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Research Article

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In the control subjects, there was almost complete reabsorption of arginine and ornithine, with increases in their filtered loads to 50-100 times normal. With further increases in the filtered loads of these amino acids, there was a gradual decrease in their fractional reabsorption. Mutual competitive inhibition was suggested by the observation that an increase in the filtered load of one diamino acid was associated with a decrease in the reabsorption of the other two.

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A B S T R A C T Lysinuric protein intolerance (LPI) is a rare recessively inherited disease in which one of the fundamental physiological defects is in the mechanism by which diamino acids are transported by the kidney. The purpose of the present studies was to examine that mechanism in four controls and seven patients with LPI. Two types of studies were conducted. In the first set, the renal handling of *l*-arginine and *l*-ornithine was evaluated by gradually increasing the plasma concentration of each of these amino acids by constant infusion techniques. In the second set of studies, the possible existence of competitive inhibition between *l*-arginine, *l*-ornithine, and *l*-lysine was examined.

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In LPI, the fasting plasma diamino acid concentrations were significantly lower than in the controls. With low filtered loads, the fractional reabsorption of the diamino acids was clearly below normal. This defect diminished with higher loads. A stepwise increase in the plasma concentration of one diamino acid resulted in a biphasic response. Initially, net tubular secretion of the other diamino acids was noted, but later was followed by return to net absorption. When two diamino acids were infused simultaneously, net absorption of both took place, though less efficiently than in the controls.

We conclude that the renal reabsorption mechanism is defective in patients with LPI. With low normal filtered loads, there is increased fractional excretion of all three diamino acids resulting in low serum concentrations of these compounds. However, at higher artificially elevated concentrations of diamino acids, the capacity of the renal transport system in these patients appears normal.

INTRODUCTION

Lysinuric protein intolerance (LPI)[1] (1–3) is a well-defined recessively inherited (4) disease associated with massive renal lysinuria without cystinuria and with hyperammonemia after amino nitrogen loading. It is characterized by loss of appetite, vomiting and diarrhea after introduction of cow's milk feeding, short stature, hepato- and frequently splenomegaly, neutropenia and often thrombocytopenia, and aversion to protein-rich food. The hyperammonemic response to protein intake can be abolished by giving arginine or ornithine (3). At present, 20 patients with LPI are known in Finland and 1 in Sweden (5, 6), and some patients with very similar symptoms have been reported elsewhere (7–11).

The nature of the association between the slow synthesis of urea and the renal leakage of diamino acids in LPI has not been clarified (5, 6, 12, 13). We suggest that the availability of ornithine, the carrier molecule upon which urea is formed in the liver, is decreased because of a transport defect severe enough to impair the function of the cycle. The transport of diamino acids in LPI thus has to be studied in detail.

Several lines of evidence suggest that the diamino acids, lysine, arginine, and ornithine, share a common transport system in the human kidney tubule. Previous workers have shown that normal persons have increased urinary loss of all three diamino acids during lysine infusion (14, 15). In cystinuric subjects, the three diamino acids are selectively lost along with cystine (16–

TABLE I
Characteristics of the Patients

| Subject | Sex | Age | Weight | Height | BSA | Mean (range) plasma concentration* of | | |
						Lysine	Arginine	Ornithine
		y	*kg*	*cm*	*m²*	μM	μM	μM
Patients with LPI								
1. R. P.§	M	28.0	62.5	170.5	1.72	81.5 (67.2–104)	37.4 (28.9–50.6)	30.9 (23.5–37.9)
2. R. P.¶	F	18.9	55.8	160.0	1.56	67.6 (54.3–83.3)	42.6 (24.9–58.3)	27.8 (13.8–45.4)
3. E. R.	F	16.2	35.5	147.7	1.22	97.1 (82.6–113)	28.6 (19.0–45.3)	18.0 (12.5–26.6)
4. H. H.	F	12.0	16.6	110.5	0.71	40.8 (32.0–52.5)	19.8 (12.4–24.1)	11.7 (9.0–14.6)
5. M. R.	F	4.8	11.8	87.5	0.52	46.1 (39.3–50.1)	17.0 (12.7–19.2)	13.5 (12.0–16.3)
6. J. S.	F	1.5	7.5	69.8	0.36	60.6 (56.9–64.3)	26.6 (22.0–31.2)	20.2 (18.6–21.7)
7. S. V.	F	1.0	7.0	66.0	0.34	87.3 (36.7–179)	23.1 (17.8–28.5)	16.9 (15.5–18.9)
Controls								
8. P. R.	F	13.6	28.5	134.0	1.04	140 (87.7–195)	119 (103–137)	54.6 (51.2–57.5)
9. T. W.	M	9.8	32.0	151.0	1.20	126 (94.7–157)	53.0 (45.4–60.5)	51.8 (41.4–62.1)
10. E. K.	M	5.3	14.0	106.0	0.64	138 (118–169)	67.8 (51.2–87.9)	56.1 (46.1–68.4)
11. T. N.	M	1.1	7.4	71.0	0.37	94.4 (78.1–110)	63.3 (39.7–108)	37.1 (31.9–42.2)

