0.958$, $p = 0.0026$) to 3-hydroxyisobutyrate excretion after administration of both isoleucine and valine, and the excretion of urinary lactate was also positively

Table 1. *Effects of valine and isoleucine on concentrations in plasma and urine**

	Isoleucine challenge (h)					Valine challenge (h)				
	0	1	2	3	4	0	1	2	3	4
Patient (S. B.)										
Glucose (mmol/L)	4.77	4.33	4.33	4.16	4.39	3.83	2.78	2.61		
Lactate (mmol/L)	2.02		2.09			2.00		2.89		
3-OH-butyrate (μ mol/L)	300	320	360	460	390	1800		2640		
Bicarbonate (mmol/L)						22		10		
Valine (μ mol/L)						333		1528		
Isoleucine (μ mol/L)	122		686							
Control (J. A.)										
Glucose (mmol/L)						4.33	4.61	4.61	4.66	4.39
Bicarbonate (mmol/L)						22	24	23	24	23
3-OH-butyrate (μ mol/L)						560	300	400	640	700
Valine (μ mol/L)						243	1641	1363	1016	863
Control (S. C.)										
Glucose (mmol/L)						5.16	4.50	4.88	4.44	4.11
Lactate (mmol/L)						2.12		1.71		
3-OH-butyrate (μ mol/L)						60	80	80	80	70
Valine (μ mol/L)								1349		
Control (Z. M.)										
Glucose (mmol/L)						5.77	5.05	5.50	4.83	4.72
Lactate (mmol/L)						0.66		0.66		0.83
3-OH-butyrate (μ mol/L)						180	80	175	220	460
Bicarbonate (mmol/L)						24	25	27	25	27
Valine (μ mol/L)						74	1554	1311	1067	889

* J. A. and S. C. were both patients under study for recurrent episodes of acidosis in whom no metabolic abnormality was found. Z. M. was a boy with a disorder of isoleucine metabolism in whom the assay for 2-oxothiolase was normal but the fibroblast conversion of ^{14}C -2-methylbutanoate to macromoles was abnormal (7).

Fig. 1. The effects of valine and isoleucine on the urinary excretion of 3-hydroxyisobutyric acid, lactic acid, and 3-hydroxybutyric acid.

Fig. 2. Pathway of catabolism of valine.

correlated ($r = 0.935$, $p = 0.006$) with 3-hydroxyisobutyrate excretion.

DISCUSSION

The clinical phenotype of this patient was typical of disorders of organic acid metabolism, the hallmark of which is the occurrence of recurrent episodes of vomiting, ketosis, and acidosis leading to dehydration and lethargy and requiring admission to hospital and vigorous resuscitation with large amounts of fluids containing sodium bicarbonate. Secondary lactic acidemia is common in such acute episodes of organic acidemia, but this patient had consistent elevations of blood and urinary concentrations of lactate and alanine. The lactic acidemia was usually mild, and the anion gap was sometimes normal; however, anion gaps were observed as high as 22 and 27 (defined as $\text{Na} - \text{CO}_2 + \text{Cl}$). The normal should approximate 15. Gluconeogenesis and the oxidation of pyruvate *in vitro* could not be shown to be disturbed. However, the lactic acidemia was regularly accom-

Table 2. *Disposition of valine load*

	Urinary excretion		% Administered valine
	μmol	mmol	
Patient S. B. (dose 14.87 mmol)			
First 8 h			
Valine	18.89		
3-Hydroxyisobutyrate	1820.00		
3-Aminoisobutyrate	trace		
Carnitine ester	20.64		
Total 8 h		1.86	12.5
Second 8 h			
Valine	16.49		
3-Hydroxyisobutyrate	2035.00		
3-Aminoisobutyrate	3.31		
Carnitine ester	47.16		
Total		2.10	14.1
Third 8 h			
Valine	16.68		
3-Hydroxyisobutyrate	1169.00		
3-Aminoisobutyrate	6.11		
Carnitine ester	129.36		
Total 8 h		1.32	8.9
Total 24 h		5.28	35.5
Patient Z. M. (dose 16.36 mmol)			
First 8 h			
Valine	1.37		
3-Hydroxyisobutyrate	17.86		
3-Aminoisobutyrate	0.34		
Carnitine ester	216.41		
Total 8 h		0.24	1.4

panied by the concomitants hyperalaninemia and lactic aciduria. The highest blood alanine recorded was 1259 $\mu\text{mol/L}$. The urinary lactate was usually 100 times normal, and blood lactates as high as 6.8 mmol/L were recorded. The elevation of plasma and urinary lactate in response to the administration of valine was striking. The level of urinary lactate increased in proportion to the accumulation of 3-hydroxyisobutyric acid. For these reasons, this disorder should be considered in the differential diagnosis of chronic lactic acidemia.

