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# Ornithine Loading Did Not Prevent Induced Hyperammonemia in a Patient with Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome

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**ABSTRACT. Impairment of urea cycle function in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome is presumably caused, in some patients, by deficient transport of ornithine from cytoplasm into mitochondria. We studied the effect of L-ornithine on L-alanine-induced hyperammonemia in a French-Canadian proband with the syndrome by giving: i) a 90-min intravenous alanine load (6.6 mmol/kg) together with ornithine (1.1 mmol/kg); ii) an intravenous ornithine bolus (0.3 mmol/kg) followed by ornithine infusion (1.1 mmol/kg) 90 min prior to loading with alanine and ornithine; iii) ornithine supplementation per os (1 g, four times daily × 2 wk) prior to loading with alanine and ornithine. Blood ammonia increased from high normal values to 975, 990, and 750 μmol/liter (normal <70) and urinary orotic acid from trace to 539, 494, and 1296 μmol/mmol creatinine (normal 5–11) after the respective loads. Plasma alanine peaked at 1.56–4.24 mmol/liter and ornithine at 1.29–1.95 mmol/liter, but other amino acids were stable. Therefore, ornithine loading did not protect this hyperornithinemia-hyperammonemia-homocitrullinuria patient from hyperammonemia induced by amino-nitrogen loading. Renal fractional excretion of citrulline, lysine, ornithine, glycine, alanine, and tyrosine increased more than 3-fold during ornithine priming, whereas all amino acids were excreted in excess after alanine + ornithine loads; homocitrulline excretion remained unchanged; some urine collections indicated "negative reabsorption" (*i.e.* apparent secretion) of lysine, histidine, and citrulline. Dietary supplementation with ornithine could deplete lysine pools by impairing lysine reabsorption. (*Pediatr Res* 19: 1283–1287, 1985)**

### Abbreviations

**HHH, hyperornithinemia-hyperammonemia-homocitrullinuria**
**LPI, lysinuric protein intolerance**

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Ornithine, an amino acid not incorporated into protein, is an important precursor of polyamines, γ-aminobutyric acid, Δ'-pyrroline-5-carboxylate, proline, and glutamate, and it primes the Krebs-Henseleit urea cycle (Fig. 1). Shih *et al.* (1) were the first to describe, in 1969, a patient with a disorder comprising hyperornithinemia, hyperammonemia, and homocitrullinuria (designated the HHH syndrome). Case histories of ten published patients indicate that the disease is inherited as autosomal recessive with manifestations of intermittent hyperammonemia, vomiting, lethargy, or coma after protein feeding, and retarded somatic and mental development (2–7). The syndrome clearly differs from gyrate atrophy of choroid and retina in which hyperornithinemia is caused by deficiency of ornithine aminotransferase (8–11).

During the formation of urea in mammalian liver, ornithine is transported from cytoplasm into the mitochondrial matrix where it is combined with carbamyl phosphate to form citrulline (12–14). Citrulline is then transported back to the cytoplasm for further processing to form urea. Recent studies suggest that mitochondrial ornithine transport is deficient in the HHH syndrome leading to accumulation of ornithine in cytoplasm and extracellular fluids (5–7, 15, 17). Furthermore, mitochondria in hepatocytes (4, 18, 19) and fibroblasts (20) have abnormal morphology, and their carbamyl phosphate synthetase activity is decreased (4), whereas ornithine aminotransferase activity is normal (4, 5, 21, 22). Ornithine decarboxylase, the first enzyme in polyamine synthesis, has low activity (23).

Supplementation with urea cycle intermediates enhances function of the new cycle in animals (24, 25), possibly in man (26, 27), in patients with LPI (28–30), and in some with urea cycle enzymopathies (31–33).

In theory, a further increment in cytoplasmic ornithine concentration, achieved by expanding the extracellular pool, might benefit HHH patients if cytoplasmic ornithine could then diffuse into mitochondria. It was reported (3, 4, 34) that ornithine supplementation decreases fasting blood ammonia values and leads to clinical improvement in some HHH patients. We have studied the effect of acute and prolonged ornithine supplementation on alanine-induced hyperammonemia in our previously unreported patient. We also measured renal excretion of homocitrulline and other amino acids. We report herein no benefit from ornithine loading in this patient.

