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Long-term oral lysine supplementation in lysinuric protein intolerance

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Abstract

In lysinuric protein intolerance (LPI), defective transport of cationic amino acids at the basolateral membrane of the polar epithelial cells in the intestine and renal tubules leads to decreased intestinal absorption and excessive renal loss of lysine, arginine, and ornithine. Citrulline supplementation partially restores the function of the urea cycle that is impaired by deficiency of arginine and ornithine, but does not correct the chronic lysine deficiency. Previous attempts to supplement lysine orally have been hindered by profuse diarrhea, probably caused by excess lysine remaining unabsorbed in the gut. However, individually adjusted minute doses of L-lysine hydrochloride at mealtimes are tolerated well, but the long-term benefits of this therapy remain unknown. The aim of the study was to investigate the long-term benefits and possible adverse effects of oral lysine supplementation in patients with LPI. Supplementation of meals with low doses of oral lysine improved fasting plasma lysine concentrations in 27 Finnish patients with LPI without causing hyperammonemia or other recognizable side effects during 12 months of follow-up. In conclusion, low-dose oral lysine supplementation is potentially beneficial to patients with LPI and can be started safely at an early age.

1. Introduction

Lysinuric protein intolerance (LPI) is an autosomal recessive transport disorder of the cationic amino acids lysine, arginine, and ornithine [1]. It is caused by mutations in the *SLC7A7* (solute carrier family 7, member 7) gene encoding the y^+L amino acid transporter (y^+LAT-1) [2,3]. Lysinuric protein intolerance is more prevalent in Finland (1 in 60 000) than elsewhere in the world [1]. All Finnish patients with LPI share the same mutation 1181-2A?T (LPI_{Fin}) not found in non-Finnish patients with LPI [2,4].

In LPI, the transport defect is located at the basolateral cell membrane of polar epithelial cells in the intestine and renal tubules [5]. Urinary excretion of lysine, arginine, and ornithine is massively increased and their absorption from the intestine is decreased. The deficiency of urea cycle intermediates arginine and ornithine causes urea cycle dysfunction and episodes of hyperammonemia after ingestion of protein. The characteristic symptoms and signs of LPI include aversion to dietary protein, postprandial vomiting, failure to thrive, growth retardation, osteoporosis, hepatosplenomegaly, and muscle weakness [6]. Mental development is normal if hyperammonemia can be avoided. Several patients with LPI have developed pulmonary alveolar proteinosis and renal insufficiency [7].

The treatment of LPI consists of dietary protein restriction and oral supplementation with citrulline, a neutral amino acid and urea cycle intermediate that is effectively absorbed in LPI [8]. It improves protein tolerance and helps to prevent hyperammonemia. However, citrulline supplementation does not correct the chronic deficiency of lysine, which is further aggravated by the aversion to protein-rich foods and the therapeutic restriction of protein intake. Some clinical symptoms of LPI such as poor growth and prominent osteoporosis have not been corrected by citrulline and may be causatively linked with chronic lysine deficiency.

In an earlier study by Rajantie et al [9], 17 Finnish patients with LPI received daily four to six 0.2-g tablets of lysine-HCl according to body weight in addition to citrulline supplementation. Eight of them discontinued the lysine supplementation after 6 to 18 months because of frequent abdominal cramps and loose stools. After 2 years on treatment, there was no difference in growth or any chemical values including plasma and urinary lysine concentrations between the patients with or without lysine

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supplementation. On the other hand, Awrich et al [10] reported a case where the combination of oral citrulline and lysine nearly normalized the plasma amino acid profile and prevented postprandial hyperammonemia in the one patient with LPI studied.

Lysine is an essential amino acid, but high lysine concentration in liver cells may also inhibit the function of the urea cycle [11]. In patients with LPI, however, a massive transient increase in plasma lysine concentration during intravenous load of L-lysine caused no hyperammonemia or increased orotic acid excretion [12]. It is thus likely that citrulline supplementation preserves sufficient urea cycle function also in the presence of high concentrations of lysine.

