Effect of Lysine Infusion on Urea Cycle in Lysinuric Protein Intolerance

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Poor intestinal absorption and excessive renal loss of dibasic amino acids result in low plasma concentrations in patients with lysinuric protein intolerance (LPI). Arginine and ornithine deficiency impair the function of the urea cycle and cause hyperammonemia after protein intake, while chronic lysine deficiency may cause growth failure and lead to reduced bone density in such patients. Since high lysine concentrations inhibit several enzymes of the urea cycle in the liver, lysine supplementation may induce hyperammonemia in LPI. We thus studied how LPI patients tolerate high plasma lysine by intravenous (IV) infusion of 3.3 mmol/kg lysine hydrochloride over 90 minutes in 6 adult patients and 4 healthy controls. The plasma lysine concentration (mean ± SD, range) peaked in the patients (9,114 ± 1,864, 7,156 to 12,044 μmol/L) and controls (10,185 ± 2,253, 7,714 to 13,122 μmol/L) at 90 minutes. Urinary lysine excretion peaked in the second 2-hour urine collection in the patients (4,582 ± 1,276, 3,018 to 6,315 μmol/m² body surface area per hour) and in the first 2-hour collection in the controls (5,373 ± 1,766, 3,551 to 7,286 μmol/m²/h). Two patients had mild nausea but no hyperammonemia and one patient had moderate hyperammonemia (peak, 112 μmol/L) at the end of the infusion. Orotic acid excretion increased in 2 subjects with a peak excretion rate of 33 and 251 μmol/m²/h in the third 2-hour collection after starting the load. All other subjects remained asymptomatic and showed no change in plasma ammonia or urinary orotic acid excretion. We thus conclude that an acute increase in plasma lysine caused minimal clinical or biochemical untoward effects in patients with LPI. Moderate increases in plasma lysine after low-dose oral supplementation with lysine or well-absorbed lysine derivatives are probably well tolerated in LPI.

LYSINURIC PROTEIN INTOLERANCE (LPI) is an autosomal recessive defect in the transport of the dibasic amino acids lysine, arginine, and ornithine caused by mutations in the SLC7A7 gene encoding the transmembrane cationic amino acid transporter y^+LAT-1. The disorder, localized to the basolateral membrane of the renal tubules and intestinal epithelium and to the plasma membrane of fibroblasts and possibly also hepatocytes, leads to excessive urinary losses and low plasma concentrations of the dibasic amino acids. A deficiency of arginine and ornithine causes a malfunction of the urea cycle and hyperammonemia after a dietary protein load or intravenous (IV) infusion of L-alanine.[1] The number of LPI patients known to us now exceeds 120, and 45 of these patients are Finns.

After well-tolerated breastfeeding, patients with LPI characteristically develop a strong aversion to dietary protein, fail to thrive, grow poorly, and have short stature, hepatosplenomegaly, muscle weakness, and osteoporosis. Recurrent episodes or prolonged hyperammonemia may lead to psychomotor delay, while individuals protected by a strong protein aversion show normal mental development.[2] LPI treatment consists of dietary protein restriction and oral supplementation with citrulline, which is a neutral amino acid and urea cycle intermediate that is effectively absorbed in LPI. It improves protein tolerance and helps to prevent hyperammonemia.[3] However, citrulline therapy fails to correct the shortage of lysine, and the restricted protein intake further aggravates lysine deficiency. Patients with LPI may thus have a chronic deficiency of essential lysine, as suggested by the almost constant combination of a subnormal plasma lysine concentration, poor growth, and prominent osteoporosis.

A high lysine concentration in liver cells may inhibit the function of the urea cycle[4] and thus increase the risk of hyperammonemia in these patients. Low-dose oral lysine supplementation did not cause hyperammonemia in the patients but led to profuse diarrhea and abdominal cramps, and was soon abandoned.[3] Today, more accurate monitoring of the metabolic control of LPI is possible, and potentially well-absorbed lysine precursors are becoming available. We have thus studied the effects of an acute IV lysine load on plasma amino acid concentrations, urinary amino acid excretion, and urea cycle function in LPI.

