0031-3998/88/2401-0009$02.00/0
PEDIATRIC RESEARCH

Vol. 24, No. 1, 1988
Printed in U.S.A.

The Treatment of Isovaleric Acidemia with Glycine Supplement[1]

MARY NAGLAK, RINO SALVO, KEVIN MADSEN, PHILIP DEMBURE, AND LOUIS ELSAS

Emory University, Department of Pediatrics, Division of Medical Genetics, Atlanta, Georgia 30322

ABSTRACT. Although dietary leucine restriction and supplemental glycine are used to treat patients with isovaleric acidemia [deficient isovaleryl-CoA-dehydrogenase (E.C. 1.3.99.10)], little quantitative information is available regarding their optimum relationship. Herein we compare different glycine supplements and quantitate isovalerylglycine produced in two patients with clinically different forms of isovaleric acidemia during restricted leucine intake and during oral leucine loading. We found that under stable conditions of leucine restriction, 150 mg glycine/kg/day is an optimum glycine supplement and that glycine supplements of more than 250 mg/kg/day may result in reduced isovalerylglycine production; that when isovaleric acid accumulation is increased, glycine supplements to 600 mg/kg/day will increase isovalerylglycine production; and that the phenotype of isovaleric acidemia is related not only to the extent of impaired isovaleryl-CoA dehydrogenase, but also the ability to detoxify accumulated isovaleryl CoA to isovalerylglycine. (*Pediatr Res* 24: 9–13, 1988)

Abbreviations

IVA, isovaleric acid
IVG, isovalerylglycine
MSUD, maple syrup urine disease

Isovaleric aciduria, first described in 1966 by Tanaka *et al.* (1) was subsequently detected in numerous cases (2–18). It is an inborn error of metabolism due to a defect of isovaleryl-CoA dehydrogenase (E.C. 1.3.99.10) (19). Heterogeneity for this enzyme in patients with isovaleric acidemia has been noted (20). The gene for isovaleryl-CoA dehydrogenase has been cloned and assigned to the long arm of human chromosome 15 (21). The pathophysiology of this condition results from an accumulation of IVA which is toxic to the central nervous system (Fig. 1). An alternative pathway through glycine-N-acylase (E.C. 2.3.1.13) allows detoxification by producing isovalerylglycine which is excreted (17). Isovaleric acidemia is divided into two clinical categories: acute and chronic intermittent (18). The acute form (2–8) presents during the first days of life with poor feeding, tachypnea, and vomiting. If untreated, death may occur. Patients with the chronic, intermittent form (1, 9–16) present later with similar episodes of vomiting, acidosis, stupor, and the characteristic sweaty-feet odor of isovaleric acid. In this latter type, symptoms may begin as early as the first 2 wk of life (10). The frequency of attacks decreases with age with both clinical phenotypes (9–11, 14, 15).

Isovaleryl-CoA dehydrogenase is impaired in fibroblasts cultured from affected patients (19). A tritium release assay (19, 22) measured ^3H_2O formed by mitochondria from [2,3-^{3}H]isovaleryl-CoA but demonstrated 13% residual activity in both acute and chronic-intermittent forms (19). Dubiel *et al.* (23) also found no difference in the residual activity of 12 isovaleric acidemia cell lines using macromolecular-labeling techniques. An improved tritium release assay using an acyl-CoA dehydrogenase inhibitor (methylenecyclopropylacetyl-CoA) (24) gave an unexpected finding. The acute, early-onset form had residual activity of 0.41 pmol ^3H_2O/min/mg protein compared to a normal of 19.4 ± 8.0 whereas three milder clinical forms had no enzyme activity. The authors suggested that age of onset and the severity of clinical presentation were not caused by differences in impaired isovaleryl-CoA dehydrogenase, but by the competency of the glycine-N-acylase pathway to use accumulated isovaleric acid (24). When [2-^{14}]C-leucine conversion to $^{14}CO_2$ by intact cells was used as the enzyme assay, less than 2% residual activity was seen in acute isovaleric acidemia (25). Comparisons of clinical phenotype using this assay are not published.

The treatment for isovaleric acidemia follows the general concepts used in treating inborn errors of amino acid metabolism (26). Leucine is given in amounts sufficient for normal growth while minimizing the formation of isovaleric acid. In 1976, Krieger and Tanaka (10) used supplemental glycine to treat isovaleric acidemia and demonstrated increased excretion of IVG (Fig. 1). Glycine was subsequently used primarily to avert acute ketotic episodes (3, 9, 10, 12, 13) with little information available regarding the chronic use of glycine.

