Oral Supplementation Corrects Plasma Lysine Concentrations in Lysinuric Protein Intolerance

Mari Lukkarinen, Kirsti Näntö-Salonen, Kari Pulkki, Maija Aalto, and Olli Simell

In lysinuric protein intolerance (LPI), intestinal absorption and renal tubular reabsorption of arginine, ornithine, and lysine are impaired due to a defective cationic amino acid transporter. Deficiency of arginine and ornithine restricts the function of the urea cycle, leading to hyperammonemia after protein load, and to strong protein aversion. Mealtime supplements of citrulline, another urea cycle intermediate that uses other transport mechanisms, prevent postprandial hyperammonemia and improve protein tolerance. Deficiency of lysine, an essential amino acid, most probably also contributes to the symptoms of LPI. We investigated possibilities to improve the availability of lysine for tissues by increasing plasma lysine concentration. Six patients with LPI were started on short-term oral lysine supplementation that was administered with their regular citrulline doses and standard low-protein meals. L-Lysine in consecutive doses of 0.55 and 1.1 mmol/kg caused profuse diarrhea in first 3 patients. To avoid gastrointestinal side effects, the 3 other patients were started on smaller lysine supplements of only 0.05 mmol/kg per dose, given 3 times daily for 3 days. All pre- and postprandial plasma lysine concentrations remained within normal range in 2 of the 3 patients studied. Even after the larger doses, no significant effects on the urea cycle were seen. We conclude that low-dose oral lysine supplementation normalizes plasma lysine concentration in patients with LPI, and is safe and well tolerated at least in short-term use.

MUTATIONS IN THE SLC7A7 gene encoding a transmembrane cationic amino acid transporter y^+LAT-1 cause lysinuric protein intolerance (LPI), an autosomal recessive disorder in the transport of the dibasic amino acids lysine, arginine, and ornithine.[1] The transport defect affects the basolateral membrane of the renal tubules and the intestinal epithelium, as well as the plasma membrane of fibroblasts.[2-4] Poor intestinal absorption and renal tubular reabsorption lead to deficiency of arginine and ornithine, essential urea cycle intermediates, explaining why the patients develop hyperammonemia after high-protein meals. As a result, strong protein aversion develops early, and poor protein intake further aggravates the deficiency of the cationic amino acids.

The symptoms and signs of LPI comprise aversion to dietary protein, failure to thrive, short stature, muscle weakness, osteoporosis, and hepatosplenomegaly. Some patients are slightly to moderately mentally retarded, particularly if they have experienced prolonged episodes of hyperammonemia.[5] The patients with LPI are also prone to upper respiratory tract infections and often develop severe, life-threatening varicella infection.[6] Current therapy consists of dietary protein restriction and supplementation with citrulline, another urea cycle intermediate which is well absorbed and effectively prevents hyperammonemia, improving protein tolerance of the patients.[7] The present therapy, however, fails to correct the shortage of lysine.[7,8]

Citrulline supplementation has ameliorated the growth failure of children with LPI only slightly despite the fact that it has essentially improved their protein tolerance and protein nutrition. Relative deficiency of lysine, an essential amino acid, may in fact be the growth-limiting factor in LPI. Most patients with LPI have radiological signs of osteoporosis, and especially in childhood, several patients suffer from fractures after minor trauma.[9] Adequate calcium and vitamin D supplementation has failed to correct osteoporosis in LPI. Thus, defective synthesis of bone matrix proteins due to deficiency of the essential cationic amino acids may play a role.[10] The problem still remains as severe as before the era of citrulline therapy. Interestingly, Civitelli et al have shown that addition of L-lysine to the diet of healthy subjects enhanced intestinal calcium absorption and improved renal calcium conservation.[11] Lysine supplementation might thus improve bone mineral density also by increasing calcium retention.