## CASE REPORT

The patient is the only child of a nonconsanguineous French-Canadian couple. Feeding difficulties were prominent in infancy, and developmental milestones were delayed. Occasional periods of lethargy, poor appetite, decreased head control, and vomiting

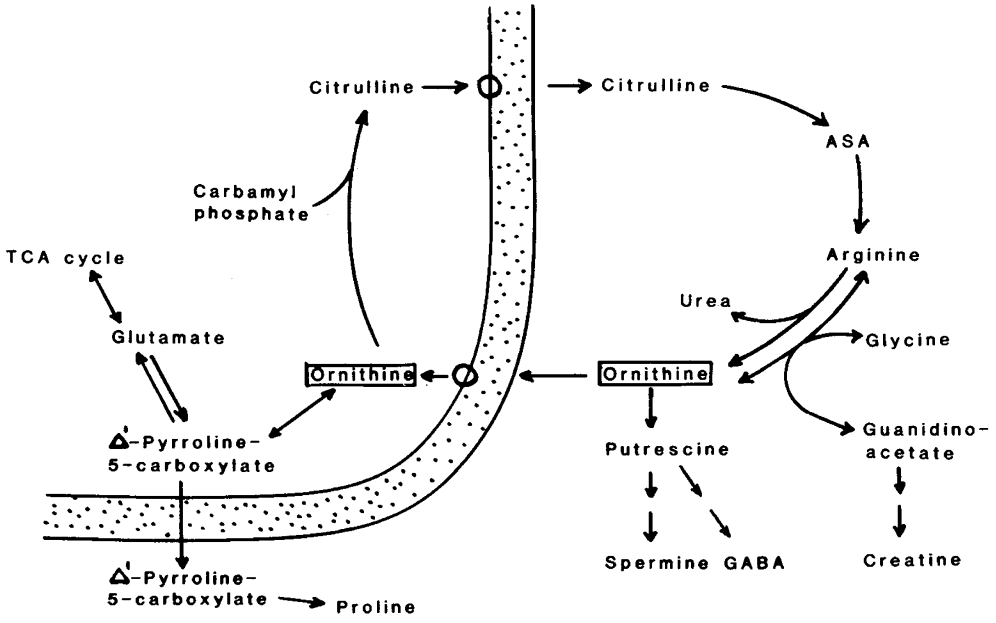


Fig. 1. Metabolism of ornithine. Events in the mitochondrial matrix (*left*) are separated from those in the cytoplasm by the mitochondrial membranes (*dotted area*).

were noted throughout childhood, especially during intercurrent infections.

At age 5 yr, during investigation of a hematoma, deficiencies of coagulation factors VII (1% of control), IX (28%), and X (11%) were found. Liver and spleen were not palpable. An EMG at the time suggested myopathy with fragmented units, excessive numbers of motor-unit potentials, and diminished mean amplitude and duration of impulses. A muscle biopsy was normal by light microscopy. EEG showed mildly disturbed cerebral activity with diffuse arrhythmia. Serum alkaline phosphatase, GOT, GPT, and CPK activities were elevated. Normal values were found for serum electrolytes, calcium, phosphate, urea, creatinine, uric acid, protein, ceruloplasmin, and protein electrophoresis; values for serum cholesterol, triglycerides, and free fatty acids were slightly below normal.

At age 8 yr, the patient had a generalized convulsion. His postprandial blood ammonia, measured then for the first time was 216 μmol/liter (normal <70). Plasma and urinary ornithine levels were increased (plasma: 534–700 μmol/liter, normal 27–86; urine: 2–253 μmol/mmol creatinine; normal 2–4). Urine also contained large amounts of homocitrulline (92–101 μmol/mmol creatinine, normal 0-trace).

The following investigations were performed in 1977 when the patient was 11 yr of age. He was 26 kg (3rd centile), 132 cm (3rd centile), with hyperactive behavior, poor short-term memory, intelligence quotient below the 3-yr level, and poor speech with few words and only short sentences. His eye fundi were normal. He is now 19 yr old and attends a special center for the mentally retarded; he can speak fluently and make himself understood, but is unable to follow abstract thinking or anything but simple commands. He is hypotonic with poor posture but takes care of his own daily activities.

## METHODS

*Infusion protocols.* Infusions were given after an overnight fast. Three protocols were used: i) L-alanine, (6.6 mmol/kg) plus L-ornithine (1.1 mmol/kg) were given as 5% aqueous solutions in 90 min followed by 0.9% saline (200 ml/m²/h) for the next 3 h; ii) ornithine was infused as a bolus (0.3 mmol/kg) followed by infusion of ornithine (1.1 mmol/kg in 90 min), before the infusion of alanine + ornithine (see protocol i); iii) ornithine was given by mouth (1 g, 4 times daily with meals) for 2 wk, followed by the infusion of alanine + ornithine (protocol i). The alanine infusion povided the nitrogen equivalent of 0.6 g protein/kg body weight, whereas 1.1 mmol ornithine/kg was equivalent to 0.2 g protein/kg. Healthy subjects have no symptoms with these loads, and they have been used safely to study patients with various forms of impaired ammonia homeostasis.