Herein we determine the optimum glycine supplement required to treat two clinically different patients with isovaleric acidemia during stable periods of restricted dietary leucine, and during leucine loading.

METHODS

Case report 1. A.W. is a 9-yr-old girl with no other affected family members and no known consanguinity. During the first month of life she required a diluted formula because full-strength formula resulted in vomiting, irritability, and diarrhea. Her mother noted a peculiar odor when she was sick. She preferentially avoided meat and other high protein foods. Developmental milestones were met normally and she was in the 25th percentile for weight. At 4½ yr of age she was admitted to a local hospital for vomiting, dehydration, and extreme lethargy. This episode responded to intravenous fluids. A seizure-like episode was noted at age 5 yr. At 5½ yr of age, after excessive protein intake at a family holiday meal, she became lethargic, vomited, and comatose. She was hospitalized for treatment of dehydration, hypoglycemia, and acidosis. Recovery was rapid with intravenous fluids and protein restriction. Urine organic acid analysis by GC/MS

Received August 19, 1987; accepted February 25, 1988.
Correspondence Louis J. Elsas, II, M.D., Emory University, Division of Medical Genetics, 2040 Ridgewood Drive, Atlanta, GA 30322.
Supported in part by a Clinical Research Center Grant RR00039.
[1]Presented at the Southern Society for Pediatric Research, and published in part in Clin. Res. 35:60A(abstr), 1987.

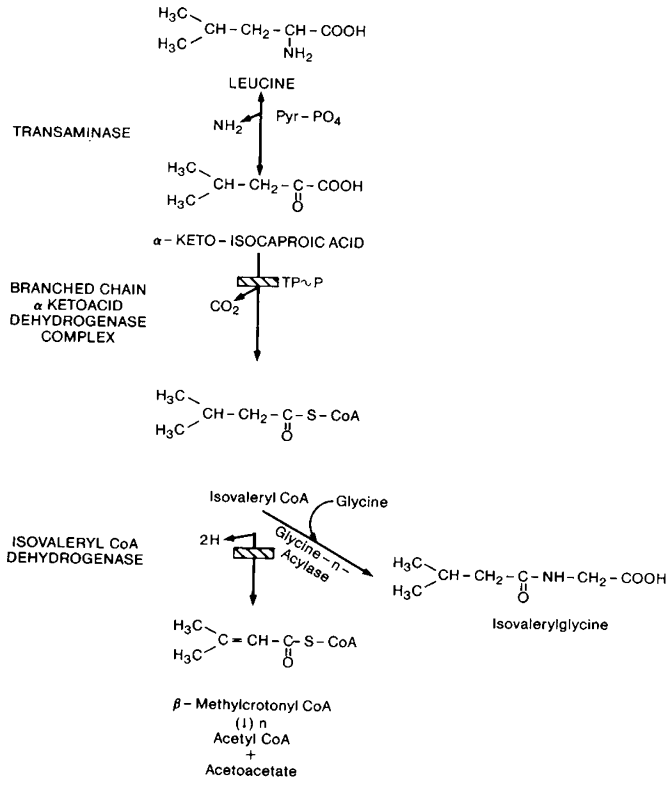

Fig. 1. Catabolism of leucine in isovaleric acidemia and the anabolism of isovalerlyglycine. *Hatched bars* represent the impaired reactions in MSUD and isovaleric acidemia, respectively.

revealed large amounts of IVG and 3-hydroxy-isovaleric acid. She was treated with protein restriction (1.25 g/kg/day) supplemented with 2 g of glycine per day orally. After 6 months of this therapy, her weight/age increased from the 25th to the 75th percentile whereas her height/age remained at the 50th percentile. After 1 yr of treatment her height also reached the 75th percentile. There was a noticeable improvement in her behavior and academic performance and she had no further episodes of acidosis.

