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# Isovaleric acidemia: Therapeutic response to supplementation with glycine, L-carnitine, or both in combination and a 10-year follow-up case study

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ABSTRACT

Isovaleric acidemia (IVA) is an organic acid disease caused by a deficiency of isovaleryl-CoA dehydrogenase. Deficiency of this enzyme leads to accumulation of organic acids, such as isovalerylcarnitine and isovalerylglycine. The proposed IVA treatments include leucine restriction and L-carnitine and/or glycine supplementation, which convert isovaleric acid into non-toxic isovalerylcarnitine and isovalerylglycine, respectively. We examined the therapeutic response using the leucine load test and performed a 10-year follow-up in the patient.

*Methods:* We evaluated the patient with IVA beginning at 5 years of age, when he presented with a mild to intermediate metabolic phenotype. Ammonia, free carnitine, isovalerylcarnitine, and isovalerylglycine were analyzed in the urine and blood after a meal consisting of 1600 mg leucine with glycine alone (250 mg/kg/day), L-carnitine alone (100 mg/kg/day), or both glycine and L-carnitine for four days each.

*Results:* (Leucine load test) Three hours after the meal, serum ammonia levels increased most dramatically with glycine treatment alone, then with both in combination, and least with L-carnitine alone. Urinary isovalerylglycine levels increased 2-fold more with glycine supplementation than those following supplementation with both agents or with L-carnitine alone. Treatment with both agents resulted in a gradual increase in urinary acylcarnitine levels during the 6-h period following the leucine load, reaching concentrations comparable to those observed with L-carnitine alone. (Clinical course) After initiation of both glycine (200 mg/kg/day) and L-carnitine (100 mg/kg/day) supplementation at 5 years of age, doses were gradually reduced to 111.7 mg/kg/day and 55.8 mg/kg/day, respectively, at 15 years of age. His mind and body had developed without any sequelae.

*Discussion:* We concluded that L-carnitine conjugated isovaleric acid earlier than glycine. Additionally, during the 10-year follow-up period, the patient displayed no clinical deterioration.



## 1. Introduction

Isovaleric acidemia (IVA; MIM 243500) is an autosomal recessive disorder of organic acid metabolism caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD). IVD catalyzes the conversion of isovaleryl-CoA to 3-methylcrotonyl-CoA during leucine catabolism [1]. IVD deficiency results in an accumulation of derivative organic acids, including isovaleric acid, 3-hydroxyisovaleric acid, isovaleryl (C5)-carnitine (IVC), and isovalerylglycine (IVG) [2,3]. Clinical and laboratory findings in patients with IVA include episodic vomiting, metabolic acidosis, ketosis, hyperammonemia, dehydration, lethargy, distinctive "odor of sweaty feet" because of isovaleric acid buildup, and mental retardation. IVA manifestation varies widely. The severe neonatal onset form presents with early onset of metabolic decompensation. The chronic intermittent form with onset in infancy or childhood presents with developmental delays and/or failure to thrive. Finally, the asymptomatic form is identified through newborn screening of blood spots by tandem mass spectrometry [4,5].

The proposed IVA treatments include dietary leucine restriction and dietary supplementation with L-carnitine and/or glycine to conjugate isovaleric acid, resulting in its conversion into non-toxic IVC and IVG that are subsequently excreted in urine [6,7]. As the glycine dose increases in patients with IVA, IVG excretion in the urine concomitantly increases. However, one study reported that an increase in the glycine dose from 300 to 600 mg/kg/day led to a decrease in IVG excretion

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[8]. Thus, they suggested that glycine overdose might inhibit glycine conjugation by glycine N-acyltransferase (E.C. 2.3.1.13). Fries et al. [9] determined that combined glycine (250 mg/kg/day) and L-carnitine (100 mg/kg/day) therapy maximally increased IVA and IVG excretion in 12-h urine specimens collected overnight after a 2000 mg leucine load in an 8-year-old patient with IVA. Previous studies have indicated that combined therapy with carnitine and glycine maximized the total excretion of isovaleryl-CoA conjugates, but the clinical benefits of combined versus single therapy have not been established in controlled studies [7,10–11]. The relative merits of the two therapies either singly or in combination in patients with IVA remain unclear. Concern has been raised about potential glycine toxicity, though no reports of such an occurrence have been published.

We examined the acute biochemical response, including serum ammonia levels, to a leucine load test following supplementation with glycine, L-carnitine, or both glycine and L-carnitine in combination. Additionally, we followed the patient with IVA for ten years under stable conditions.

## 2. Methods and clinical course

### 2.1. Clinical course (until IVA diagnosis)

The patient had an uneventful delivery after a 39 week-gestation period with a birth weight of 3088 g. Ten days after birth, he presented with anemia and thrombocytopenia. At 3 years of age, mild mental retardation was identified. As he exhibited symptoms, such as repetitive vomiting with hyperammonemia, he was diagnosed with IVA at 5 years and 6 months of age. His height was 119 cm (+1.8 SD), and weight was 19.2 kg (mean). Tsumori's Mental Development Test identified mild mental retardation (DQ = 69). Molecular analysis of the IVD gene revealed a compound heterozygosity of missense mutations consisting of p.Y403C and p.E411K [12]. The biochemical phenotype of the patient was metabolically mild or intermediate type, based on a C5 acylcarnitine concentration of 0.8–6 mmol/L and a urine isovalerylglycine concentration of 15–195 mmol/mol creatinine [3,4].