Blood samples were drawn before the ornithine infusion in protocol ii and at 0, 120, 180, and 270 min during the combined (alanine + ornithine) infusions (all protocols). Urine was collected by spontaneous voiding during three or four consecutive periods (1½ to 3 h each).

The protocol and its relevance to possible treatment later was explained to the parents. They offered informed consent and the investigation was approved by the institution's peer review group. Whereas no adverse effects were observed in our study, there is a potential for inducing severe hyperammonemia; access to emergency therapeutic measures should exist when undertaking the procedure.

*Anaytical measurements.* Amino acids were measured in plasma and urine by elution chromatography on a modified Beckman-Spinco analyzer (35). Ammonia was measured using an ammonia-specific electrode (Orion Research, Cambridge, MA, model 95-10). Glucose, urea, creatinine, calcium, and phosphate were measured by standard automated techniques. Renal fractional excretion values for amino acids were calculated by using plasma and urinary creatinine and amino acid values. Because plasma concentrations of some amino acids changed considerably during the loads, calculations were based on mean plasma concentrations measured during each urine collection period. Thus, the fractional excretion values, especially of alanine and ornithine, are approximations.

## RESULTS

*Blood ammonia.* Fasting sample blood ammonia values ($n$ = 3, mean 51 μmol/liter, range 35–80) were within or near the reference range (<70 μmol/liter). Blood ammonia increased markedly during the alanine + ornithine infusion (protocol i) [peak value 975 μmol/liter; increment (Δ) + 940 μmol/liter] (Fig. 2). Ammonia remained stable during priming with ornithine (Δ + 40 μmol/liter), but increased following the combined (alanine + ornithine) infusion (protocol ii) (peak value 990 μmol/liter; Δ + 951 μmol/liter). Prolonged ornithine priming by oral

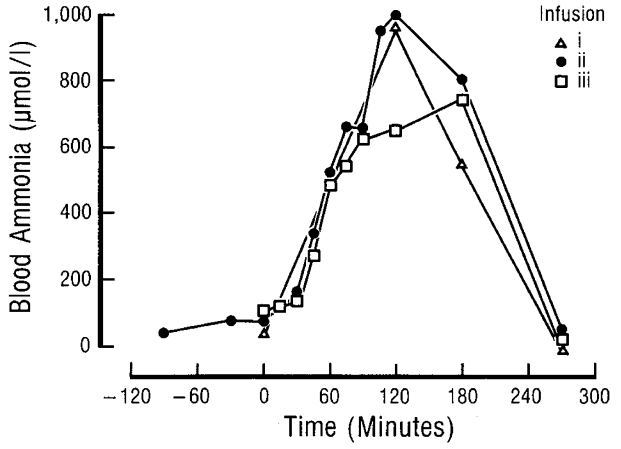


Fig. 2. Blood ammonia during the 90-min alanine + ornithine infusions in the patient with the HHH syndrome. Δ—Δ 1, L-alanine, 6.6 mmol/kg, + L-ornithine, 1.1 mmol/kg were infused in 90 min starting at time 0. ●—●, an ornithine bolus, 0.3 mmol/kg, and 90-min ornithine infusion, 1.1 mmol/kg, preceded the combined (alanine + ornithine) infusion. Ornithine bolus was given at time −90 min, followed immediately by ornithine infusion; alanine + ornithine began at time 0 min. □—□, alanine + ornithine infusion was given after 2 wk of oral ornithine supplementation. For experimental details, see "Materials and methods."