Case report 2. K.H. is a 24-month-old Caucasian boy. A sibling had recurrent severe acidosis, coma, and died at 2 wk of age. He was diagnosed postmortem as having isovaleric acidemia by enzyme assay. K.H.s gestation was therefore monitored prenatally. Cultured amniotic fluid cells assayed for isovaleryl-CoA dehydrogenase had low activity. There was also a 400-fold increase in amniotic fluid IVG concentration during the late second trimester. He was delivered by cesarian section at 42 wk weighing 4.98 kg and was placed on 250 mg/kg of oral glycine at birth in addition to breast milk. Despite total protein restriction at 3 days of life, he began to vomit, hyperventilate, and became acidotic. Breast-feeding was discontinued and a leucine-free diet providing 125 kcal/kg was supplemented with oral glycine (380 mg/kg/day). His clinical status quickly improved. Dietary leucine (45 mg/kg/day) was reintroduced at age 5 days with a total protein intake of 2.0 g/kg/day and glycine intake was reduced to 250 mg/kg/day. His urine contained large amounts of IVG. He continued to gain weight and was discharged at 16 days of life. He has been maintained on leucine restriction and glycine supplements as an outpatient with one hospitalization for vomiting and dehydration that quickly reverted with intravenous fluids and additional oral glycine. He is developmentally normal and his growth is >95th percentile for height and weight.

Quantitation of amino acids and organic acids. Deproteinized plasma samples were analyzed for amino acid content by ion exchange chromatography on a Beckman System 6300 High Performance Analyzer using lithium buffers. A standard of isovalerylglycine was synthesized using a modified method of Bondi and Eissler (27). Plasma and urinary isovalerylglycine and β-hydroxyisovaleric acid were quantitated by gas chromatography/mass spectrometry (26, 28). There was 65% recovery of IVG and β-hydroxyisovaleric acid. The minimum concentration of IVG detectable in the plasma was 377 μM and in the urine was 0.9 mmol/g creatinine.

An adaptation of the method described by Tedesco (unpublished observations) was used to determine the concentration of plasma and urinary IVA. No extraction was required for either urine or plasma samples and there was 100% recovery of IVA. The minimum detection level of IVA in the urine and plasma was 245 μM.

Culture of skin fibroblasts and enzyme assay. Skin biopsies were obtained from K.H. and A.W. and cultured in monolayers from explants using previously described methods (29, 30). Cell lines from a control and a patient with classical MSUD were matched for culture age. $^{14}CO_2$ release [1-^{14}C] and [2-^{14}C] labeled leucine by cultured fibroblasts from all three phenotypes were performed simultaneously by previously described methods (29, 30). [1-^{14}C]-leucine and [2-^{14}C]-leucine were purchased from New England Nuclear, Boston, MA.

Diet and clinical research protocols. The formula for both patients consisted of MSUD-1 (Milupa Corporation, Darien, CT), MSUD-Diet Powder (Mead Johnson Nutritional Division, Evansville, IN), and Protein-Free Diet Powder (Mead Johnson Nutritional Division) supplemented with L-valine, L-isoleucine, and L-glycine (Ajinomoto, USA, Inc., New York, NY). The children maintained a constant intake of isoleucine, leucine, valine, protein, and energy from solid foods before and during the study. Supplemental carnitine was not given; intake ranged from 5–10 mg/day.

During A.W.'s first admission to Emory University Clinical Research Facility, her glycine supplements were increased weekly from 0, to 50, 100, 150, 300, and 600 mg/kg/day while on a constant leucine intake of 54 ± 3.6 mg/kg/day (Table 1). Semi-fasting plasma samples and 24-h urine collections were obtained

Table 1. *Summary of dietary intake for A.W. and K.H. during constant leucine restriction and for A.W. during leucine tolerance tests*

Diet (per kg)	Average intake*	% recommended intake (31)	% usual intake†
During leucine restriction			
Patient A.W.			
Isoleucine (mg)	44 ± 1.0	200	96
Leucine (mg)	54 ± 3.6	180	86
Valine (mg)	46 ± 1.7	250	87
Protein (gm)	1.1 ± 0.02	95	94
Energy (kcal)	38 ± 1.8	58	68
Patient K.H.			
Isoleucine (mg)	45 ± 1.5	111	100
Leucine (mg)	46 ± 0.6	81	88
Valine (mg)	44 ± 0.9	136	94
Protein (gm)	1.7 ± 0.01	105	100
Energy (kcals)	72 ± 4.5	101	101
During leucine tolerance tests			
Patient A.W.			
Isoleucine (mg)	31 ± 1.7	164	90
Leucine (mg)	38 ± 2.5	147	87
Valine (mg)	33 ± 1.6	212	90
Protein (gm)	0.9 ± .03	87	91
Energy (kcal)	34 ± 2.3	61	95

* During leucine restriction mean ± SD of six, weekly triplicate observations. During leucine tolerance test, mean ± SD of two test days.