The patients with LPI are prone to upper respiratory tract infections and often develop severe, life-threatening varicella infections.[5,6] As lysine was recently found effective in suppressing the reactivation of herpes simplex virus infection,[12] subnormal plasma lysine concentrations may be one of the factors that make patients with LPI susceptible to unusually severe varicella infection.[6] Lysine deficiency together with subclinical protein-energy malnutrition may at least partly explain the immune abnormalities in LPI.

In some patients with hyperdiaminoaciduria or specific lysine transport defects, mental retardation has been suggested to result from lysine deprivation at some critical stage of brain development.[5,13] Chronic lysine deficiency may thus contribute to the cognitive problems of individual patients with LPI that have been attributed solely to recurrent episodes of hyperammonemia.

In theory, the availability of lysine for tissues could be improved by increasing its plasma concentration by sufficient oral supply of lysine itself or a better-absorbed lysine derivative. One possible drawback is that large amounts of extra lysine will also contribute to the total nitrogen load, further straining the already limited capacity of the urea cycle. More-

From the Department of Pediatrics, University of Turku; and the Department of Clinical Chemistry, Turku University Central Hospital, Turku, Finland.

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Address reprint requests to Mari Lukkarinen, MD, c/o Dr Kirsti Näntö-Salonen, Department of Pediatrics, University of Turku, PL 52, FIN-20521, Turku, Finland.

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Table 1. Characteristics and Daily Citrulline Doses of the Patients

Patient No.	Gender	Age at the Time of Study (yr)	Age at the Time of Diagnosis (yr)	Weight at the Time of Study (kg)	Height at the Time of Study (cm)	Daily Citrulline Dose (mmol/kg)
1	M	25	1.5	54.2	161.5	0.52
2	M	30	0.2	64.5	172	1.25
3	M	32	2.2	64.1	172	0.44
4	F	35	12.0	49.5	142	0.43
5	F	40	14.0	42.0	159	0.84
6	F	42	12.0	62.0	151.5	0.50

over, the enzymes of urea cycle are inhibited by high lysine concentration.[14] The membrane transport defect in LPI may particularly predispose the patients to enrichment of lysine in certain intracellular compartments, where high local concentrations might be harmful. We investigated whether it is possible to overcome the intestinal transport defect by sufficient amounts of oral L-lysine to normalize plasma lysine concentration in the patients, without inducing hyperammonemia or gastrointestinal side effects.

PATIENTS AND METHODS

Patients

Six adult patients with LPI (3 females) with the Finnish founder mutation 1181-2A→T in the SLC7A7 gene were investigated (Table 1). All had characteristic clinical and biochemical findings of LPI, and had been on protein-restricted diet and individually tailored oral citrulline supplementation (0.43 to 1.25 mmol/kg/d) for several years. They continued this regimen during the study period.

The study was approved by the Joint Commission on Ethics of Turku University and Turku University Central Hospital. Written informed consent was obtained from all the subjects.

Short-Term Oral Lysine Supplementation

We selected L-lysine-HCl for the supplementation because of its superior solubility compared with lysine base. After an overnight fast, patients no. 1, 2, and 3 received 0.55 mmol/kg of L-lysine-HCl together with a standard protein-restricted hospital breakfast and their regular citrulline supplement (0.11 to 0.15 mmol/kg). A larger dose of lysine (1.1 mmol/kg) was administered at 4 PM together with a standard protein-restricted hospital meal and the regular citrulline dose (0.11 to 0.22 mmol/kg). Within an hour, all 3 patients developed profuse watery diarrhea.

To avoid gastrointestinal adverse effects, a smaller dose was selected for further studies. Patients no. 4, 5, and 6 received 3 daily doses of 0.05 mmol/kg of L-lysine-HCl (up to a maximum dose of 2.5 mmol) for 3 days. The lysine supplement was administered together with standard low-protein hospital meals at 8 AM, 11 AM, and 4 PM. The patients continued their regular pre-meal citrulline supplementation (from 0.43 to 0.84 mmol/kg) through the study. Plasma amino acid and ammonia concentrations and serum glucose, γ-glutamyltransferase, urea, and creatinine concentrations were measured before and 90 minutes after the lysine dose. Spot urine samples were collected for amino acid and orotic acid analysis when the blood samples were drawn.

