Intestinal absorption in lysinuric protein intolerance: impaired for diamino acids, normal for citrulline

J RAJANTIE,* O SIMELL, AND J PERHEENTUPA

From the Children's Hospital, University of Helsinki, Helsinki, Finland

SUMMARY Lysinuric protein intolerance (LPI) is an autosomal recessive defect of diamino acid transport characterised by massive diaminoaciduria, especially lysinuria, with hyperammonaemia after heavy nitrogen intake. The defect has previously been demonstrated in the kidney, and is probably present in the liver cells. To evaluate the effect of the LPI gene on the net intestinal absorption of the diamino acids and citrulline, separate oral loads of each were given to controls, and to subjects heterozygous and homozygous for LPI. In the affected subjects the plasma concentrations of the loaded diamino acids showed lower increments after the loads than in the controls, the difference being marked in the homozygotes and moderate in the heterozygotes. Urinary excretion failed to explain these differences. Thus, the diamino acid transport defect of LPI is also present in the intestine. After citrulline loads, in contrast, plasma citrulline levels rose similarly in controls and homozygotes. Thus, LPI is associated with intact citrulline absorption. The ornithinopenic hyperammonaemia of LPI is probably preventable by supplementing dietary protein with the ornithine precursor citrulline.

Lysinuric protein intolerance (LPI) is an autosomal recessive defect in diamino acid transport. It appears clinically as failure to thrive, vomiting, diarrhoea, protein aversion, severe growth retardation, hepatomegaly and splenomegaly, osteoporosis, and sometimes mental retardation. Its chemical pathognomonic features are increased urinary excretion and decreased plasma concentrations of the diamino acids lysine, arginine, and ornithine, and hyperammonaemia after protein intake. The diamino acids are not adequately reabsorbed by the renal tubuli, and their entry into the hepatic cells seems also to be impaired. Evidently, this results in deficiency of urea cycle intermediates. Hyperammonaemia can be prevented by giving ornithine or arginine intravenously. Dietary arginine supplementation has been used for long-term therapy.[1] Reports on intestinal diamino acid absorption in LPI have been conflicting.[2-7] Plasma arginine and ornithine concentrations have remained low during arginine supplementation, and the long-term results of this therapy have not been fully satisfactory.[1] Intestinal absorption of diamino acids has been subnormal even in protein-tolerant subjects with

*Address for correspondence: Dr J Rajantie, Children's Hospital, SF-00290 Helsinki 29, Finland.

hyperdiaminoaciduria.[8 9] In LPI, because of its ornithinopenic hyperammonaemia, one would expect the diamino acid transport defect to be more severe and also present in the intestine.

We have now studied intestinal absorption of oral amino acid loads in healthy controls, and in subjects heterozygous and homozygous for LPI. The diamino acid absorption was defective in the homozygotes and intermediate in the heterozygotes. Citrulline absorption appeared to be normal.

Methods

Seventy-three oral amino acid loads were given to 10 controls (one infant, two pubertal girls, seven young adults; eight females), six obligate heterozygotes (age 25·2–41·5 years; four females), 14 homozygotes (one infant, eight prepubertal children, five young adults; eight females), and two siblings of the homozygotes (16·6 and 26·5 years; one female).

Past histories and clinical findings in all homozygotes were typical of LPI.[1] The diagnosis was confirmed by measurements of plasma and urinary amino acids and with an intravenous alanine test.[10] The homozygotes were on a low-protein diet, in good nutritional condition and free of gastrointestinal symptoms. Their jejunal mucosae (examined in 10 patients) and faecal fat excretions (six patients) were normal.

The obligate heterozygotes were six parents of five patients. They and the siblings and controls (members of staff and their relatives) were healthy subjects without medication. All subjects and/or their guardians had given informed consent to the studies.