† During leucine restriction and leucine tolerance tests comparison made to average intake three days prior to the study.

on the last 3 days of each week for quantitation of amino acids and organic acids.

K.H. was admitted for 1 wk on 0 mg/kg/day glycine supplements and a constant leucine intake of 46 ± 0.6 mg/kg/day (Table 1). Semifasting plasma samples were obtained every 3rd day and 24-h urine collections were obtained daily. On an outpatient protocol, his glycine supplements were increased weekly to 100, 150, 300, and 600 mg/kg/day. During this time, 24-h urine collections were obtained on the last 2 days of each week. Because of his age, blood was not collected for plasma amino acids analysis.

Only patient A.W. was given a leucine load, after informed consent, and under constant surveillance. She was given two leucine tolerance tests (120 mg/kg load of leucine) on each of two supplemental glycine doses (190 and 600 mg/kg) which she received for the week before and during each of the two leucine tolerance tests. During each test, blood samples were collected before the oral leucine load and at 1, 2, 3, 6, 9, and 12 h after the load. Urine was collected and analyzed throughout the 12-h tests in two successive, 6-h periods.

RESULTS

Oxidation of radioactive substrates. The production of $^{14}CO_2$ by cultured skin fibroblasts from [1-^{14}C]-leucine and [2-^{14}C]-leucine substrates is summarized in Table 2. The data represent the mean of two different experiments. In the first experiment A.W.'s cells produced 0.115 nmol $^{14}CO_2$/mg protein/hour from [2-^{14}-C]-leucine which was 7.1% of control and significantly more than background. K.H.'s cells were not analyzed in the first experiment. In the second experiment cell proteins were less (670 *versus* 1100 μg), background was higher (340 *versus* 200 cpm) and A.W.'s cells produced $^{14}CO_2$ from [2-^{14}C]-leucine similar to cell-free blanks. In this experiment K.H.'s cells produced no $^{14}CO_2$ above background. Table 2 incorporates data from both experiments. There was mild reduction of $^{14}CO_2$ production from [1-^{14}C]-leucine by both patients' cells and the MSUD cell line converted only 3%. The moderate reduction in oxidative decarboxylation of [1-^{14}C]-leucine by cells from the patients may be caused by IVA accumulation and consequent, product-inhibition of the preceding reaction, branched chain α-ketoacid dehydrogenase.

Response to glycine supplements during leucine restriction. Without glycine supplements, A.W.'s mean plasma glycine concentration (Table 3) was slightly above the normal concentration of 219 ± 33 μM (32). Her plasma glycine concentrations increased gradually when glycine supplements of 50 and 100 mg/kg/day were given. Marked increases in plasma glycine were noted thereafter with a maximum concentration of 2547 ± 591 μM when the largest dose of glycine was given (Table 3). Plasma glycine concentrations for K.H. were 377 ± 25 μM without glycine supplement. Both patients had normal and constant plasma leucine concentrations throughout the study which reflected their controlled intake of leucine. Note that A.W. tolerated more dietary leucine (54 mg/kg/day) than K.H. (46 mg/kg/day) (Table 1). On these leucine-restricted diets, neither patient had detectable amounts of IVA, IVG, or β-hydroxy-IVA in the plasma. There was no detectable β-hydroxy-IVA in the urine from either patient. IVA excretion by patient A.W. was constant at 4.3 to 6.2 mmol/g creatinine, and unrelated to glycine supplementation.

Table 2. *Diagnosis of isovaleric acidemia using [1-^{14}]C and [2-^{14}C]-leucine decarboxylation by intact cultured skin fibroblasts*

		Leucine decarboxylation (nmol CO_2-released/mg protein/h)*		
Cell line	[1-^{14}C]-leucine	% control	[2-^{14}C]-leucine	% control
Patient A.W.	13.04 ± 3.36 (4)	56	0.04 ± 0.04 (4)	0–7.1
Patient K.H.	16.05 ± 2.33 (2)	69	0.00 (2)	0
MSUD	0.64 ± 0.04 (2)	3	0.02 ± 0.00 (2)	2
Control	23.3 ± 4.7 (4)	100	0.87 ± 0.18 (4)	100

* Data are presented as the mean ± 1 SD with the number of observations in parentheses from two experiments.