Biochemical Analyses

Plasma and urinary amino acids were measured with an LKB-Alpha Plus amino acid analyzer (Pharmacia, Uppsala, Sweden) using reference values of Parvy et al.[15] Plasma ammonia concentrations were measured with an automatic analyzer (Hitachi, Model 717; Boehringer-Mannheim, Mannheim, Germany) using an enzymatic assay, and urinary orotic acid concentrations were measured with high-performance liquid chromatography (HPLC) cation exchange chromatography of pretreated samples (Aminex HPX-87H column, Bio-Rad Laboratories, Watford, UK).

RESULTS

Clinical Symptoms

Patients no. 1, 2, and 3 tolerated well the first dose of L-lysine-HCl (0.55 mmol/kg), but the second dose of 1.1 mmol/kg caused severe abdominal cramps and profuse watery diarrhea within an hour. The symptoms disappeared in the next 2 to 3 hours without intervention. Patients no. 4, 5, and 6 tolerated the repeated smaller lysine dose without any symptoms.

Plasma Amino Acids

Plasma lysine, arginine, and ornithine concentrations of all 6 patients were at or below the lower reference limits at the beginning of the study after an overnight fast. The concentrations of lysine, arginine and ornithine (mean ± SD; range) were 94 ± 22 (61 to 120) μmol/L, 23 ± 10 (10 to 38) μmol/L, and 36 ± 15 (21 to 59) μmol/L, respectively; the reference ranges of the three amino acids were 114 to 289 μmol/L, 15 to 183 μmol/L, and 22 to 115 μmol/L. At baseline, plasma glycine concentrations were above the upper reference limit in 5 patients and glutamine + glutamic acid concentrations in 1 patient [553 ±186 (335 to 627) μmol/L and 829 ± 164 (613 to 1,053) μmol/L, respectively; reference ranges, 145 to 356 and 324 to 971 μmol/L].

During the first day of the study period, all tested lysine doses corrected postprandial plasma lysine concentrations up to normal range. Maximal concentrations were measured 3 hours after ingestion. Mean increments after the lysine doses of 0.55 and 1.1 mmol/kg were 95 μmol/L (range, 80 to 112 μmol/L) and 50 μmol/L (range, 49 to 51 μmol/L), respectively. As plasma lysine concentrations did not decrease to baseline between the 2 doses, the peak concentrations were almost equal (mean values, 185 and 189 μmol/L, respectively). The first lysine dose of 0.05 mmol/kg increased mean lysine concentration by 21 μmol/L (range, 8 to 28 μmol/L) and the second doses by 29 μmol/L (range, 9 to 53 μmol/L), respectively (Fig 1). Mean values for peak concentrations after these doses were 118 μmol/L and 145 μmol/L.

During the 3-day study period all plasma lysine concentrations of patients no. 4 and 6 remained steadily within normal range. For the first day, the lysine values of patient no. 5 were also normal, but during the rest of the study period, subsequent