SUBJECTS AND METHODS

Patients

Six adult LPI patients (4 women) and 4 age-matched healthy subjects (2 women) received an IV lysine load. All patients had the characteristic clinical and biochemical findings of LPI and the Finnish mutation in the SLC7A7 gene (A1181-2T) and were on a protein-restricted diet and daily oral citrulline supplementation. The supplementation was stopped on the evening prior to lysine infusion, and continued 6 to 7 hours after beginning the infusion, before ingestion of the next meal. The fasting serum creatinine concentration, creatinine clearance, and serum liver enzyme activity were normal in all subjects. One patient received therapy for hypertension (Table 1). All subjects provided informed consent to the study.

IV Infusion of L-Lysine

After an overnight fast, the test subjects emptied their bladder, and 3.3 mmol/kg L-lysine hydrochloride was infused as a 5% aqueous solution IV over 90 minutes, followed by infusion of physiological saline 200 mL/m² body surface area per hour for 270 minutes. The subjects fasted throughout the 6-hour total infusion period, after which they ate a light, low-protein lunch. Samples for plasma ammonia, serum creatinine, plasma amino acids, and blood pH were drawn at the

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Submitted April 23, 1999; accepted November 4, 1999.

Supported by grants from the Sigrid Juselius Foundation, Emil Aaltonen Foundation, and Ulla Hjelt Fond of the Foundation for Pediatric Research, Finland.

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0026-0495/00/4905-0022$10.00/0

Table 1. Patients With LPI Receiving Daily Citrulline Supplementation

Patient No.	Gender	Age at Diagnosis (yr)	Age at Study (yr)	Serum Creatinine (μmol/L)	Other Diseases	Medication
1	F	0.3	28.9	63		
2	M	7.6	30.5	118	Hypertriglyceridemia	
3	F	10.8	38.7	90		
4	F	11.5	39.3	65		
5	F	15.6	43.5	91		
6	M	25.0	51.0	88	Arterial hypertension	Enalapril maleate

Abbreviations: M, male; F, female.

beginning of lysine infusion and at 60, 90, 120, 180, 270, and 360 minutes. Serum glucose, insulin, and growth hormone levels were measured before starting the lysine infusion and at 120 minutes. Amino acid and orotic acid excretion were measured in urine collected between 0 and 2 hours, 2 and 4 hours, 4 and 6 hours, 6 and 12 hours, and 12 and 24 hours after the start of the infusion. No preinfusion urine samples were collected.

Analyses

Plasma and urine amino acid levels were measured with an LKB-Alpha Plus amino acid analyzer (Pharmacia, Uppsala, Sweden), and reference values from Parvy et al[5] were used. Plasma ammonia concentrations were measured with an automatic analyzer (Hitachi model 717; Boehringer, Mannheim, Germany) using an enzymatic assay, and urinary orotic acid concentrations were analyzed by cation-exchange high-performance liquid chromatography of pretreated samples (Aminex HPX-87H column; Bio-Rad Laboratories, Watford, UK).

Statistical Analysis

Statistical comparisons were made using ANOVA for repeated measurements or Friedman's test as appropriate.

RESULTS

Plasma Amino Acids

In the LPI patients, the baseline concentration of plasma lysine was at or below the lower reference limit (range, 63 to 128 μmol/L; reference range, 114 to 289 μmol/L).[5] Patient no. 3 had exceptionally high plasma lysine at the beginning of the study (616 μmol/L) which was probably caused by a technical error in the analysis, as her plasma lysine values measured during years of systematic follow-up evaluation had always been less than 100 μmol/L. Unfortunately, the rest of the sample was lost, preventing a reanalysis. The baseline plasma ornithine and arginine concentrations were in the low-normal range (mean concentration in the patients, 35.2 μmol/L [range, 27 to 52] and 26.8 μmol/L [range, 13 to 40], respectively; reference ranges, 22 to 115 and 15 to 183 μmol/L).[5] Fasting plasma amino acid concentrations in all control subjects were normal. Lysine infusion increased plasma lysine in the patients to peak values (mean ± SD, range) of 9,114 ± 1,864 μmol/L (7,156 to 12,044), and in the controls to 10,185 ± 2,253 μmol/L (7,714 to 13,122) at 90 minutes (Fig 1). The mean plasma arginine level tripled in the patients and controls during the infusion, but since the fasting value was markedly higher in the controls, the absolute peak value in the controls clearly exceeded that in the patients. Plasma ornithine slightly declined during the study in both groups, while plasma glycine remained at the baseline and plasma glutamine and glutamic acid concentrations slightly increased or remained stable. Plasma alanine decreased clearly after lysine infusion in LPI patients, but remained unchanged in the controls (Fig 2).