In the study performed by Lukkarinen et al [13], low-dose oral L-lysine-HCl (0.05 mmol/kg) at mealtimes together with citrulline for 3 days was able to normalize plasma lysine concentrations in 6 patients with LPI. The small dose was well tolerated and did not induce hyperammonemia. After this finding, low-dose oral lysine supplementation has been started to most of the Finnish patients with LPI. To investigate the long-term effects of oral lysine supplementation, we have now followed up a group of Finnish patients with LPI receiving oral lysine supplementation for a mean of 30 months (range, 6-60 months) and analyzed changes in fasting plasma lysine concentrations and other laboratory parameters at 6 and 12 months after the beginning of the supplementation.

2. Patients and methods

2.1. Subjects

Twenty-seven Finnish patients with LPI (18 women and 9 men; age range, 3-56 years; mean age, 27.1 years) were included in this study. All patients had been on a protein-restricted diet and individually tailored oral citrulline supplementation (58-182 mg/kg per day) before the onset of oral lysine supplementation. They continued this basal regimen also during the study. Seventeen patients also received sodium benzoate (n = 12), sodium phenylbutyrate (n = 2), or both (n = 3) as ammonia-scavenging drugs (Table 1).

2.2. Methods

2.2.1. Study design

L-Lysine hydrochloride was administered 3 or 4 times a day at mealtimes. The dose was titrated individually to avoid gastrointestinal side effects (8-46 mg/kg per day; mean dose, 22.7 mg/kg per day). Plasma concentrations of amino acids, ammonia and alkaline phosphatase, creatinine-corrected urinary orotic acid concentration, and serum

Table 1
Characteristics and lysine doses of the 27 patients with LPI included in this study

ID	Age at the beginning of supplementation (y)	Maximum tolerated dose of L-lysine (mg/kg)	Total duration of lysine supplementation (y)	Dose of citrulline (mg/kg)	Dose of sodium benzoate (mg/kg)	Dose of sodium phenylbutyrate (mg/kg)
1	3	20	4	140	102	76
2	4.5	24	4.5	175	210	
3	5	29	5	182		352
4	8	8	5	130	108	288
5	10.5	20	5	85		224
6	11.5	20	1.5	99	81	163
7	17	18	1.5	130	72	
8	17	30	4.5	102		
9	21	30	0.75	80		
10	22	25	1.5	64		
11	25	46	1.5	85	204	
12	28	14	1	108	112	
13	28	22	3.5	91	117	
14	28	26	2.25	100		
15	30	29	1.75	121	98	
16	33	26	1.5	108	125	
17	34	13	1.5	87	105	
18	34	33	2	61		
19	35	20	4.5	87		
20	35	20	0.5	80	80	
21	37	31	0.75	86		
22	37	19	2	127	123	
23	38	9	2	162		
24	40	21	4	94		
25	47	25	1.5	58	70	
26	48	17	0.75	104		
27	56	19	4	73	102	

The maximum tolerated lysine dose was also used for daily therapy for the entire follow-up period with a few exceptions.

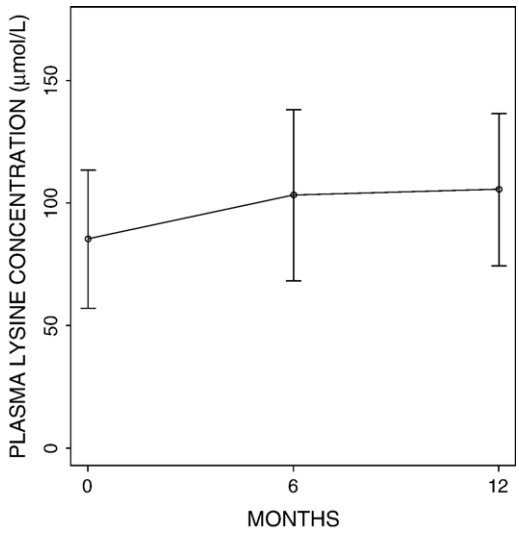

Fig. 1. Mean ± SD of plasma concentration of lysine (μmol/L) before oral low-dose lysine supplementation was started (0) and at 6 and 12 months of supplementation.

concentrations of calcium and phosphate were measured both before the initiation of lysine supplementation and at 6 and 12 months of follow-up. The patients were interviewed about possible gastrointestinal side effects and episodes of acute infection at each control visit.