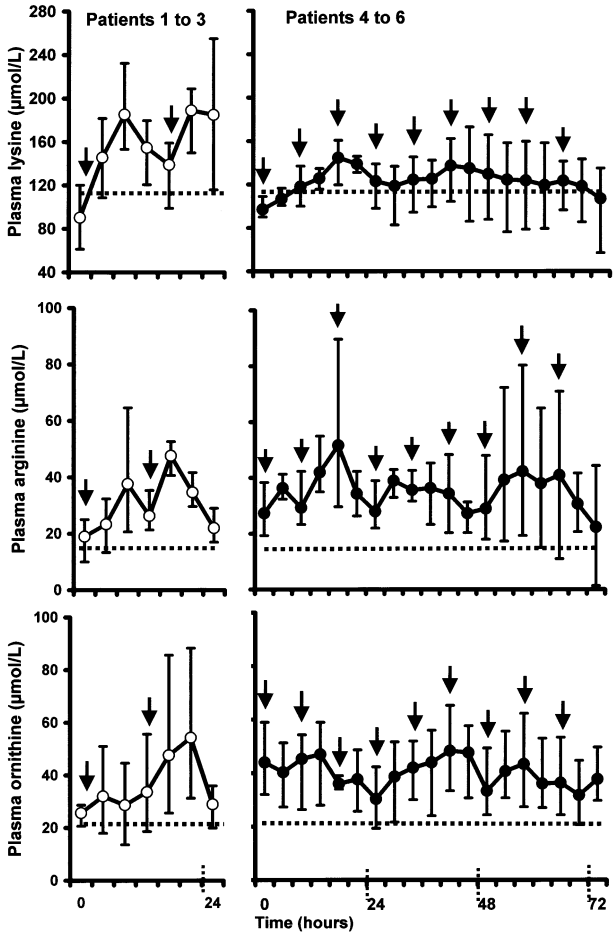

Fig 1. Plasma concentrations of lysine, arginine, and ornithine (mean and range) in patients with LPI during the 2 different short-term oral L-lysine-HCl supplementation periods (for details, see Methods). Data of patients no. 1 to 3 are shown in the left panel, and data of patients no. 4 to 6 in the right panel. Horizontal broken lines indicate the lower reference limits. Arrows indicate intake of oral L-lysine-HCL.

lysine doses failed to increase her plasma lysine concentrations, which slowly returned back to baseline.

During the 3-day lysine supplementation, plasma arginine and ornithine concentrations (range, 15 to 90 μmol/L and 19 to 65 μmol/L) remained steadily within the reference range, fluctuating normally with meals. Plasma glycine and glutamine + glutamic acid concentrations remained stable during the study except for a short unexplained peak in plasma glycine from 827 to 2,140 μmol/L in patient no. 6 during the second day of the study. Plasma alanine concentrations were around the upper reference limit in all patients (range, 219 to 895 μmol/L; reference range, 231 to 580 μmol/L). The plasma concentrations of all other amino acids fluctuated very slightly during the study (data not shown).

Amino Acid Excretion

At baseline, the mean urinary excretions of lysine, arginine, and ornithine (expressed as μmol/mmol creatinine; mean and range) were 1,217 (591 to 1,598), 137 (4 to 297), and 34 (2 to 72), respectively. The respective reference ranges were 7 to 58, 0 to 5, and 0 to 5 μmol/mmol creatinine. Baseline excretions of glycine, glutamine + glutamic acid, and alanine were slightly above the reference values in 2 of the patients (data not shown).

In patients no. 1 to 3, mean lysine excretion (μmol/mmol creatinine) increased from 882 to 1,333 after 2 doses of L-lysine-HCl. However, in patients no. 4 to 6 who received the smallest lysine dose, urinary lysine excretion declined slightly from 1,551 to 1,210 μmol/mmol creatinine. Arginine and ornithine excretions increased markedly in all patients, especially during the first day of lysine supplementation: in patients no. 1 to 3 from 146 to 310 μmol/mmol creatinine and in patients no. 4 to 6 from 127 to 740 μmol/mmol creatinine. In patients no. 4 to 6, arginine and ornithine excretion then remained at values 2 to 4 higher than baseline. No clear changes occurred in urinary excretion of other amino acids (data not shown).

Nitrogen Metabolism

There were no clinical symptoms or changes in biochemical parameters that would have suggested abnormal accumulation of lysine. The plasma ammonia concentrations of the patients fluctuated between 17 and 60 μmol/L (reference range, <50 μmol/L), with the exception of a single asymptomatic peak (108 μmol/L) 90 minutes after the first lysine dose in patient no. 2. Serum urea concentrations varied randomly between 2.2 and 6.5 mmol/L (reference range, 2.5 to 7.4 mmol/L). Lysine supplementation did not alter orotic acid excretion profiles of the patients. Eight hours after the first lysine dose (0.55 mmol/kg) patient no. 3 showed a single orotic acid excretion peak (36 μmol/mmol creatinine) but simultaneously measured plasma ammonia concentration was normal.