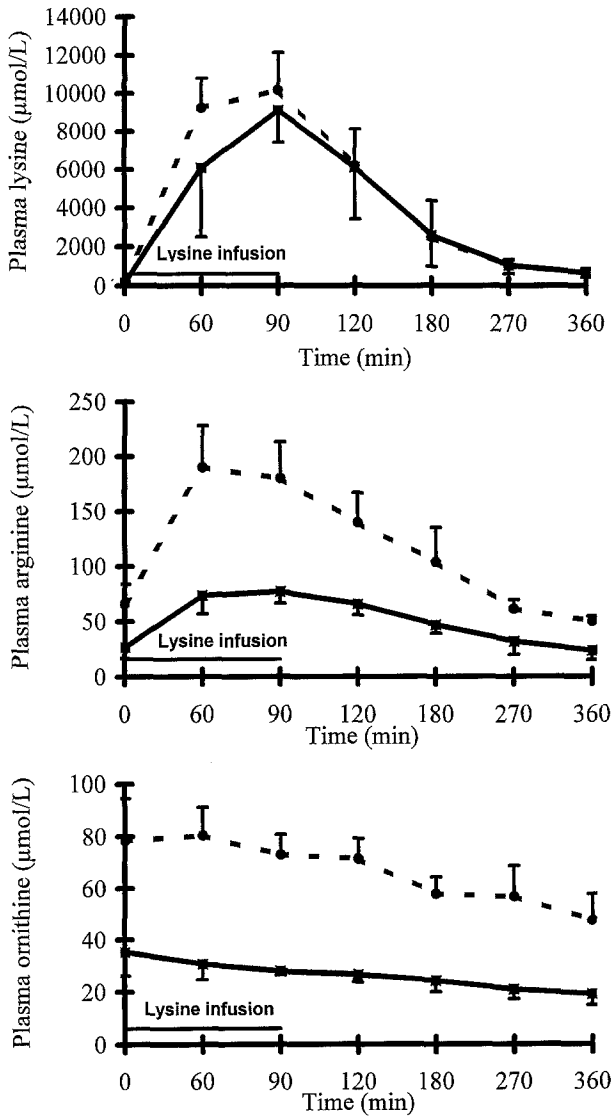

Fig 1. Plasma lysine, arginine, and ornithine (mean ± SD) in patients with LPI (■) and controls (●) in the lysine infusion test. Plasma arginine increased more during the test in the controls *v* the patients ($P < .0001$).