### 2.2. Leucine load test

We examined the initial biochemical response for 6 h following a leucine load in the patient with IVA. We used a meal containing 1600 mg of leucine. At diagnosis, the patient typically consumed meals containing 1800 mg of leucine. He received a supplement of glycine (250 mg/kg/day), L-carnitine (100 mg/kg/day), or both glycine and L-carnitine for four days each. Following the 1600 mg leucine meal, we measured ammonia, free carnitine, acylcarnitine, IVC, and IVG levels in dry blood spots and urine samples for 6 h. The study was performed in accordance with the standards of the Ethics Committee in the Ryukyus Graduate School of Medicine (Okinawa, Japan).

### 2.3. Clinical course (following IVA diagnosis)

Following the leucine load test, the patient received both glycine (200 mg/kg/day) and L-carnitine (100 mg/kg/day) supplementation and had been followed carefully with an almost constant dose of glycine and L-carnitine for ten years. Magnetic resonance imaging (MRI) of the brain and echocardiography were examined. We measured serum ammonia, serum glycine, free carnitine, and IVG levels from dry blood spots 3 h after lunch during an outpatient visit. We analyzed the relationship between ammonia and glycine, ammonia and free carnitine, and ammonia and IVC levels using Pearson's correlation coefficient in Microsoft Excel to determine the effective quantity of glycine and L-carnitine.

## 3. Results

### 3.1. Leucine load test

Three hours after the test meal, serum ammonia levels displayed the smallest increase with L-carnitine supplementation only, followed by both agents in combination, and the largest increase with glycine supplementation only (Fig. 1a and Supplementary Fig. 1). Blood free carnitine levels were highest following supplementation with both agents (Fig. 1b). Initially, blood IVC levels were the highest following supplementation with L-carnitine only; however, 4 h after the leucine load, IVC levels following supplementation with L-carnitine only or both agents were comparable (Fig. 1c). The increase in urinary acylcarnitine was the highest following L-carnitine supplementation. Supplementation with both agents, however, resulted in a gradual increase over 6 h post-leucine load to concentrations comparable to those observed with L-carnitine alone (Fig. 1d). Treatment with a combination of both agents was associated with a dramatic increase in urinary free carnitine levels (Fig. 1e). The increase in urinary IVG levels following glycine supplementation was twice that of both agents in combination and of L-carnitine alone (Fig. 1f).

### 3.2. Clinical course (following IVA diagnosis)

Following initiation of treatment with both glycine (200 mg/kg/day) and L-carnitine (100 mg/kg/day) in combination with protein restriction to 50 g per day (1.6 g/kg) at five years and 7 months of age, daytime sleepiness was reduced. He had been infected with the influenza virus three times notwithstanding vaccination. During each hospitalization, he was administered L-carnitine (100 mg/kg/day) intravenously and recovered without metabolic acidosis, hyperammonemia, or any sequelae. An MRI of the brain presented no abnormal findings at 6, 10, or 14 years of age. An echocardiogram exhibited normal findings. At 13 years of age, an examination of the Wechsler Intelligence Scale for Children (WISC) III revealed mild mental retardation (IQ = 70). The growth curve indicated that the patient was in the normal range for both height and weight (Supplementary Fig. 2). At 15 years and 7 months of age, his height was 179.6 cm (+1.85 SD), and his weight was 58.2 kg (−0.2 SD). A WISC IV examination revealed mild mental retardation (Full Scale IQ, 66; Verbal Comprehension Index, 66; Perceptual Reasoning Index, 74; Working Memory Index, 73; and Processing Speed Index, 76). He was a student of a technical high school. During the ten-year follow-up period, the serum ammonia concentrations 3 h after a meal at the time of outpatient consultation were within an almost normal range, except when he forgot to take both agents following a meal. There were no significant correlations ($p > 0.05$) between ammonia and glycine, ammonia and free carnitine, or ammonia and IVC levels (Supplementary Fig. 3).

## 4. Discussion

We described clinical observations following an initial leucine load test and during a 10-year follow-up period in a patient with IVA that was treated with both glycine and L-carnitine. We estimated the patient's ammonia levels for 6 h following a leucine load and during the basal state before the leucine load following supplementation with glycine, L-carnitine, or both glycine and L-carnitine for four days each. The patient's baseline ammonia levels might vary as a result of the amount of isovaleric excretion caused by these supplements over the 4-day treatment period. During the 6 h following a leucine load, changes in ammonia levels might result from an early reaction to these supplements.

Glycine conjugation of toxic acyl-CoAs derived from organic acids, such as IVA, by glycine N-acyltransferase in mitochondria is an important metabolic pathway responsible for maintaining mitochondrial energy metabolism [13]. Carnitine acyltransferases are important