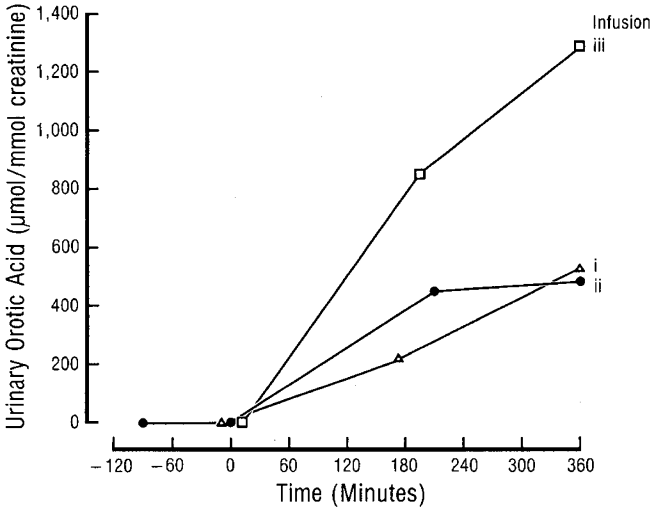


Fig. 3. Urinary orotic acid excretion during and after alanine + ornithine infusions in the patient with the HHH syndrome. For details of the graphs see legend of Figure 2. Each *symbol* represents a 1.5- to 3-h urinary collection; *symbols* have been placed on *time axis* according to the end of the collection periods.

feeding did not prevent hyperammonemia during the combined infusion (protocol iii) (peak value 750 μmol/liter; Δ + 670 μmol/liter). Ammonia values returned rapidly to basal levels in all tests after the infusion. In healthy controls an alanine (or alanine + ornithine) load is followed by a rapid increase in serum urea, but blood ammonia remains unchanged (28). Serum urea increased only minimally in the patient, peak values occurring in the last sample of each protocol.

*Urinary orotic acid.* Excretion of orotic acid was minimal during the basal collection periods, remained unchanged during ornithine infusion (protocol ii), and increased steadily during the combined amino acid infusions (Fig. 3). The later urine collections always had the highest orotate content suggesting that nitrogen loading affected cellular metabolism for several hours. Orotic acid increments after protocol iii were 2-fold as compared with other protocols.

*Plasma amino acids.* Plasma alanine increased from low-normal values (0.11, 0.14, and 2.0 mmol/liter; normal range for age 0.17–0.32 mmol/liter) to 2.74, 1.56, and 4.24 mmol/liter, respectively, in the three protocols. Plasma ornithine concentration increased to 2.02 mmol/liter during infusion of ornithine alone (protocol ii) and to 1.29 and 1.95 mmol/liter during the combined infusions (protocols i to iii) (Fig. 4). Plasma citrulline concentration more than doubled during protocols i and iii but decreased in protocol ii (Fig. 4); the molar citrulline increments represented only 4% of the infused ornithine. Mean basal and 120 min plasma concentrations of glutamine and proline in the three loads were 987 and 929; and 48 and 85 μmol/liter, respectively. Changes in other amino acids including lysine, proline, glutamine, and glutamic acid were minimal.

*Urine amino acids.* Homocitrulline excretion was 79 μmol/mmol creatinine (range 18–106) during the collection periods. Amino acid infusions had no apparent effect on homocitrulline excretion. The fractional excretion values for all other amino acids increased during the infusions. When we infused ornithine alone (protocol ii), fractional excretion values for citrulline, lysine, ornithine, glycine, alanine, and tyrosine (in decreasing order) increased more than 3-fold (data not shown). During the combined (alanine + ornithine) infusions, the largest increments in fractional excretion were for lysine, citrulline, histidine, glycine, and ornithine. Single collections suggested "negative reabsorption" (net "secretion") of lysine, histidine, and citrulline during the alanine + ornithine infusion in protocol ii.

*Other measurements.* Blood glucose remained stable during all infusions. Serum calcium was stable; phosphate decreased slightly. Fractional excretion (×100) of calcium was constant (0.26–1.89) whereas that of phosphate increased from 1.14–11.44 to 22.92–32.22.

To estimate the effect of acute hyperammonemia on coagulation factor activities, we measured them before and after an alanine + ornithine infusion. Factor VII was 9% of control before and 7% after the load; factor IX increased from 17% to 34%.

*Clinical symptoms.* Unlike healthy subjects, who remain symptom free with this amino acid loading protocol (27), pallor, dizziness, nausea, and vomiting occurred at 90–110 min during the alanine + ornithine infusions in the patient.