The patients had different responses to glycine as measured by urinary IVG excretion. Patient A.W. had 2-fold higher excretion than K.H. on no glycine supplements (12.3 ± 5.8 *versus* 5.5 ± 2.8 mmol/g creatinine). A.W.'s IVG excretion rose nearly 3-fold to 33.6 ± 14.2 mmol/g creatinine after 50 mg/kg of glycine supplement was started (Fig. 2). This rise did not change significantly until glycine supplements of 300 and 600 mg/kg were provided. Unexpectedly at this time IVG excretion decreased from ranges between 24.1 ± 5.8 and 33.6 ± 14.2 to 13.8 and 16.3, a fall of 50% (Fig. 2). For patient K.H., IVG excretion increased gradually as glycine supplements were introduced. When glycine supplements of 300 and 600 mg/kg were given, he produced maximal IVG of 10.8 ± 0.3 and 10.6 ± 0.8 mmol/g creatinine, respectively (Fig. 2). A. W. excreted three times as much IVG as K.H. when optimally supplemented with oral glycine (Fig. 2).

Response to glycine supplementation during leucine loading. The response to low and high glycine supplements were then compared during leucine load (Fig. 3; Table 4). When receiving a 190 mg/kg glycine supplement, A.W.'s plasma glycine concentration was twice normal (Table 4). When 600 mg/kg of glycine supplement was given, plasma glycine concentration increased nearly 8-fold (Table 4). During the first tolerance test at lower glycine supplementation A.W. became nauseated within ¾ h of the administration of the leucine load, and began vomiting 1½ h after the load. Nausea was not present during the second leucine tolerance test when she had received 600 mg/kg glycine. The 34% lower mean plasma leucine concentration during the first tolerance test reflects losses of oral leucine through emesis (Table 4; Fig. 3). Her initial plasma leucine concentrations for both tolerance tests were normal (Fig. 3). Plasma leucine concentrations peaked 2 h after the leucine load regardless of glycine supplementation (Fig. 3).

The urinary excretion of IVG increased from 193.8 mmol/g creatinine during the first tolerance test to 418.6 mmol/g creatinine during the second test (Table 4). To normalize for the difference in leucine loading between the two tolerance tests, the amount of IVG excreted (mmol/g creatinine) during each 12-h test was normalized by the mean plasma leucine concentration (μM) during each test. This ratio of urinary IVG/mean plasma leucine concentration increased from 1.2 during tolerance test one to 2.2 during tolerance test two. Thus, there was approximately a 2-fold increase in IVG excretion when glycine supplementation was 600 mg/kg as compared to 190 mg/kg whether

Table 3. *Plasma amino acids during constant leucine restriction in patient A.W.*

	Glycine supplement					
	0	50	100	150	300	600
Plasma (μM) Patient A.W.						
Glycine	293.9 ± 57	345.9 ± 35	393.4 ± 33	547.1 ± 129	978.2 ± 117	2547.5 ± 591
Leucine	92.9 ± 13	69.2 ± 10	70.2 ± 34	71.1 ± 2	84.5 ± 15	66.5 ± 4