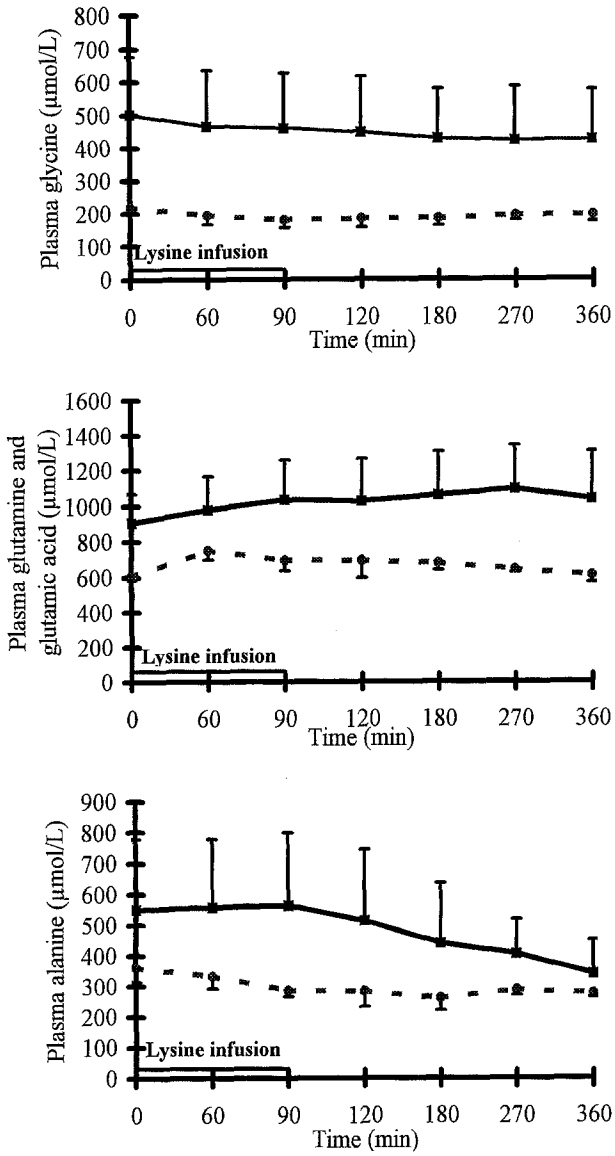

Fig 2. Plasma glycine, glutamine + glutamic acid, and alanine (mean ± SD) in patients with LPI (■) and controls (●) in the lysine infusion test. Responses for glutamine + glutamic acid and alanine in patients and controls differed at $P \leq .002$.

Amino Acid Excretion

Urinary lysine excretion (mean ± SD, range) peaked in the second 2-hour urine collection (4,582 ± 1,276, 3,018 to 6,315 µmol/m^2 body surface area per hour), while the lysine peak in the controls occurred already in the first 2-hour collection (5,373 ± 1,766, 3,551 to 7,286 µmol/m^2/h). LPI patients excreted 4 times more lysine versus baseline even at 12 to 24 hours after the load.[6] As expected, arginine and ornithine excretion in patients with LPI increased markedly after lysine, reaching mean excretion rates of 237 and 29 µmol/m^2/h. The excretion values remained above those measured in daily urine collections for at least 24 hours, while arginine and ornithine excretion normalized in 6 hours in the controls (Fig 3).

Lysine infusion caused generalized aminoaciduria in the first (0 to 2 hours) and second (2 to 4 hours) urine collections in both groups. Threonine, methionine, leucine, phenylalanine, and histidine excretion increased similarly in both groups, but the increments in other essential amino acids were greater in the patients versus the controls (data not shown). Also, urinary alanine and glycine excretion increased more in the patients than in the controls. However, the excretion of these two amino acids decreased rapidly in the patients after the infusion, even below the baseline values reported previously for the LPI patients.[6] Urinary citrulline excretion peaked in the patients first in the fourth collection which lasted for 6 hours and thus was influenced by the postload meal and the patient's first postload