2.2.2. Laboratory analyses

Plasma amino acid concentrations were measured with automatic ion-exchange chromatography (Biochrom 20 Amino Acid Analyzer, Biochrom, Cambridge, UK) with norleucine as an internal standard. The plasma samples were deproteinized with sulfosalicylic acid and then centrifuged. The patients collected random urine samples at home and sent them via mail for orotic acid analysis. Both morning and evening samples were required for each analysis. The samples were purified by solid-phase extraction using Bond Elut strong anion-exchange (SAX) cartridge (Varian, Palo Alto, CA). The analysis was made with Agilent 1100 high-performance liquid chromatography system (Agilent Technologies, Palo Alto, CA). Other analyses were performed using routine laboratory methods.

2.3. Statistics

Statistical analyses of the data were performed using the Statistical Analysis System Enterprise Guide 3.0 program (SAS Institute, Cary, NC). Shapiro-Wilk test was used to test for gaussian distribution, and logarithmic or exponential transformation was used with the variables with non–gaussian distribution. Comparisons between time points were made with analysis of variance mixed models analysis with Satterthwaite correction for degrees of freedom and Dunnett post hoc adjustment. The data were presented as means ± SD and a $P < .05$ was considered statistically significant.

3. Results

The mean plasma concentration of lysine after an overnight fast increased from 85 ± 28 (proportion of subjects exceeding the lower limit of the reference range is 16%) to 105 ± 31 μmol/L during the 12-month period, reaching the lower limit of the reference range at 12 months in 55% of the patients (Fig. 1). The difference was statistically significant (Table 2).

Plasma ammonia concentrations in random fasting or postprandial samples remained normal with a few peaks (Table 2). The creatinine-corrected concentrations of urinary orotic acid, another indicator of the function of the urea cycle in LPI, varied considerably over time. The values showed no clear changes during the study.

No statistically significant changes occurred during the follow-up in the mean plasma concentrations of glutamine, glutamic acid, glycine, alanine, and citrulline, the amino acids that to some extent reflect urea cycle function (Table 2). The mean plasma concentrations of leucine and

Table 2
Plasma concentrations (mean ± SD) of lysine, ammonia, leucine, isoleucine, glutamine and glutamic acid, glycine, alanine, citrulline, albumin, calcium, phosphate, and alkaline phosphatase before and at 12 months of oral lysine supplementation

	Unit	Before supplementation	Range	At 12 mo	Range	Reference range (for adults)	*P*
Lysine	μmol/L	85 ± 28	34-154	105 ± 31	47-164	114-289	.014
Ammonia	μmol/L	26.6 ± 20.8	0-91	26.7 ± 14	6-58	<50	NS
Leucine	μmol/L	89 ± 22	42-135	93 ± 23	44-142	70-232	NS
Isoleucine	μmol/L	51 ± 12	25-72	52 ± 12	30-71	23-212	NS
Glutamine + glutamic acid	μmol/L	1116 ± 559	466-2824	1090 ± 430	670-2086	324-971	NS
Glycine	μmol/L	472 ± 187	198-914	457 ± 134	189-715	145-356	NS
Alanine	μmol/L	510 ± 385	226-2166	467 ± 122	324-679	231-580	NS
Citrulline	μmol/L	67 ± 33	25-150	70 ± 36	0-144	8-52	NS
Albumin	g/L	40.6 ± 6.2	21-47	42.4 ± 5.4	26-50	36.1-47.5 (M), 34.8-46.1 (F)	NS
Calcium	mmol/L	2.31 ± 0.12	2.03-2.49	2.36 ± 0.12	2.13-2.68	2.17-2.47	NS
Phosphate	mmol/L	1.14 ± 0.28	0.63-1.82	1.10 ± 0.25	0.71-1.76	0.7-1.3	NS
Alkaline phosphatase	U/L	370 ± 332	108-1354	360 ± 324	74-1280	60-270	.036

NS indicates not statistically significant.

isoleucine were also closely similar before and at 12 months of lysine supplementation. Interestingly, the proportion of very high and very low concentrations of alanine decreased during the supplementation. The plasma concentrations of other amino acids remained stable during the follow-up. The mean plasma albumin concentration remained unaltered (Table 2).

Five patients, all of whom were girls, were younger than 11 years (3, 4.5, 5, 8, and 10.5 years) when lysine supplementation therapy was initiated. In the analysis of growth, 2 of them were excluded because they also received growth hormone therapy. The remaining 3 children showed no improvement in the linear growth during the 12 months of lysine supplementation.