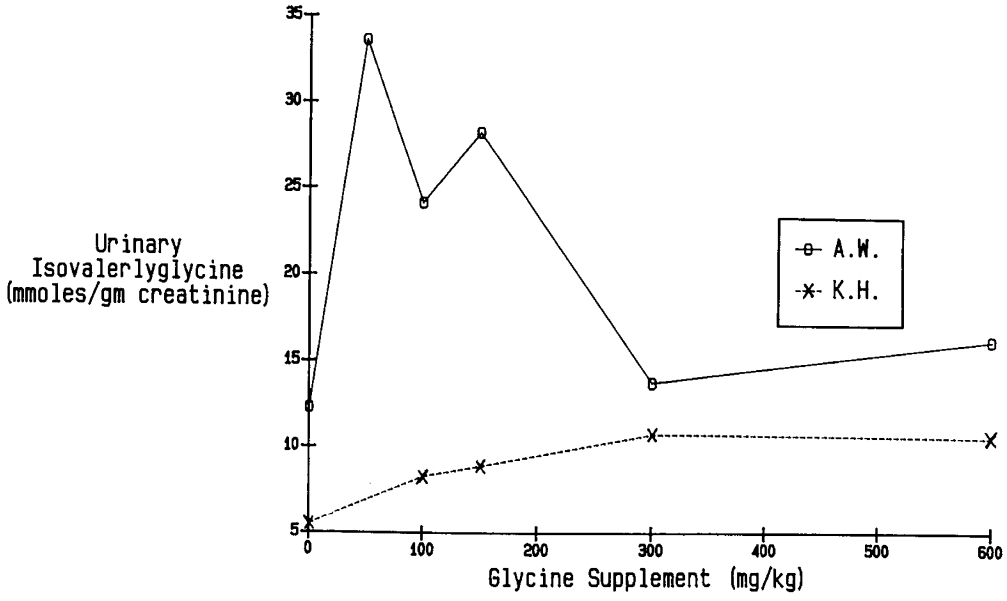

Fig. 2. Isovaleryglycine excretion by A.W. and K.H. while receiving constant leucine restriction and incremental glycine supplements. Each *point* represents the mean of at least duplicate observations on two sequential days.

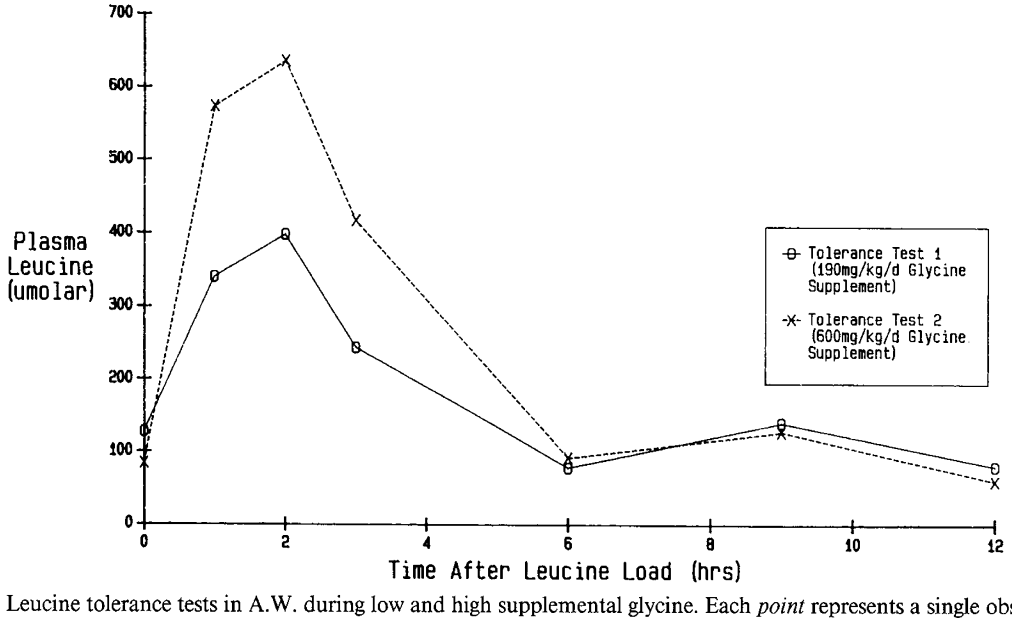

Fig. 3. Leucine tolerance tests in A.W. during low and high supplemental glycine. Each *point* represents a single observation.

Table 4. *Plasma and urine amino acids and organic acids during leucine tolerance tests*

Glycine supplement (mg/kg)	Tolerance test I 190	Tolerance test II 600
Plasma (μM)*		
Glycine	389.8 ± 50.5	1729.7 ± 513.2
Leucine	213.5 ± 123.9	317.7 ± 234.2
Urine† (mmol/g creatinine)		
Isovalerylglycine	193.8	418.6

* Mean ± SD of six consecutive plasma samples.
† Mean of two 6-h urine collections.

or not it was normalized to the higher plasma leucine concentration attained during the second test.

DISCUSSION

Several observations suggest a clinical classification of A.W. as "chronic intermittent" and for K.H. as "acute" isovaleric acidemia (18). The phenotypes presented in each family were different. A.W. survived to mid-childhood without treatment and with minimal symptoms. By contrast, K.H.'s sibling died of isovaleric acidemia in infancy, and K.H. had neonatal symptoms despite protein restriction and glycine supplementation. A.W. tolerated more dietary leucine than K.H. She could ingest 54 mg leucine/kg/day without evidencing free urinary IVA, whereas he required

leucine restriction of less than 46 mg/kg/day. These different phenotypes may also have different detoxification capacities for accumulated isovaleryl-CoA through glycine-N-acylase. A.W. produced considerably more urinary isovalerylglycine than K.H. (Fig. 2). This observation supports the view of Hyman and Tanaka (24) that the ability of glycine-N-acylase to conjugate IVA also determines severity of disease.