* Measured during infusion of isotonic mannitol solution, 12 ml/kg body wt/h.
‡ Mean excretion, calculated from four to six 30-min urine collections during infusion of isotonic mannitol solution, 12 ml/kg body wt/h.
§ The case presented in reference no. 3.
‖ Number of clearance periods.
¶ A case presented in reference no. 2.

18). In addition, in vitro experiments with kidney cortex slices gave results consistent with the existence of a common transport mechanism (19). Cystine is obviously not transported by the same mechanism (18, 19). Rosenberg, Albrecht, and Segal (20) found evidence for two different lysine transport systems in human kidney cortex slices: one with weak affinity but large capacity, and the other with strong affinity but small capacity. Christensen and Liang (21) suggested that in Ehrlich ascites tumor cells, lysine transport is mediated by three different receptor sites, one of which is possibly also used by the leucine-preferring transport system.

Using the constant infusion technique, we have studied the handling of lysine, arginine, and ornithine by the kidneys of patients with LPI and of controls. In these studies, we have quantitated the tubular reabsorption of the diamino acids, defined the defective system in LPI, and demonstrated competition for transport sites.

METHODS

Subjects (Table I). Renal reabsorption of arginine and ornithine was measured in seven patients with LPI. All had the typical aminoaciduria, with hyperammonemia after *l*-alanine loading and other typical signs (2). Arginine and ornithine infusions were given twice to one patient with a 2-yr interval.

Of the four controls, three were hospitalized for examination because of retarded growth. Normal plasma growth hormone and cortisol responses and normal thyroid hormone concentration were found, without evidence of malabsorption or other metabolic abnormalities, and the diagnosis of familial delayed growth was made. Another child (no. 9) had well-substituted hypoparathyroidism. Only one control was female, and only one patient male. No sex-dependent difference has been reported in the metabolism of amino acids, nor was there any suggestion of such a difference in the present groups.

Informed consent for these studies was obtained from all subjects or their guardians (in the case of children).

Experimental procedure. Conventional clearance methods were used. Steady plasma concentration of the infused amino acid was achieved with a constant-flow infusion pump (Infusion/Withdrawal Pump, model 957, Harvard Apparatus Co., Inc., Millis, Mass.). A balloon catheter was inserted in the bladder and a venous catheter (Venflon intravenous catheter, Viggo AB, Sweden) in a cubital vein. This was used for both the infusion and the drawing of blood samples; an infusion pause of 45 s and 12 wasted drops of blood preceded the drawing of each 6-ml blood sample. This gave the same plasma concentration of the infused amino acid as blood from another vein, as ascertained in separate experiments. Care was taken to maintain sterility, and prophylactic sulfafurazole was given to all subjects. One patient with LPI became nauseated at the end of an arginine infusion, but no other adverse effects were noticed.

The infusions were started after a 10-h fast, with a priming injection of 10% mannitol and ⁵¹Cr-EDTA (From the Radiochemical Centre, Amersham, England), 5.6 ml and 0.25 µCi/kg body wt, respectively. A constant infusion of an isotonic solution (12 ml/kg body wt per h) was then maintained throughout the test, only interrupted by further priming injections and blood sampling. The solution con-