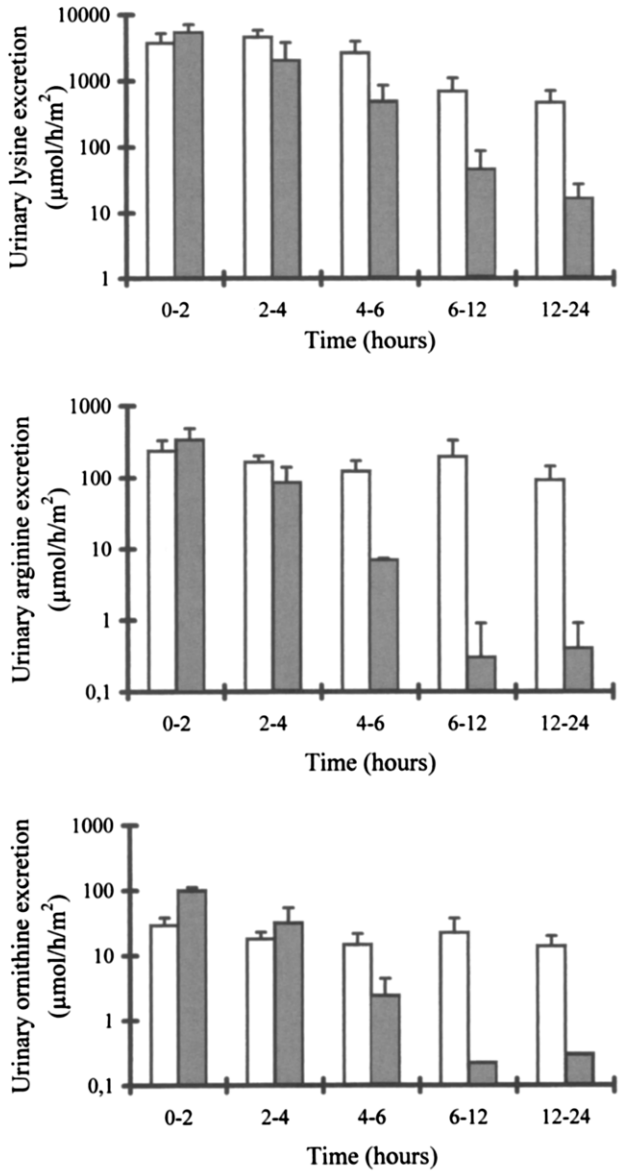

Fig 3. Urinary excretion of lysine, arginine, and ornithine (mean ± SD) in the lysine infusion test in patients with LPI (□) and controls (■). Excretion patterns of the 3 dibasic amino acids differed in the patients and controls ($P \leq .003$). Note the logarithmic scale on the y-axis.

citrulline dose, while the peak occurred in the first collection in the controls (0 to 2 hours; Fig 4).

Clinical Symptoms

All control subjects tolerated L-lysine infusion without any symptoms, but 2 LPI patients (no. 4 and 5) complained of transient mild nausea at the end of infusion.

Plasma Ammonia

Plasma ammonia concentrations remained within the reference range in all controls and in 5 LPI patients. Two LPI patients who had mild nausea at the end of the infusion showed no increase in plasma ammonia, but the values for 1 asymptomatic patient (no. 6) increased slightly (peak, 112 μmol/L at 270 minutes; Fig 5).

Orotic Acid Excretion

Four LPI patients had similar urinary orotic acid excretion rates after the lysine load compared with the controls, but 2 patients showed markedly increased excretion (patient no. 1, peak 33 μmol/m²/h, and patient no. 6, peak 251 μmol/m²/h, both in the third 2-hour collection; Fig 6). Patient no. 1 showed no increase in the blood ammonia concentration, while plasma ammonia in patient no. 6 peaked at 270 minutes (112 μmol/L).

Serum Glucose and Insulin

The mean serum glucose concentration decreased between 0 and 90 minutes from 5.2 ± 1.0 mmol/L (range, 4.2 to 6.7) to 4.1 ± 0.8 mmol/L (range, 3.5 to 5.4) in the patients and from 4.7 ± 0.3 mmol/L (range, 4.4 to 5.2) to 3.6 ± 0.3 mmol/L (range, 3.3 to 3.9) in the controls. Serum insulin increased in the patients during the lysine infusion from 15 ± 4.6 mU/L (range, 4 to 14) to 21 ± 5.5 mU/L (range, 8 to 40) at 0 and 90 minutes, while the respective values in the controls increased from 6 ± 4.2 mU/L (range, 5 to 7) to 11 ± 3.8 mU/L (range, 8 to 15). In both groups, serum insulin returned to baseline values by 120 minutes. The insulin peak of patient no. 3 was higher that that of the other subjects (39 mU/L at 0 minutes and 24 mU/L at 120 minutes; reference range, 5 to 20 mU/L), but her serum glucose values were normal throughout the study.