The loads were given in the morning, after a 12 hour fast and at least two days without arginine supplement. The interval between the loads was at least two days—exceptionally, only one. The dose of each amino acid was 0·5 mmol/kg of body weight, given in 200 ml of dilute apple juice. The diamino acids were monohydrochlorides. Only water was allowed during the next six hours. Blood samples were drawn into tubes pre-chilled in an ice-water bath, through a catheter inserted into an antecubital vein, before the load (time zero) and at 60 minute intervals. After centrifugation, 2·0 ml plasma was deproteinised with 0·20 ml 50% (w/v) sulphosalicylic acid. Urine was collected for six to 24 hours before and 4·5 to six hours after the loads.

All specimens were stored at –23°C. The amino acids were measured with an automatic amino acid analyser (Beckman Unichrom or Beckman 121 M, with citrate or lithium buffers, respectively). For comparisons, urinary excretion was expressed as rates per six hours. To evaluate the reproducibility of the plasma curves five loads were repeated in the same homozygotes, after an interval ranging from one week to 3·3 years.

For evaluation of differences between groups, Student's *t* test was used.

Results

One to two hours after the diamino acid loads most homozygotes had abdominal cramps and flatulence. Some of them passed loose stools. Mild diarrhoea after lysine was also observed in one of the controls. The mixture of citrulline and lysine provoked loose stools in the one control tested as well as in all the patients. After citrulline all subjects remained free of symptoms.

The five homozygotes, who had the same test twice, responded to both with almost identical plasma curves (Fig. 1).

The plasma responses did not correlate with the age, weight, or height of the subjects.

PLASMA CONCENTRATIONS AFTER DIAMINO
ACID LOADS (Fig. 2)
After arginine loads, the increments in plasma arginine and ornithine concentrations were roughly equimolar (the mean maximal increment in plasma arginine was 16·5 μmol/l in the homozygotes and 90·2 μmol/l in the controls). Evidently, about half the arginine absorbed was rapidly converted into ornithine. Thus, the sum of the plasma arginine and
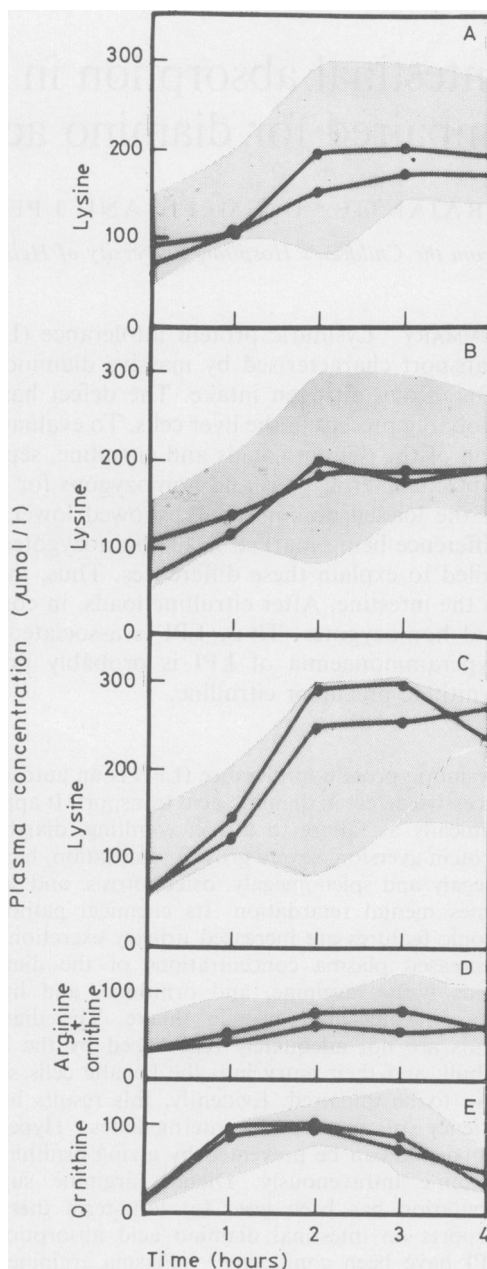

Fig. 1   *Intraindividual variation (curves from loading tests given twice to the same homozygotes) compared with interindividual variation (the shaded areas indicate the range of the curves of all homozygotes) in the plasma concentration of the given diamino acid. Lysine (curves A, B, C), arginine (D) or ornithine (E), 0·5 mmol/kg, was given orally to the homozygotes for LPI. The intervals between the loads were 3·3 years, 10 months, 7 days, 3·3 years and 10 months, respectively.*

ornithine concentrations was used in the comparisons.