Mean (range) renal clearance* of			Urinary excretion‡ of			⁵¹Cr-EDTA clearance (mean±1 SD)
Lysine	Arginine	Ornithine	Lysine	Arginine	Ornithine	
ml/min	*ml/min*	*ml/min*	*μmol/h*	*μmol/h*	*μmol/h*	*ml/min*
48.5 (43.7–52.8)	12.7 (4.62–22.2)	5.90 (4.11–8.85)	237.17	28.50	10.96	76.5±18.7 (n = 28)‖
41.3 (34.3–45.6)	14.0 (9.48–18.1)	8.47 (4.57–12.6)	167.50	35.79	14.13	68.4±7.2 (n = 30)
27.2 (25.5–30.0)	14.3 (8.09–19.8)	4.00 (2.62–5.10)	158.46	24.54	4.33	63.5±12.5 (n = 26)
20.8 (17.1–25.6)	0.42 (0.26–0.66)	0.75 (0.57–1.06)	50.92	0.50	0.54	38.8±3.9 (n = 28)
15.9 (13.8–17.6)	5.38 (4.37–6.32)	2.26 (1.65–2.75)	44.00	5.50	1.83	24.5±2.8 (n = 24)
16.6 (16.1–17.1)	9.82 (7.87–11.8)	4.53 (4.02–5.04)	60.38	15.67	5.50	16.4±1.2 (n = 16)
11.6 (3.4–15.9)	5.58 (5.00–6.13)	4.54 (2.45–7.46)	60.75	7.75	4.58	16.0±1.4 (n = 23)
0.24 (0.17–0.33)	0.07 (0.05–0.10)	0.39 (0.10–0.76)	2.00	0.50	1.29	46.4±10.0 (n = 23)
0.52 (0.49–0.55)	0.36 (0.18–0.55)	0.75 (0.67–0.83)	3.92	1.13	2.33	55.5±5.5 (n = 12)
0.38 (0.24–0.50)	0.31 (0.08–0.60)	0.87 (0.23–1.83)	3.17	1.25	2.92	36.9±7.9 (n = 24)
0.17 (0.13–0.21)	0.05 (0.02–0.08)	0.07 (0.05–0.09)	0.96	0.21	0.17	16.8±1.9 (n = 24)

tained the amino acid, Cr-EDTA, 10.0 μCi/l, and 25 mM of both NaCl and KCl and was made isotonic with mannitol. The two oldest subjects tended to become hyperkalemic with the regular solution and had 40 mM NaCl and 10 mM KCl. A shift to a higher concentration of the amino acid was made every 2 h. Besides the 0-level, two, three, or four of the following concentrations were used in each study: 17.8, 35.6, 53.4, 71.2, and 89.0 mM. Before every shift, a priming dose of the amino acid was given (0.50 ml of 5% *l*-ornithine-HCl, 0.60 ml of 5% *l*-lysine-HCl, or 0.63 ml of 5% *l*-arginine-HCl solution/kg). 1 h was enough to give a steady concentration of the infused amino acid in the plasma. After this equilibration period, the urine was collected for two 30-min periods, and a blood sample was drawn at the change of the urine collection. The plasma amino acid concentration of this sample was used for clearance calculations for both collection periods. In one experiment, a blood sample was drawn at both the start and the end of each collection, and a negligible difference was found in the plasma amino acid concentration. The bladder was rinsed with 15 or 20 ml of 0.9% NaCl solution and with air before and after each collection.

The competition of arginine and lysine for reabsorption was measured in five patients with LPI (nos. 1, 3–5, 7) and in three controls (nos. 8, 10, 11) having a constant concentration of arginine, 35.6 mM, in the infusion solution, while lysine concentration was increased stepwise from 0 to 35.6 and 57.5 mM, as described above.

Analytical. The renal clearance of plasma ⁵¹Cr-EDTA was used to estimate glomerular filtration rate (GFR). Radioactivity was measured in 3-ml urine and plasma samples (Wallac Decem-GTL³⁰⁰⁻¹⁰⁰⁰ gamma counter, LKB-Wallac, Turku, Finland). Plasma proteins were precipitated with 0.100 ml of 100% (wt/vol) sulfosalicylic acid before

counting. The precipitate had a negligible quenching effect (less than 1%). As a rule, 10,000 counts were recorded.

The plasma samples were centrifuged immediately after counting, and the supernates stored at −23°C with samples of the urines. The basic amino acids were measured with an automatic amino acid analyzer (Beckman Unichrom Automatic Amino Acid Analyzer, with Beckman M 72 ion exchange resin, Beckman Instruments Inc., Fullerton, Calif.). Statistical calculations were made with Student's *t* test.

RESULTS

Glomerular filtration rate. The GFR of the subjects, measured as ⁵¹Cr-EDTA clearance, is given in Table I. It tended to increase slightly during each infusion. The mean (±1 SD) for all periods was 48.0 (±5.1, n = 175) and 48.4 (±6.9, n = 83) ml/min per m² for the patients with LPI and for controls, respectively. This is slightly less than is usually reported for inulin clearance. The clearance of ⁵¹Cr-EDTA has previously been found to be about 15% less than that of inulin (22). We may thus have underestimated the GFR's and, consequently, the filtered loads of amino acids by approximately 15%. The absolute rates of tubular reabsorption would then be underestimated by the same absolute amounts as the filtered loads, and observations of tubular secretion would be false when less than 15% of the filtered load. None of the differences observed between the patients with LPI and the controls would be false because of such underestimation.