The mean serum concentrations of calcium and phosphate did not show statistically significant changes during the follow-up. However, the mean plasma concentration of alkaline phosphatase decreased slightly (Table 2). The number of clinically detectable acute infections remained unchanged during the lysine supplementation. The patients reported no gastrointestinal complaints while using the doses mentioned at Table 1.

4. Discussion

In this study, individually adjusted low-dose oral lysine supplementation increased the plasma lysine concentrations of the patients with LPI without inducing hyperammonemia.

During supplementation, no clinical symptoms suggested abnormal accumulation of lysine. Urinary orotic acid excretion is an even more sensitive measure of the sufficiency of the urea cycle function than blood ammonia in revealing effects of an oral protein load in LPI [14]. However, the values show marked variation over time due to small changes in the diet or in the general condition of the patient, making the comparison of the values between patients difficult. Lysine supplementation had no effect on the mean creatinine-corrected concentration of urinary orotic acid in the patients. During the observation period, the values remained mostly within the reference range with some individual peaks. The mean plasma ammonia concentrations also remained unaffected. The long-term lysine therapy was well tolerated by the patients, and no significant gastrointestinal side effects were observed.

The plasma concentrations of leucine and isoleucine, reflecting the intake of essential amino acids, remained at the same low level during the follow-up period as before. The mean plasma albumin concentration remained unaltered during the supplementation. Plasma concentrations of glutamine + glutamic acid, glycine, citrulline, and alanine, thought to reflect the nitrogen load, were also closely similar before and at 12 months of lysine supplementation, suggesting further that the daily protein intake of the patients probably remained rather stable during the follow-up.

In rats, lysine-deficient diet causes moderate growth retardation with decreased levels of serum growth hormone [15]. In patients with LPI, birth weights and lengths are usually normal for gestational age, and postnatal growth is normal for as long as the infants are breast-fed. After weaning, the growth curves begin to deviate progressively from the normal mean and the skeletal maturation is delayed [6]. In this study, the effect of lysine supplementation on linear growth could not be reliably assessed because of the small number of children included in this study and the simultaneous growth hormone therapy received by some of the patients. No apparent changes in growth were observed in the 3 children receiving oral lysine without growth hormone.

Osteopenia and a pronounced tendency to fractures are constant complications of LPI, and osteopenia may even be the presenting sign of LPI [16]. Most patients have structural abnormalities in the skeleton, but there is no correlation between fracture incidence and radiologic bone structure [17]. The patients do not usually show clinical or biochemical signs of rickets. Osteopenia in LPI may thus reflect defective extracellular matrix protein synthesis due to dietary protein deprivation and functional deficiency of cationic or other essential amino acids [18]. In rats, lysine-deficient diet leads to a failure in bone growth and subsequent decrease in the skeletal deposition on radiocalcium [19]. On the other hand, Civitelli et al [20] have shown that dietary L-lysine enhances intestinal calcium absorption and improves renal conservation of the absorbed calcium in man, suggesting that lysine may have a beneficial effect in patients with osteopenia. In the current study, the mean concentration of serum alkaline phosphatase, which to some extent reflects the rate of bone turnover, decreased slightly during the lysine supplementation, whereas serum calcium and phosphate concentrations remained unchanged. However, the bone density of patients with LPI before and after lysine supplementation was not systemically surveyed in this study, partly because most of the patients were adults and had already reached their peak bone mass before the lysine supplementation was started.

Many patients with LPI show signs of disturbed immune function. Humoral immune responses are defective in some patients [21] and varicella infections may be exceptionally severe [22]. Some patients have leukocytopenia, high serum immunoglobulin G values, and abnormal distribution of lymphocyte subpopulations. During the lysine supplementation, no alteration in the clinical susceptibility to infections was observed in the patients with LPI.

In summary, low-dose oral lysine supplementation improves long-term plasma lysine concentration in patients with LPI without causing hyperammonemia or gastrointestinal side effects. However, the long-term effects of lysine supplementation on growth rate, bone density, and immunologic abnormalities are difficult to estimate because relevant conclusions require much longer follow-up periods, and several other factors such as growth hormone therapy received by some of the children with LPI may as well contribute to the outcome. The amount of L-lysine tolerated

by the patients may also be insufficient for correcting the lysine deficiency in the body, although it may be able to normalize lysine concentrations in plasma. Thus, low-dose oral lysine supplementation is probably beneficial and safe to the patients with LPI and can be started safely at an early age.

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