Failure to thrive and anorexia, other features of the presentation, are also typical of organic acidemia. Treatment with a diet restricted in protein and with carnitine led to an appreciable reduction in the frequency and severity of episodes of acidosis. However, anorexia was continuing and the patient's height and weight remained considerably below the norms for age. The occurrence of features of the Russell-Silver syndrome is further evidence for the nonspecific nature of these dysmorphic features (8).

Reproduction of the episode of acute illness by the administration of valine and the accumulation of 3-hydroxyisobutyric acid indicate that this is a disorder of valine catabolism. The conditions, 3 h postprandial, were the same for the valine and isoleucine loads. There is nothing in the record that explains the fact that the blood 3-hydroxybutyrate at time zero was 6 times higher for the valine load than that from the isoleucine load the previous day. The patient looked no different clinically. On the other hand, unexplained, or even cryptic, ketosis is common in this disorder, in which the worst consequence is the development of ketosis. The valine load could be followed for only 2 h because of the acute illness of the patient, which required intervention.

The pathway of valine catabolism is shown in Figure 2. The absence of accumulation of 3-hydroxypropionate, methylcitrate, or methylmalonate in the urine indicates that the defect lies before these steps in the pathway. Those steps are also shared with the catabolism of isoleucine, and the absence of reaction to isoleucine would also be against an abnormality at that level. An

inborn error has been postulated in methacrylyl CoA hydratase (9) in a patient with multiple malformations who died at 3 mo of age, but the defect was found to be in 3-hydroxyisobutyryl CoA deacylase (10). That patient excreted the cysteine conjugates S-(2-carboxypropyl)cysteine and S-(2-carboxypropyl)cysteamine. These compounds were not found in our patient. The fundamental defect could be in the 3-hydroxyisobutyrate dehydrogenase, which catalyzes the conversion of 3-hydroxyisobutyrate to methylmalonic semialdehyde, or in the conversion of the semialdehyde to propionyl CoA. We speculate that the defect is at the latter step. This would be consistent with the increase observed in the urinary excretion of 3-aminoisobutyrate after valine. Methylmalonic semialdehyde could not be found in urine samples, but this compound would be expected to be unstable in samples not treated promptly with reagents capable of making stable derivatives of the aldehyde. The interconversion of methylmalonic semialdehyde and 3-hydroxyisobutyrate would be expected to be under redox-controlled equilibrium and highly skewed toward 3-hydroxyisobutyric, as is the case with the interconversion 2-ethylhydracrylic acid and ethylmalonic semialdehyde. Propionate as the first metabolite in common in the catabolism of isoleucine and valine should be clearly distal to the metabolic defect, inasmuch as loading with isoleucine produced no illness and no increase in the excretion of 3-hydroxyisobutyrate or 3-aminoisobutyrate.

A patient with 3-hydroxyisobutyric aciduria has recently been reported (11). This patient had malformations, massive acidosis, and hypotonia and also excreted large amounts of 3-hydroxypropionic acid, ethylhydracrylic acid, and 3-hydroxyisovaleric acid. In our patient, small amounts of 3-hydroxypropionate and 3-hydroxyisovalerate (23 to 126 mmol/mol of creatinine) were found in the urine after valine loading and the amounts were similar after isoleucine loading. All of these compounds increased after loading with valine in the patient recently reported (11). Therefore, it appears that this was a very different disorder. It is possible to miss the presence of 3-hydroxyisobutyric acid with many systems of organic acid analysis. In fact, in the analytic system previously in use in this laboratory (2), 3-hydroxyisobutyric acid could not be distinguished from 3-hydroxybutyric acid except by gas chromatography-mass spectrometry and, in the presence of ketosis when large amounts of 3-hydroxybutyric acid are excreted, it would not be recognized. Thus, this disorder should be included in the differential diagnosis of organic acidemia in which there is ketoacidosis.

The compounds along the pathway from isobutyryl CoA to methylmalonic semialdehyde or 3-aminoisobutyric acid would be expected to be in the form of the S isomers. Accumulated methylmalonic semialdehyde could undergo spontaneously oxo-enol tautomerism yielding a racemic mixture of the S and R forms. Reduction would yield some (R)-3-hydroxyisobutyric acid and amination some (R)-3-aminoisobutyric acid. Therefore, determination of the nature of these compounds would be of considerable interest.

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