Plasma diamino acid concentrations. The plasma diamino acid concentrations in fasting subjects were logarithmically distributed. The log scale means (95% confidence interval) of the μM concentrations in patients with LPI and controls were 63.1 (27.2–146.6) and 119.7 (67.0–213.8) for lysine, 26.2 (11.2–61.5) and 71.3 (29.2–174.2) for arginine, and 18.5 (8.0–43.0) and 48.5 (30.6–76.9) for ornithine, respectively. The differences between the two groups of subjects are all significant at $P < 0.0005$. The low plasma concentration of diamino acids in the patients with LPI is clearly re-

flected by the small filtered loads in the basal state (Fig. 1).

Arginine infusion was accompanied by an increase in plasma ornithine concentration. This was 30–100% of the increase in arginine concentration, in molar units, in both patients with LPI and controls. The same effect has been observed in animals (23, 24). Ornithine infusion influenced the plasma arginine concentration variably but to a lesser degree; the largest infusion used produced only a twofold increase in plasma arginine. The lysine concentration remained quite stable during arginine and ornithine infusions.
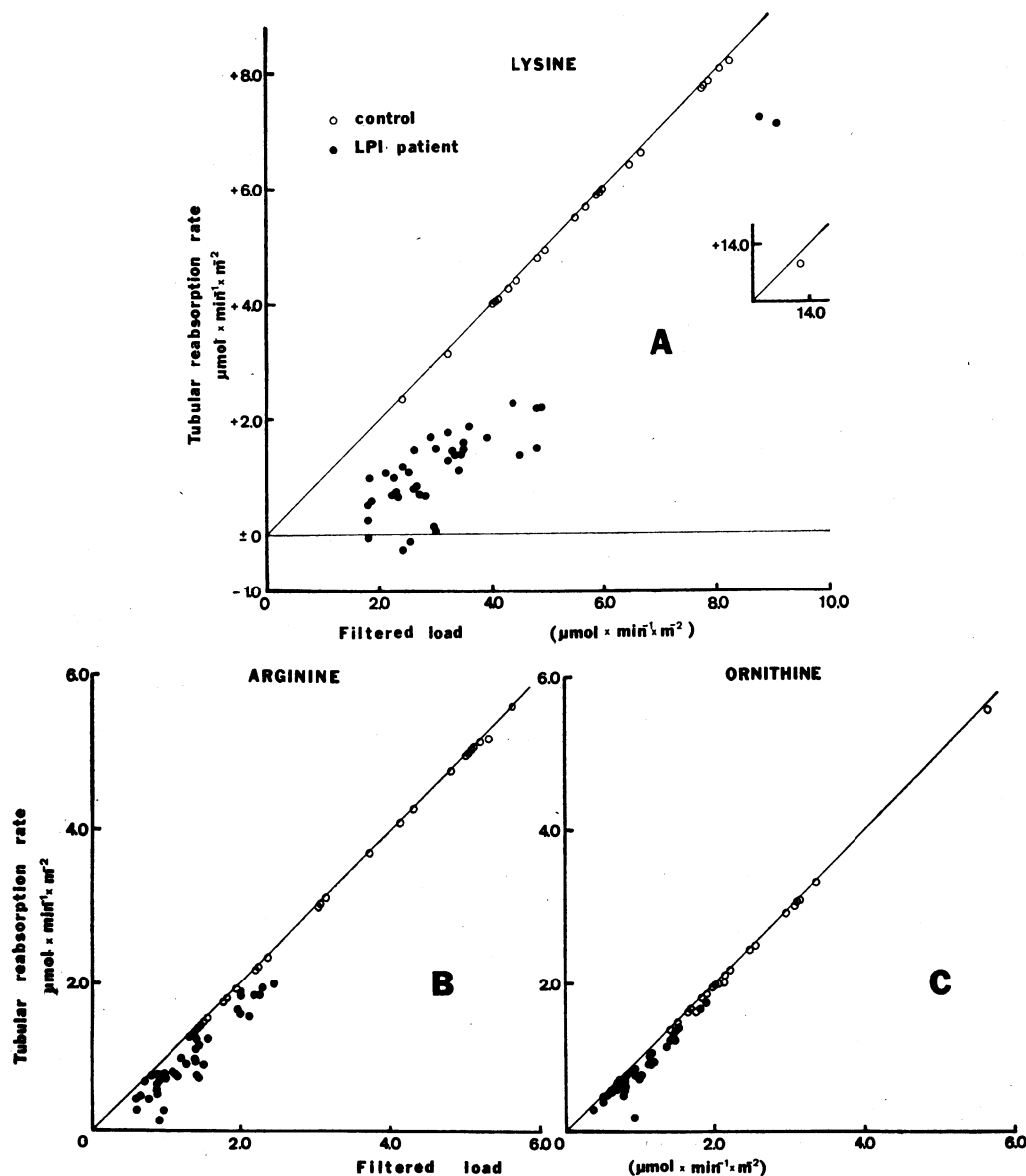

FIGURE 1 Plots of the tubular reabsorption rates versus filtered loads of lysine (A), arginine (B), and ornithine (C) in fasting patients with LPI and in controls. Each point presents a 30-min clearance period.