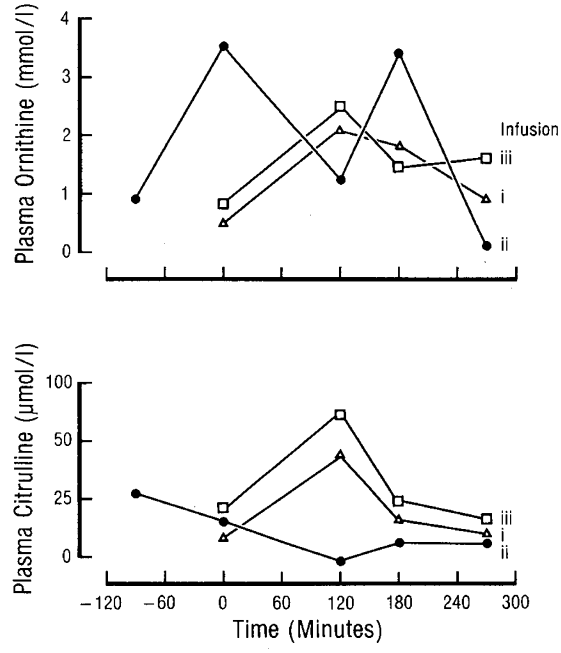


Fig. 4. Changes in plasma ornithine and citrulline during the alanine + ornithine infusions in the patient with the HHH syndrome. For details see legend of Figure 2.

## DISCUSSION

Our patient has the typical clinical and biochemical features of the HHH syndrome. Blood ammonia values were, as expected, within the normal range during fasting, but were elevated after high protein meals.

L-Alanine loading intravenously is used to measure nitrogen tolerance in LPI patients (28–30). Simultaneous infusion of 1.1 mmol/kg of ornithine prevents hyperammonemia which otherwise develops during alanine loading in LPI (28). In three previous reports oral ornithine supplementation in HHH decreased fasting blood ammonia values (3, 7, 34). Ornithine did not prevent hyperammonemia in our patient with HHH. Moreover, further filling of the ornithine pool by 90-min ornithine preload intravenously or by prolonged feeding of ornithine prior to the alanine + ornithine load was also without effect.

Orotic acid excretion is usually increased in deficiencies of the urea cycle enzymes (except carbamyl phosphate synthetase deficiency) and in lysinuric protein intolerance when the capacity of the impaired urea cycle is exceeded. Carbamyl phosphate effluxes from the mitochondria into the cytoplasm, where it is utilized for pyrimidine synthesis. Orotic acid, an intermediate in this pathway, has a high renal clearance. The nitrogen loads in our patient were followed by orotic aciduria suggesting that carbamyl phosphate was adequately produced, but its utilization for citrulline synthesis was impaired. Contrary to what we expected, prolonged ornithine supplementation (protocol iii) aggravated orotic aciduria and may thus in fact be deleterious for the nitrogen tolerance of the patient. The increase in orotic acid excretion suggests that any partial deficiency of carbamyl phosphate synthetase I in the HHH syndrome (4) is of little functional importance.

Two infusions (protocols i and iii) were followed by a rise in plasma citrulline. This response may reflect a change in citrulline flux between plasma and tissues, altered citrulline metabolism, or induced "synthesis" from (infused) ornithine. However, citrulline synthesis accounted for only 4% of the infused ornithine even if one assumes that the total increment in plasma citrulline was derived from infused ornithine. The changes in other plasma amino acid (except alanine and ornithine) were small and inconsistent showing that the infused amino acids do not greatly affect metabolism of the other amino acids.

The hyperammonemia and other changes produced by the combined load (alanine + ornithine) did not decrease coagulation factor concentrations in our patient. The coagulation factor deficiencies may be inherited separately; a similar defect has been found in some members of a large Canadian pedigree with the HHH syndrome (4), but not in other patients.

The alanine + ornithine infusion increased fractional excretion of several amino acids. Lysine and histidine were exceptionally affected. These changes (and changes in phosphate excretion) were associated with increased filtered load of alanine and/or ornithine. The demand of alanine for $Na^+$ cotransport may have dissipated the transmembrane gradient used for neutral amino acid transport. The effect of ornithine would not be by this mechanism but it would compete on the lysine carrier. Thus, continuous feeding of extra ornithine to HHH patients with marginal protein intake might induce lysine deficiency.

In summary, our findings suggest that further elevation of plasma ornithine in the HHH syndrome does not necessarily prevent hyperammonemia and might induce lysine deficiency. *In vitro* studies by others (5, 17) indicate that the apparent Km for ornithine uptake and metabolism in mitochondria is elevated in some HHH patients; an increase in the cytosolic concentration of ornithine *in vivo* should benefit these patients. On the other hand, mutation that severely decreases Vmax of the processes, would yield a phenotype unresponsive to ornithine loading *in vivo* and *in vitro*. Our patient may have the latter form of the HHH phenotype. Genetic heterogeneity in the HHH syndrome is a likely explanation why ornithine supplementation benefits only some patients (3, 4, 34).

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