What are the optimum doses of glycine required to maintain patients with these varying forms of isovaleric acidemia? Both patients responded differently to incremental glycine supplements when leucine intake was restricted. For the reasons stated above, patient A.W. probably accumulated less intracellular isovaleryl-CoA than patient K.H. On these restricted diets A.W. excreted two to three times more IVG than K.H. even without glycine supplementation. With 50 to 150 mg glycine supplements A.W. increased IVG production over 3-fold that of K.H. (Fig. 2). Unexpectedly, when her glycine supplements were raised further, her IVG excretion decreased by 50%. This effect could either be due to depletion of isovaleryl CoA or cosubstrate inhibition by glycine of glycine-N-acylase. This negative response was not observed under leucine loading conditions. When this pathway of detoxification was stressed in A.W. using oral leucine tolerance tests, she used excess glycine (600 mg/kg) to increase her IVG excretion by 83%. Thus there was no evidence of substrate inhibition of glycine-N-acylase under presumed conditions of high intracellular cosubstrate, isovaleryl-CoA. Glycine inhibition of glycine-N-acylase must occur when there is insufficient cosubstrate present relative to glycine.

Thus, the optimal amount of glycine for the treatment of isovaleric acidemia is dependent on at least three factors: the degree of impaired isovaleryl-CoA dehydrogenase, the amount of isovaleryl-CoA available for conjugation, and the capacity of glycine-N-acylase. Inasmuch as the long-term neurological effects of hyperglycinemia are not well understood, glycine supplements in excess of 300 mg/kg/day should be given cautiously while monitoring plasma glycine concentrations and urinary IVG production. This amount is probably not warranted when patients are stable but may be beneficial during catabolic episodes or during excessive leucine ingestion when intracellular accumulation of isovaleryl-CoA occurs. The amount of leucine that can be tolerated and the optimum glycine supplement should be determined for each patient using these clinical and biochemical parameters as guides only, because each patient will probably have different requirements under different clinical conditions.