Reabsorption rates in fasting subjects (Fig. 1). The reabsorption of lysine in patients with LPI was markedly impaired, the threshold being very low (Fig. 1a). Though the filtered load of lysine was lower in the LPI patients, only 37.2±20.2% (mean±SD) was reabsorbed, in contrast to 99.0±0.4% in the controls. The patients' plot of reabsorption rate versus filtered load (Fig. 1a) concentrates around an oblique straight line, with the regression equation $y = 0.87x - 1.46$ ($r = 0.92$). This finding is compatible with either of two explanations. First, the defect may be that little or no reabsorption takes place at low filtered loads, but once the filtered load has risen above a minimum, the amount reabsorbed will increase linearly with it. Second, tubular secretion of lysine may be substantial and mask reabsorption at low filtered loads. Slight tubular secretion was actually measured in three instances, but this was within the limits of technical error.

The reabsorption of arginine and ornithine was clearly diminished, though to a lesser degree than that of lysine. Secretion was not observed. Of filtered arginine, 76.0±17.8% was reabsorbed in the patients and 99.6±0.3% in the controls, and of ornithine, 87.4 ±12.5% and 98.6±2.0% was reabsorbed, respectively. Both differences are significant at $P < 0.0005$. One patient (no. 4) reabsorbed 99.2% of the arginine and 98.3% of the ornithine, which was well above the level in the other patients with LPI. At increased filtered loads (see below), this subject behaved like the other patients. The child with hypoparathyroidism (no. 9) reabsorbed 99.2, 99.4, and 98.7% of lysine, arginine, and ornithine, respectively, and did not differ from the other controls.

Three explanations can be offered for the failure of the kidney to retain arginine and ornithine adequately in LPI. First, the unreabsorbed tubular lysine may compete with the other diamino acids for normal reabsorption. As its filtered load is decreased in LPI, lysine could conceivably interfere with the normal arginine and ornithine reabsorption mechanism only if this mechanism is located distally to the normal reabsorption site of lysine. Second, an abnormality, tubular secretion of arginine and ornithine, may be present in LPI. Third, the reabsorption of all three diamino acids may be impaired because of a defect in their common transport system, though to a variable degree depending on their different affinity for that system. Whatever the mechanism is, lysine, arginine, and ornithine are lost into the urine in that decreasing order.

Arginine and ornithine reabsorption at increased filtered loads. When the filtered load of arginine or ornithine was increased by infusion, the reabsorbed fraction decreased (Fig. 2). In the controls, close to 100% was reabsorbed up to the filtered load (mean)
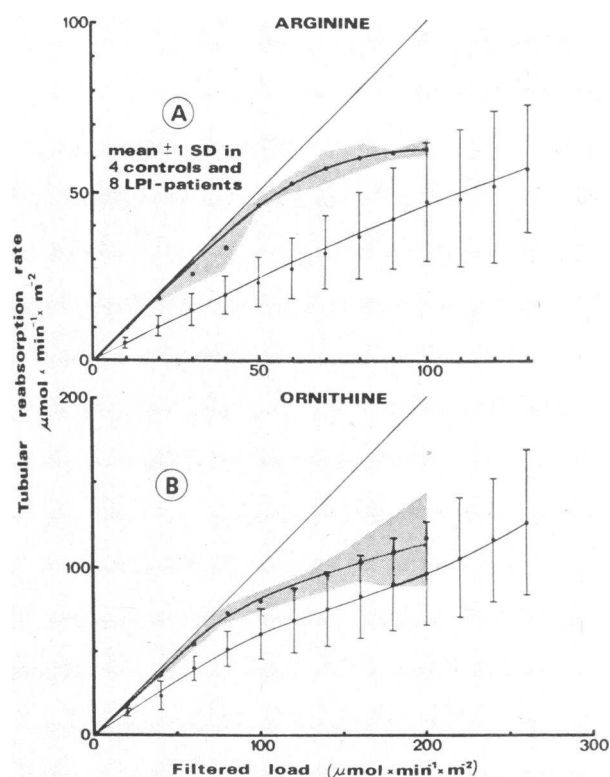