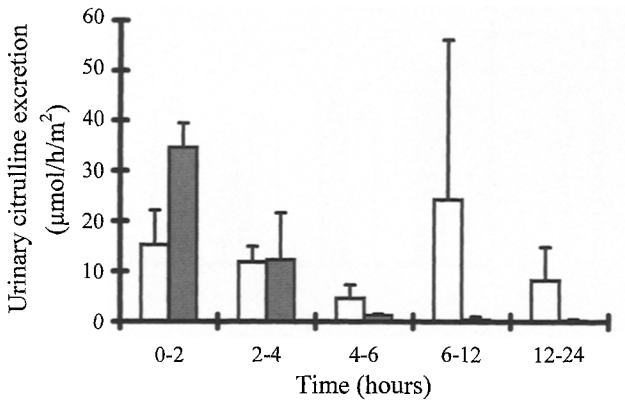

Fig 4. Urinary citrulline excretion (mean ± SD) in the lysine infusion test in patients with LPI (□) and control (■). Excretion patterns differed between the 2 groups ($P = .005$).

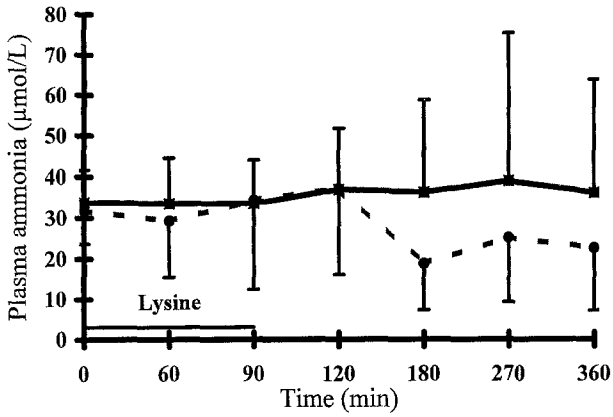

Fig 5. Plasma ammonia (mean ± SD) in the lysine infusion test in patients with LPI (■, SD values shown only upward) and controls (○, SD values shown only downward).

Other Biochemical Measures

Serum creatinine values fluctuated slightly in both groups. Mild metabolic acidosis (base excess from 0.3 to −7.8 mmol/L in patients and from 1.2 to −3.3 mmol/L in controls in capillary samples at the end of the infusion) developed in all test subjects. In all LPI patients, the serum growth hormone concentration peaked after lysine infusion (mean increase between the 0-minute and 120-minute values, 9.3 μg/L [range, 3.2 to 28 μg/L]) but a growth hormone peak (22 μg/L at 120 minutes) was recognizable only in 1 control subject after the lysine load.

DISCUSSION

The role of lysine supplementation in the treatment of LPI has remained controversial. Lysine is an essential amino acid, but it may also disturb urea cycle function,[4] and the extra nitrogen given as lysine may further aggravate hyperammonemia. In this study, the IV L-lysine load caused peak plasma lysine concentrations that exceeded the physiological values by 30-fold, and thus represented a profound but transient metabolic challenge. We monitored urea cycle function in the patients after the lysine load by measuring the plasma ammonia concentration and urinary orotic acid excretion, which are both sensitive indicators of nitrogen overload in LPI. The patients

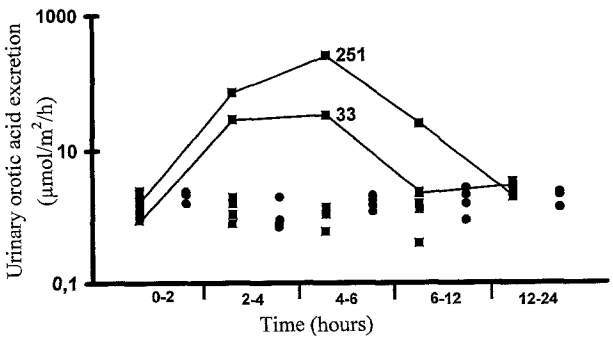

Fig 6. Urinary orotic acid excretion in the lysine infusion test in patients with LPI (■) and control (●). Values for patients no. 1 and 6 with moderately increased excretion are shown as solid lines. Note the logarithmic scale on the y-axis.

tolerated the plasma lysine concentration well and showed no signs or minimal signs of nitrogen overload. Furthermore, IV lysine infusion caused no increases in the plasma concentration of alanine, glycine, or glutamine plus glutamic acid, which usually precede hyperammonemia in LPI.