As compared with the controls the homozygotes had lower concentrations of plasma diamino acids throughout, and more sluggish and significantly smaller increments (Table 1). During the first two hours after the loads the curves for the two groups showed no overlap.

The molar increments in plasma concentration were higher after lysine than after ornithine or arginine. This was true both in the controls and in the homozygotes of LPI. This contrasts with the function of the renal tubuli, where the diamino acid transport favours reabsorption of ornithine over arginine, and arginine over lysine.[11]

In the heterozygotes as compared with the controls the increase in plasma lysine was smaller and delayed. Their mean maximal increment was intermediate between those for the control and patient groups (Table 1). Their individual curves overlapped with those of the other groups. In the only family of which all members were studied, the lysine curves of the parents were also between the curves of their affected and healthy sons (Fig. 2). The sibling of another family had a lysine curve which suggests heterozygosity. After arginine loads the plasma curves did not separate the one heterozygote tested from the controls.

The plasma levels of the other amino acids were not affected by the diamino acid loads.

PLASMA CONCENTRATIONS AFTER
CITRULLINE AND CITRULLINE + LYSINE
LOADS (Fig. 3)

The fasting plasma citrulline concentrations were higher ($p < 0.001$) in the homozygotes (49.4 ± 11.9 μmol/l, mean $\pm$SD, n=13) than in the controls (19.9 ± 3.4 μmol/l, n=4). The responses to a citrulline load showed no such clear difference: the increment areas under the plasma curves did not differ significantly. The highest concentrations were reached in the homozygotes.

Dietary supplements of citrulline + lysine have been suggested for treatment of homozygotes for LPI.[5] To find out whether these two amino acids interfere with each other's absorption, loads of

Table 1 *Maximal plasma increment of amino acids indicated after oral loads of amino acids (0·5 mmol/kg of body weight) in controls and in heterozygotes and homozygotes for LPI*

		Maximal increment (mean ± SD)			
	Loads (no.)	In plasma as concentration μmol/l	P	In ECF* as mass % of load	P
Lysine (lysine load)					
Controls	5	352 ± 66	<0·01	13·0 ± 2·4	<0·01
Heterozygotes	5	226 ± 46	<0·001	8·1 ± 1·6	<0·001
Homozygotes	13	136 ± 48		5·6 ± 2·1	
Arginine + ornithine (arginine load)					
Controls	3	135 ± 40	<0·001	5·9 ± 3·3	<0·001
Homozygotes	11	37 ± 18		1·5 ± 0·8	
Ornithine (ornithine load)					
Controls	5	260 ± 70	<0·001	9·4 ± 2·6	<0·001
Homozygotes	9	83 ± 22		3·3 ± 0·8	
Citrulline (citrulline load)					
Controls	3	542 ± 192	<0·1	19·5 ± 6·9	<0·05
Homozygotes	8	830 ± 223		32·5 ± 8·2	
Citrulline (citrulline + lysine load)			<0·3		<0·4
Controls	1	566		17·5	
Homozygotes	5	689 ± 198		27·8 ± 10·2	