FIGURE 2 Titration curves of arginine (A) and ornithine (B) reabsorption in patients with LPI and in controls. After a priming dose, the amino acid was infused in solution at two to four of the concentrations 17.8, 35.6, 53.4, 71.2, and 89.0 mM at a constant rate of 12 ml/kg body wt per h, until a steady plasma concentration was obtained. Individual titration curves were drawn by rectilinear connection of the zero point and the points obtained for filtered load versus reabsorption rate. The curves presented here were established by calculating statistics from the individual curves at increments of GFR of 10 and 20 μmol/min per m^2 for arginine and ornithine, respectively. The shaded area indicates the calculated mean ±1 SD for the controls, the vertical lines the calculated mean ±1 SD for the patients with LPI.

of 50 μmol/min per m^2 for arginine, and 80 μmol/min per m^2 for ornithine. The difference in reabsorption between the patients with LPI and the controls first increased with the tubular load and then decreased as the slope of the reabsorption curves of the controls began to decrease.

Competition studies. In controls, infusion of increasing amounts of ornithine resulted in a gradual approximately linear decrease in the fractional reabsorption of arginine and lysine (Fig. 3). Arginine infusion, which also brought about a rise in plasma ornithine concentration (see above), caused a similar but steeper decrease in the fractional reabsorption of lysine (Fig. 4). Similar infusions with lysine alone were not conducted. Infusion of arginine at a constant rate, with

stepwise increased concentrations of lysine, was followed by decreased fractional reabsorption of arginine and ornithine (Fig. 5). When arginine alone was infused, the fractional reabsorption of lysine (mean and range) was 68.6 (58.7–80.1)%. The first addition of lysine to the arginine infusion increased the filtered load of lysine to 63.4 (52.5–82.3), the second one to 117 (107–130), in μmol/min per m². The fractional reabsorption of lysine dropped first to 42.0 (34.8–53.8)% and then to 30.5 (20.4–44.3)%.

In the patients with LPI, the reabsorption curve after stepwise increase of the filtered load of ornithine differed from that of the controls (Fig. 3). At first, net tubular absorption of arginine and lysine decreased, and in many patients, even turned to unequivocal net secretion. A further increase in the ornithine load was

associated in many with an increase in the absorption of arginine and lysine and even with a shift from net secretion back to net absorption again. With high ornithine loads, absorption proceeded in several patients at rates close to those of the controls. Lysine behaved similarly during the arginine infusions (Fig. 4). Addition of lysine to the constant infusion of arginine was followed by a small increase in the fractional reabsorption of ornithine in four patients and of arginine in two of the five patients studied. A further increase in the filtered load of lysine caused a drop in the fractional reabsorption of both amino acids in all but one of the patients (Fig. 5). When arginine alone was infused, the fractional reabsorption of lysine (mean and range) was − 10.4 (− 43.5 to + 6.1)%. The first addition of lysine to the infusion increased its filtered load to 71.3
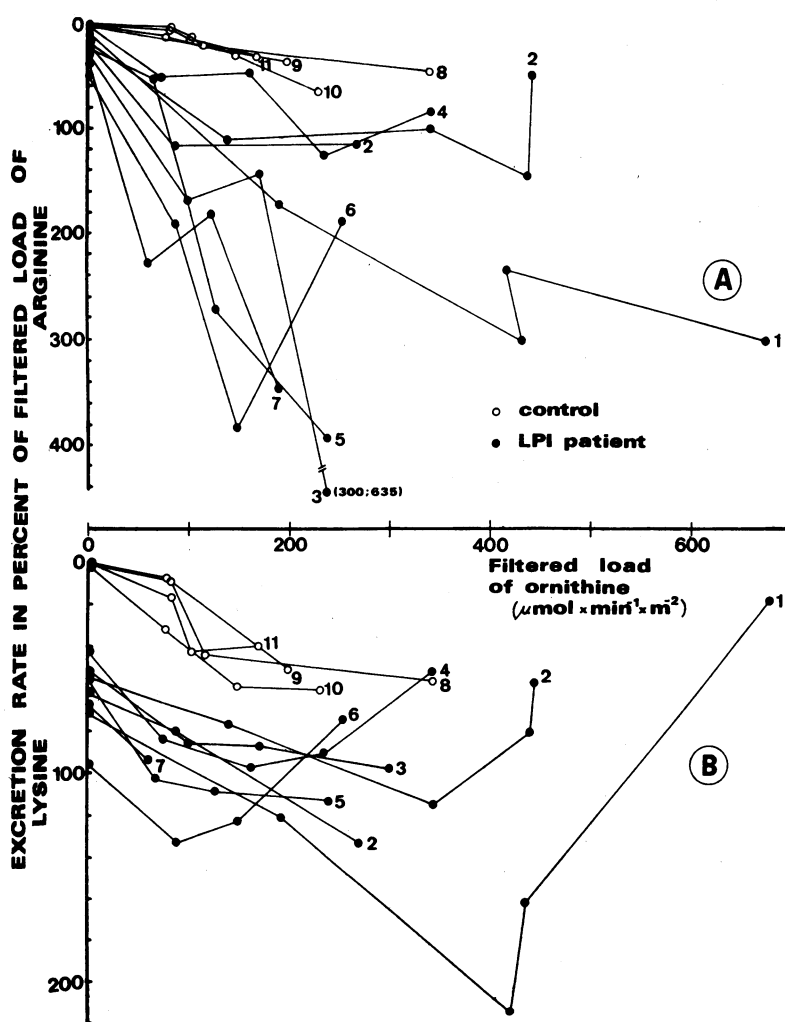