Other Measurements

Neither single doses of L-lysine hydrochloride nor 3-day supplementation induced hypoglycemia in any of the patients. Serum creatinine and γ-glutamyltransferase values remained normal in all patients.

DISCUSSION

Although the role of lysine in the treatment of LPI has not yet been established, there are strong theoretical implications for lysine supplementation. Lysine deficiency may be associated with several clinical symptoms of LPI, eg, growth failure, osteoporosis, muscular hypotonia, and immunological abnormalities. Ample follow-up data indicate that plasma lysine concentration cannot be normalized by the lysine absorbed from the restricted amounts of natural protein that the patients tolerate, even with the help of citrulline supplementation.[7,8]

In an earlier study, addition of L-lysine-HCl (5.5 to 8.2 mmol/d) to the diet of 12 children with LPI for 0.5 to 2 years did not improve their growth more than citrulline supplementation alone. The above doses of lysine provoked abdominal colics and diarrhea in several patients, and most of the patients soon abandoned the therapy. The data suggested that gastrointestinal side effects probably outweigh the potential benefits of lysine supplementation.[7] However, there are reports of successful L-lysine-HCl supplementation in individual patients.[8,16]

Awrich et al showed that the combination of citrulline plus lysine nearly was able to normalize the plasma amino acid profile and prevent postprandial hyperammonemia.[8]

In the present study, we tested different doses of L-lysine-HCl to find a dose sufficient to normalize plasma lysine concentrations in patients with LPI but not provoke any adverse effects. We confirmed that larger doses induce abdominal colics and diarrhea, while small frequent doses were well tolerated and effective. In the future the individually efficient and safe L-lysine dose probably can be found with careful titration between these tolerated doses 0.05 to 0.55 mmol/kg L-lysine-HCl.

High lysine concentration may interfere with the function of the urea cycle[14] probably because lysine competes with ornithine for a common transporter at the plasma membrane and possibly at other transport-restricting membranes between certain compartments of the cell.[17] The additional nitrogen load provided by extra lysine may also further aggravate hyperammonemia in LPI. However, we recently showed that LPI patients were able to handle extremely high plasma lysine concentrations induced acutely via intravenous lysine loads without developing hyperammonemia or increasing orotic acid excretion.[18] Urinary orotic acid is the product of the "overflow" from the urea cycle into the pyrimidine pathway via carbamyl phosphate, and thus reflects plasma ammonia concentration over a longer period of time. Hence the method is able to pick up also temporary elevations that may be missed in the serial direct measurements of plasma ammonia concentrations at certain intervals. In the present study, small oral doses of lysine markedly enhanced the amount of lysine absorbed to the body during the 3-day study period. The increase in the lysine pool led to no signs of disturbed urea cycle function, further supporting the previous findings of the safety of lysine in the treatment of LPI. It is likely that the simultaneous citrulline supplementation preserves sufficient urea cycle function also in the presence of higher lysine concentrations.

In conclusion, this study shows that L-lysine-HCl in small doses of 0.05 mmol/kg, given 3 times a day at mealtimes together with citrulline, was able to normalize plasma lysine concentration in patients with LPI. The therapy was well tolerated during a 3-day study period, and did not induce hyperammonemia. The smallest effective dose of L-lysine has probably to be titrated individually. Another cohort of Finnish patients with LPI has now been on similar L-lysine regimen for more than 1 year with normalization of plasma lysine concentrations and no apparent clinical adverse effects. Such long-term follow-up studies will show if lysine supplementation is able to ameliorate the clinical symptoms of LPI that are not corrected by citrulline supplementation, eg, poor linear growth, muscle strength, and bone mineral density.

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