*Each subject's increment in the mass of amino acid present in the extracellular fluid (ECF) was calculated by multiplying the maximal plasma concentration increment by the mean extracellular water volume for age and weight[17] and expressing the product as a percentage of the dose.
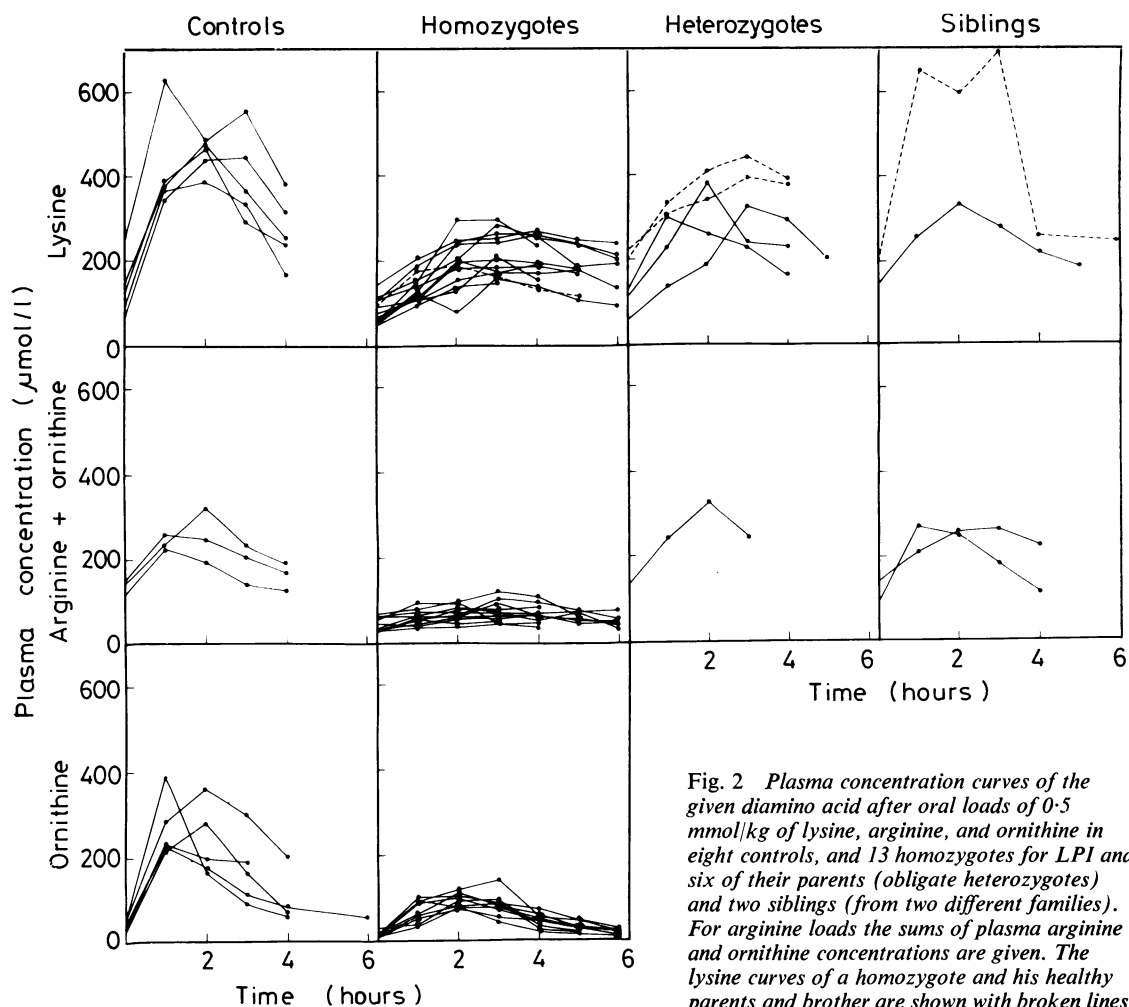
Fig. 2 *Plasma concentration curves of the given diamino acid after oral loads of 0·5 mmol/kg of lysine, arginine, and ornithine in eight controls, and 13 homozygotes for LPI and six of their parents (obligate heterozygotes) and two siblings (from two different families). For arginine loads the sums of plasma arginine and ornithine concentrations are given. The lysine curves of a homozygote and his healthy parents and brother are shown with broken lines.*