REFERENCES

1. Tanaka K, Budd MA, Efron ML, Isselbacher KJ 1966 Isovaleric acidemia: a new genetic defect of leucine metabolism. Proc Natl Acad Sci USA 56:236–242
2. Newman CG, Wilson BDR, Callaghan P, Young L 1967 Neonatal death associated with isovaleric-acidemia. Lancet 2:439–441
3. Cohn R, Yudkoff M, Rothman R, Segal S 1980 Isovaleric acidemia: use of glycine therapy in neonates. J Pediatrics 92:1023–1027
4. Kelleher JF, Yudkoff M, Hutchinson R, August CS, Cohn RM 1980 The pancytopenia of isovaleric acidemia. Pediatrics 65:1023–1027
5. Yoshino M, Yoshida I, Yamushita F, Mori M, Uchiyama C, Tatibana M 1982 Neonatal isovaleric acidemia associated with hyperammonemia. Adv Exp Med Biol 153:141–146
6. Spirer Z, Swirsky-Fein S, Zakut V, Legum C, Charles R, Gil-Av E 1975 Acute neonatal isovaleric acidemia: a report of two cases. Israel J Med Sci 11:1005
7. Wysocki SJ, French NP, Grauaug A 1983 Organic aciduria associated with isovaleric acidemia. Clin Chem 29:1002–1003
8. Mendiola J, Robotham JL, Liehr JG, Williams JC 1984 Neonatal lethargy due to isovaleric acidemia and hyperammonemia. Texas Med 80:52–54
9. Ando T, Kingbert WG, Ward AN, Rasmussen K, Myhan WL 1971 Isovaleric acidemia presenting with altered metabolism of glycine. Pediatr Res 5:478–486
10. Krieger I, Tanaka K 1976 Therapeutic effects of glycine in isovaleric acidemia. Pediatr Res 10:25–29
11. Blaskovics ME, Ng WG, Donnell GN 1978 Prenatal diagnosis and a case report of isovaleric acidemia. J Inherited Metab Dis 1:9–11
12. Yudkoff M, Cohn RM, Puschak R, Rothman R, Segal S 1978 Glycine therapy in isovaleric acidemia. J Pediatr 92:813–817
13. Velazquez A, Prieto EC 1980 Glycine in acute management of isovaleric acidemia. Lancet 1:313–314
14. Shigamatsu Y, Sudo M, Monoi T, Inoue Y, Suzuki Y, Kameyama H 1982 Changing plasma and urinary organic acid levels in a patient with isovaleric acidemia during an attack. Pediatr Res 16:771–775
15. Duran M, Bruinvis L, Ketting D, Wadman SK 1982 Isovaleric acidemia presenting with dwarfism, cataract, and congenital abnormalities. J Inherited Metab Dis 5:125–127
16. Roe CR, Millington DS, Maltby DA, Kahler SG, Bohan TP 1984 L-Carnitine therapy in isovaleric acidemia. J Clin Invest 74:2290–2295
17. Tanaka K, Isselbacher KJ 1967 The isolation and identification of N-isovalerylglycine from urine of patients with isovaleric acidemia. J Biol Chem 242:2966
18. Tanaka K, Rosenberg LE 1983 Disorders of branch chain amino acids and organic acid metabolism. In: Stanbury JB, Wyngaarden JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The Metabolic Basis of Inherited Disease, ed 5. McGraw-Hill Book Co., New York
19. Rhead WJ, Tanaka K 1980 Demonstration of a specific mitochondrial isovaleryl-CoA dehydrogenase deficiency in fibroblasts from patients with isovaleric acidemia. Proc Natl Acad Sci USA 77:580–583
20. Ikeda Y, Keese SM, Tanaka 1985 Molecular heterogeneity of variant isovaleryl-CoA dehydrogenase from cultured isovaleric acidemia fibroblasts. Proc Natl Acad Sci USA 82:7081–7085
21. Matsubara Y, Kraus JP, Ito M, Glassberg R, Barton DE, Yang-Feng T, Ikeda Y, Francke U, Tanaka K 1987 Molecular cloning, nucleotide sequence, and human chromosome assignment of cDNA encoding rat isovaleryl-CoA dehydrogenase. Am J Hum Gen 41(suppl):A228(abstr)
22. Rhead WJ, Hall CL, Tanaka K 1981 Novel tritium release assay for isovaleryl-CoA and butyryl-CoA dehydrogenase. J Biol Chem 256:1616–1624
23. Dubiel B, Dabronski C, Wetts R, Tanaka K 1983 Complementation studies of isovaleric acidemia and glutaric aciduria type II using cultured skin fibroblasts. J Clin Invest 72:1543–1552
24. Hyman DB, Tanaka K 1986 Isovaleryl-CoA dehydrogenase activity in isovaleric acidemia fibroblasts using an improved tritium release assay. Pediatr Res 20:59–61
25. Tanaka K, Mandell R, Shih VE 1976 Metabolism of [1-^{14}C] and [2-^{14}C] Leucine in cultured skin fibroblasts from patients with isovaleric acidemia: characterization of metabolic defects. J Clin Invest 58:164–172
26. Elsas LJ, Acosta PB 1987 Management of inherited metabolic disorders. In Shils ME, Young VR (eds) Modern Nutrition in Health and Disease. Lea and Febiger, Philadelphia, pp 1359–1363
27. Bondi S, Eissler F 1910 Über Lipoproteide und die Deutung der degenerativen zellverfettung. Biochem Z 23:499–509
28. Goodman SI, Markey SP 1981 Appendix A: analytical methods for urine organic acids. In: Laboratory Research Methods in Biology and Medicine, vol. 6. Diagnosis of Organic Acidemias by Gas Chromatography-Mass Spectrometry. Alan R. Liss, Inc., New York, pp 105–114
29. Elsas LJ, Pask BA, Wheeler FB, Perl DP, Trusler S 1972 Classical maple syrup urine disease: cofactor resistance. Metabolism 21:929
30. Elsas LJ, Priest JH, Wheeler FB, Danner DJ, Pask BA 1974 Maple syrup urine disease: coenzyme function and prenatal monitoring. Metabolism 23:569–579
31. American Academy of Pediatrics Committee on Nutrition 1976 Special diets for infants with inborn errors of amino acid metabolism. Pediatrics 57:786
32. Brodehl and Gellissen 1965 Children 2 to 12 years of age, studied after an overnight fast. Pediatrics 42:395–404