FIGURE 3 The excretion rates of arginine (A) and lysine (B) as fractions of their filtered loads during ornithine infusions. Each excretion rate is plotted as a function of the filtered load of ornithine. Values above 100% on the ordinate indicate net tubular secretion, values below 100% net absorption. For explanation of the infusion procedure, see legend to Fig. 2.
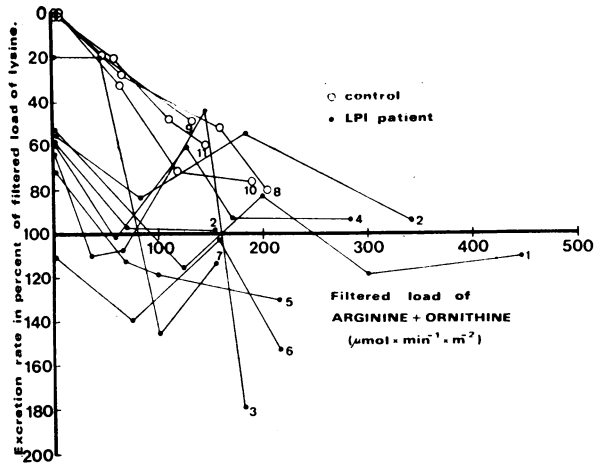
FIGURE 4 The excretion rate of lysine as a fraction of the filtered load during arginine infusion. The excretion rate of lysine is plotted as a function of the filtered load of arginine + ornithine. For explanation, see legends to Figs. 2 and 3.

(63.7–80.2), the second one to 158 (120–171), in μmol/min per m^2. Its fractional reabsorption increased first to 22.8 (15.9–40.5)% and dropped finally to 15.0 (− 3.6 to + 22.5)%.

DISCUSSION

The renal aminoaciduria of patients with LPI differs from that of classical cystinuria, in that these patients do not excrete significant quantities of cystine. This finding supports the view that cystine is at least partially transported by a mechanism separate from that for the diamino acids in the kidney tubuli. Because the reabsorption defect is more specific, LPI offers a good opportunity for studying the mechanism of tubular transport of the diamino acids in the human kidney.

Patients with LPI lose a significant amount of diamino acids in the urine even during fasting periods. Postprandially, as the plasma amino acid concentration rises, this loss must affect their diamino acid economy.

The reabsorption titration curves of arginine and ornithine in patients with LPI are splayed and differ clearly from those of the controls. Such splaying may result from a mutation altering the affinity of a transport system for its substrates. Another possibility is that at least two transport systems for the diamino acids are present in the human kidney (20). The first system, which is responsible for transport at low substrate concentrations, would then be deficient in LPI. When the substrate concentration in the tubular urine is increased to the levels at which the second weak affinity, large capacity system predominates, diamino acid reabsorption proceeds at the normal rate.