Table 2 *Urinary excretion of ingested amino acid before and after loads (0·5 mmol/kg of body weight)*

Excretion of (µmol/kg/6h)	Controls Mean (range; n)		Heterozygotes for LPI Mean (range; n)		Homozygotes for LPI Mean (range; n)
Lysine (lysine load)					
Before load	0·20 (0·15–0·28; 3)		0·57 (0·34–0·88; 4)		28·92 (1·55–63·47; 8)
After load	3·82 (3·00–5·34; 3)		2·91 (2·33–4·57; 4)		57·84 (15·58–92·15; 8)
Six hour increment, % of load	0·72 (0·57–1·01; 3)		0·53 (0·39–0·66; 4)		5·69 (0·23– 8·94; 8)
Arginine + ornithine (arginine load)					
Before load	0·10 (0·03–0·16; 2)		0·12	(1)	4·17 (0·33– 9·85; 5)
After load	0·33 (0·19–0·46; 2)		0·14	(1)	6·06 (0·48–13·75; 5)
Six hour increment. % of load	0·05 (0·03–0·06; 2)		0·01	(1)	0·38 (0·02– 0·78; 5)
Ornithine (ornithine load)					
Before load	0·04 (0·02–0·06; 4)				0·75 (0·06– 1·95; 5)
After load	0·32 (0·17–0·68; 4)				1·08 (0·09– 2·04; 5)
Six hour increment, % of load	0·07 (0·03–0·13; 4)				0·07 (0·01– 0·12; 5)
Citrulline (citrulline load)					
Before load	0·03	(1)			1·09 (0·32– 2·21; 5)
After load	0·75	(1)			16·58 (11·74–24·77; 5)
Six hour increment, % of load	0·14	(1)			3·01 (2·09– 4·51; 5)

citrulline + lysine (0·5 mmol/kg of each) were given to five homozygotes and one control. The plasma lysine curves did not differ from those obtained after loads with lysine alone. In the homozygotes the plasma citrulline increments were smaller and shorter, though the change in increment areas was not statistically significant ($p<0·1$ by the paired t test). The curve for the control subject was unchanged.
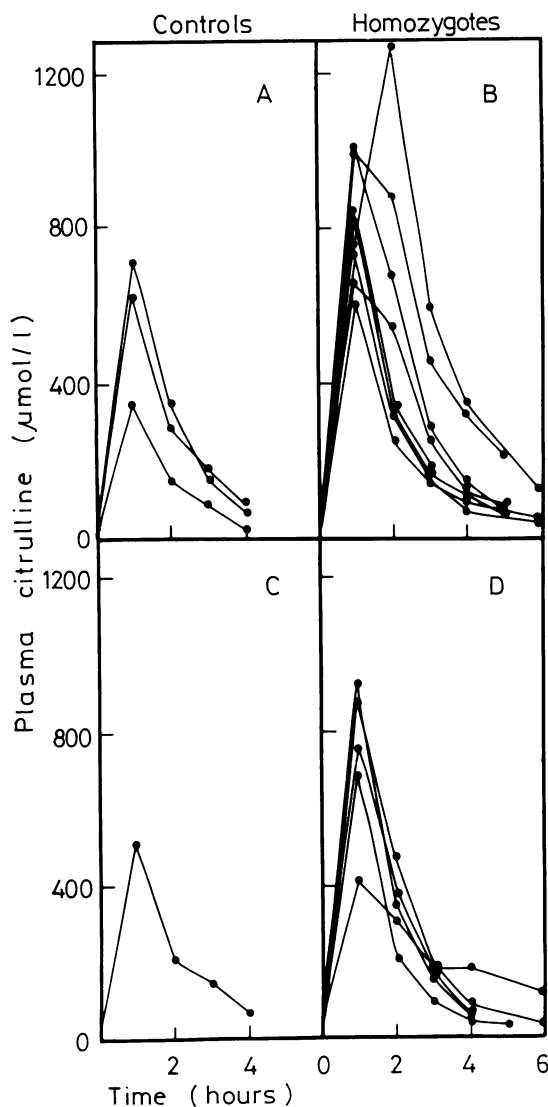