Lysine infusion increased the plasma lysine concentration similarly in LPI patients and the controls. The concentration of other amino acids in the plasma remained almost unchanged, except that plasma arginine, but not ornithine, increased clearly in the patients. It is possible that the high plasma lysine concentration during the infusion may have inhibited cell influx of the other dibasic amino acids at the cell membrane. In a previous study, there was only a minor change in the plasma arginine concentration after load of arginine or ornithine, while the plasma lysine concentration in our study increased massively. This difference may suggest that the transporters in LPI are more efficient for ornithine and arginine than for lysine.[7]

An increase in the plasma concentration of several individual amino acids stimulates the secretion of insulin and growth hormone. Serum insulin increased slightly in the patients and controls after the lysine load, but the serum glucose concentration remained unchanged. Serum growth hormone clearly increased proportionally more than serum insulin in all patients with LPI but in only 1 control subject, suggesting that the sensitive regulation of hormone secretion may be tuned differently in patients with LPI. In healthy male weight lifters, low-dose (2 g/d) oral supplementation with arginine, ornithine, and lysine had no effect on serum growth hormone and insulin secretion,[8] but in patients with LPI without citrulline supplementation, the lysine shortage may be the direct cause of the diminished or overly infrequent peaks in growth hormone secretion.

The role of chronic lysine deficiency in the development of the citrulline-incorrectable clinical symptoms of LPI remains unclear. Although citrulline supplementation ameliorates protein aversion, the minimum protein intake recommended for healthy growing subjects (0.8 to 1.2 g/kg/d) is seldom attained. Since the decreased availability of a single critical amino acid may strongly restrict protein synthesis in the body, lysine deficiency may aggravate the effects of protein deprivation. Failure to thrive, osteoporosis, and immunological defects might thus well associate with lysine deficiency. Rajantie et al[3] showed that the addition of lysine to the diet of LPI patients failed to improve growth. For the last 15 years, all diagnosed Finnish LPI patients have received regular citrulline supplementation. The therapy has improved protein intake in the patients, often after a short delay. However, several patients have shown no catch-up growth during citrulline therapy and failed to reach their inherited target height.

The incidence of fractures is increased in patients with LPI, and radiographs often show osteoporosis. Malnutrition leads to reduced bone formation in man,[9] and rats with dietary lysine deficiency show a marked retardation of bone growth.[10] Osteoporosis in LPI might thus reflect defective matrix protein synthesis due to protein deprivation and deficiency of the essential lysine.[11] As lysine is a particularly prominent amino acid in collagen, a lysine shortage might well contribute to the development of osteoporosis.

We recently showed that many patients with LPI have subnormal immunoglobulin G3 (IgG3) and IgG4 concentrations in serum and are unable to properly develop antibodies against commonly used vaccines, suggesting dysfunction of the B lymphocytes.[12] The subnormal plasma lysine concentration in LPI may thus also contribute to the immunological findings in LPI. Subclinical protein-energy malnutrition may be another factor which, via several mechanisms, may also impair immune function in LPI.

In conclusion, this study shows that a massive transient increase in the plasma lysine concentration via an acute IV load of L-lysine causes no or minimal harmful clinical or biochemical effects in patients with LPI. We propose that the patients probably tolerate normal and also supranormal plasma lysine concentrations without an excessive risk of hyperammonemia, and carefully titrated oral supplementation with lysine or well-absorbed lysine derivatives might ameliorate the symptoms probably associated with a deficiency of the essential amino acid lysine.

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