At present, quantitation of the tubular secretion of amino acids is not possible. The deviation in LPI from
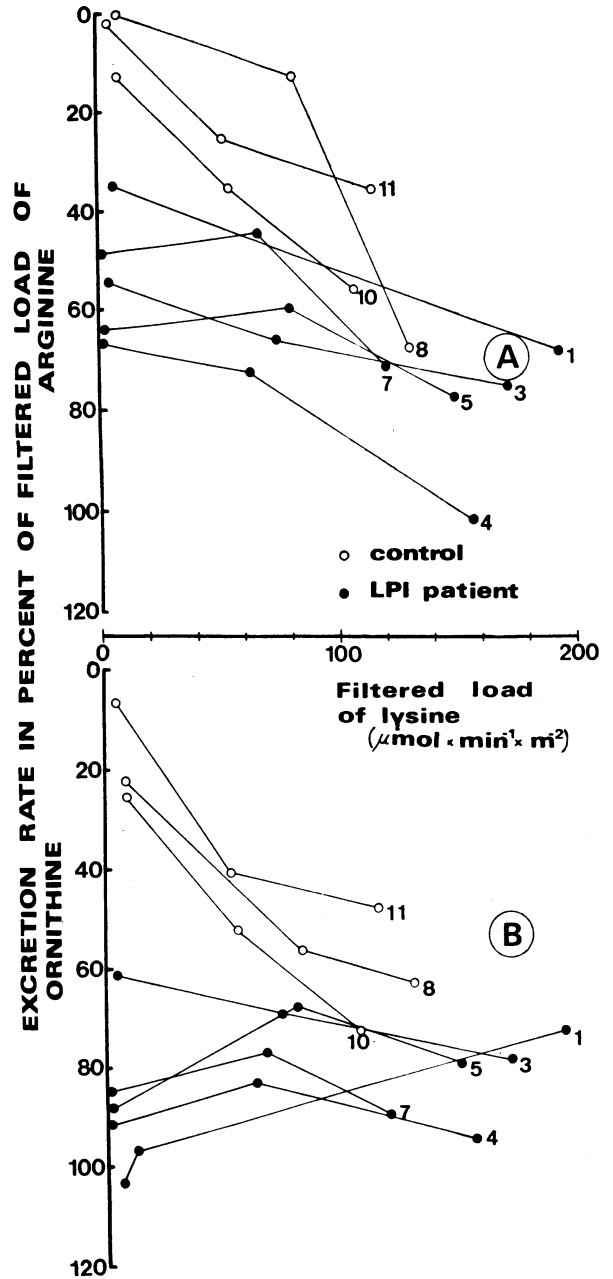

FIGURE 5 The excretion rates of arginine (A) and ornithine (B) as fractions of their filtered loads during infusion of a solution with constant arginine and stepwise increased lysine. The concentration of arginine in the infusion solution was 35.6 mM, that of lysine increased from 0 to 35.6 and 57.5 mM. Each excretion rate is plotted as a function of the filtered load of lysine. For explanations, see legends to Figs. 2 and 3.

the normal reabsorption-titration curve of arginine or ornithine could theoretically be caused by increased tubular secretion of the other diamino acids, especially lysine, as these might block the reabsorption of the infused amino acid. This is very unlikely, for the follow-

ing reasons. First, in the tubular urine, the molar concentration of the other diamino acids must be very low relative to the concentration of the infused diamino acid. This must be true even in the extreme situation in which all plasma leaving the kidney has been cleared of the other diamino acids by tubular secretion. Second, addition of lysine to an arginine infusion had only a small effect on the fractional reabsorption of arginine and ornithine, though it brought about a shift from net secretion to net absorption of lysine itself. These phenomena also refute the hypothesis that the tubular defect in LPI is limited to lysine reabsorption (6).

Net tubular secretion of arginine and ornithine occurred with high filtered loads of ornithine, and net secretion of lysine with high loads of arginine. With further increase in those loads, the net secretion induced often reverted to net absorption. This may be explained as follows. During the infusions, the grossly deficient low-concentration transport system is occupied by an excess of the infused amino acid. Normal secretion of the diamino acids out of the tubular cells continues and exceeds the influx of the other two diamino acids via the deficient transport system. This is the case, at least, when the amino acid present in excess is ornithine or arginine. When the filtered load of the infused amino acid is very high, its concentration in the tubular cells becomes high enough to inhibit the secretion of the other diamino acids, either by occupying the sites for secretion or by some other mechanism. At high concentrations, either a normal "high-concentration" transport system becomes active or the kinetically abnormal transport system reaches normal capacity, and reabsorption proceeds at a normal rate.

Our patients with LPI had net reabsorption of both arginine and ornithine during the joint infusion of arginine and lysine, in spite of the high filtered load of lysine. This is presumably due to the fact that the filtered loads of all three were markedly increased.

In conclusion, we suggest that the complicated picture of the renal handling of diamino acids in LPI is based on the interplay of two or three factors. The reabsorption system for all three diamino acids is defective with low filtered loads. Either this system attains normal capacity with higher loads, or the work is taken over by a second high-concentration system, which is unaffected in LPI. There is a large tubular efflux of all diamino acids. In normal tubuli these diamino acids are recovered by effective reabsorption, but in LPI, they partly remain in the tubular urine.

Tubular secretion of cystine in cystinuria (17, 18) and tubular secretion of two diamino acids during infusion of the third in LPI may have a similar basis. If the defective transport mechanism for cystine/cysteine (25) in cystinuria is abnormally sensitive to

competition by the diamino acids present in the tubular fluid, normal tubular efflux of cystine/cysteine will exceed the capacity for absorption, and the result will be net secretion.

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