Fig. 3 *Plasma concentration curves of citrulline after oral loads of citrulline 0·5 mmol/kg (A and B) and of a mixture of citrulline and lysine, 0·5 mmol/kg of each (C and D), in controls and homozygotes for LPI.*

URINARY EXCRETION

The basal excretion of lysine was massively increased in the homozygotes as compared with the controls and heterozygotes (Table 2). After the lysine loads, the relative increases in lysine excretion were largest in the two latter groups. The six hour increments in excretion were small in proportion to the loads. Only 0·7, 0·5, and 5·7% of the dose in the controls, heterozygotes and homozygotes, respectively, could be accounted for by this urinary loss. In the case of the arginine and ornithine loads, this fraction was even less.

The excretion of citrulline was slightly larger in the homozygotes than in the controls, both before and after citrulline loads (Table 2). Addition of lysine to the citrulline load did not increase citrulline excretion.

Discussion

After oral loads of diamino acids plasma concentration curves were markedly lower in the homozygotes for LPI than in the controls. The curves are similar to those reported in single homozygotes for LPI by Brown *et al.*, Awrich *et al.*, Kato *et al.*, and Oyanagi *et al.*[4-7] The increased urinary excretion of diamino acids in this disorder presumably lowers the plasma curves, but this effect appears to be of minor importance, because less than 1% of the dose of arginine and ornithine was excreted. However, the metabolic clearance of arginine and ornithine, and presumably also of lysine, is decreased,[12] and this would tend to raise the plasma concentrations. After an intravenous infusion of arginine or ornithine, the sum of these two opposing effects in homozygotes for LPI is a rise in the plasma level above that of controls.[12] Thus the markedly low levels of the diamino acids in plasma after oral administration constitute evidence that in LPI the intestinal absorption of arginine, ornithine, and lysine is impaired. This is additionally confirmed by our demonstration that in heterozygotes the post-loading plasma curves are intermediate in level. This is the first demonstration of a heterozygous manifestation of the LPI gene. It was already suggested by Oyanagi *et al.*'s finding in one heterozygote.[13] Because of overlap, however, it does not allow reliable identification of individual heterozygotes, except perhaps in family studies.

Evidence of the presence of the transport defect in the intestine was presented in the first description of LPI.[2] After that, however, intestinal perfusions with a double lumen tube and *in vitro* accumulation studies with mucosal specimens[3] were reported to give normal results. However, this perfusion study was inadequate with only a cystinuric child as

control. What was measured with the *in vitro* method was the net accumulation in the tissue of the radioactive diamino acid. The method might have failed in detecting a defect in the mechanism transferring the amino acid out from the cell at the basolateral membrane, or a defect which allows an increased backflux of the amino acid at the luminal membrane. In fact, our recent studies have produced evidence for the presence of a defect at the basolateral membrane in LPI.[14]

The net effects of the defect on the plasma concentration curves after oral loads of diamino acids and on the urinary loss of diamino acids are similar to what are observed in homozygotes for classical cystinuria,[15] and in subjects with hyperdiaminoaciduria without protein intolerance.[8 9] This is in agreement with the theory that the ornithinopenic defect on urea synthesis which distinguishes LPI from those other hyperdiaminoaciduric conditions is not due simply to differences in the severity of the intestinal or renal transport defects. According to previous evidence, it is associated with the presence in LPI of the same transport defect at the hepatocyte membranes.[12 16]

We have shown that in homozygotes for LPI the intestinal absorption of citrulline is normal. This was previously observed in the patient of Awrich *et al.*[5] Citrulline, in contrast to arginine and ornithine, is not a cationic amino acid and is obviously transported by a different mechanism, which is presumably efficient enough to complete absorption. Supplementation with citrulline thus clearly offers a better means for correcting the ornithinopenia of LPI than treatment with arginine or ornithine.

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