Dietary Supplementation of L-Arginine Ameliorates Renal Hypertrophy in Rats Fed a High-Protein Diet (43735)

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Abstract. **Ingestion of a high-protein diet or intravenous administration of amino acids is associated with an increase in glomerular filtration rate (GFR). It can also lead to renal hypertrophy, and, if sustained, may cause glomerular sclerosis. L-Arginine administration ameliorates the progression of renal disease in rats with subtotal nephrectomy and prevents the increase in GFR observed in rats with experimental diabetes. The present study examines the potential effect(s) of L-arginine administration (1%) in the drinking water on the renal hypertrophy that occurs in rats fed a high-protein diet for 1 month. Four groups of female Sprague-Dawley rats, six in each group, were studied (95 ± 1 g). Groups 1 and 2 were fed a low-protein diet (12% casein, 0.504% L-arginine); Group 1 was given tap water, whereas Group 2 was given tap water supplemented with L-arginine. Groups 3 and 4 were fed a high-protein diet (40% casein, 1.68% L-arginine); Group 3 was given tap water, whereas Group 4 was given tap water supplemented with L-arginine. The rats had free access to food and water during the study period. The kidney weight and the kidney to body weight ratio of rats of Group 3 were significantly greater than in the other groups of rats. Renal hypertrophy was prevented in the rats of Group 4. The excretion of orotic acid in the urine, an index of L-arginine deficiency, was significantly greater in rats of Group 3 than in rats of Group 4. Thus, the renal hypertrophy that occurs in rats fed a high-protein diet was decreased in rats given L-arginine supplementation in the drinking water. This effect was associated with less excretion of orotic acid in the urine in rats given L-arginine. A relative deficiency of L-arginine may occur during high-protein feeding that may shunt nitrogen metabolism from the urea cycle to the orotic acid pathway.** [P.S.E.B.M. 1994, Vol 206]

Administration of a high-protein diet or parenteral administration of amino acids results in significant changes in renal function characterized by increased GFR and renal plasma flow (1). Prolonged administration of a high-protein diet may cause structural changes including renal hypertrophy and glomerulosclerosis (2).

A high-protein diet causes changes in metabolic, hemodynamic, and hormonal functions that influence renal physiology. The nitrogen generated from protein metabolism is converted to urea in the liver, a metabolic pathway in which L-arginine has a key role as a donor of the two nitrogen atoms of urea (3). Thus, a relative deficiency of L-arginine may occur, which impairs the disposal of nitrogen through urea generation. This may occur when the amount of protein metabolized generates nitrogen in excess of the L-arginine available (4). This may lead to accumulation of carbamoyl phosphate, the mitochondrial precursor of urea, which may leak into the cytoplasm of the hepatocyte and promote the synthesis of orotic acid (4). Orotic acid formation is the initial step in the synthesis of pyrimidine nitrogen bases and RNA (5). The presence of orotic acid in the urine indicates a relative (during high-protein feeding in the setting of "normal" plasma levels) or absolute (low plasma levels) deficiency of L-arginine for the synthesis of urea. On the

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Received November 5, 1993. [P.S.E.B.M. 1994, Vol 206]
Accepted February 28, 1994.

0037-9727/94/2062-0157$10.50/0

other hand, higher plasma concentrations of orotic acid stimulate the synthesis of nucleic acid pyrimidines and their sugar-containing derivatives (6), all of which may promote growth.

This study examines the effect of L-arginine supplementation on renal hypertrophy and urine excretion of orotic acid in normal rats fed a high-protein diet.

We found that renal hypertrophy secondary to high-protein feeding is associated with a relative deficiency of L-arginine as assessed by the greater excretion of orotic acid in the urine. In addition, L-arginine supplementation decreased the degree of renal hypertrophy and lowered the excretion of orotic acid in the urine. We suggest that the effects of a high-protein intake on renal function and morphology may be mediated, at least in part, through a relative deficiency of L-arginine which may impair the disposal of nitrogen waste through the urea cycle, and increase the availability of orotic acid, a precursor of nucleic acid synthesis.

Materials and Methods

Animals. We used 24 female Sprague-Dawley rats obtained from Harlan (Indianapolis, IN). Upon arrival rats were housed in individual metabolic cages and for the next 4 days were fed a standard rat chow containing 22.8% protein with 1.42% L-arginine (Ralston Purina, St. Louis, MO) and were allowed tap water *ad libitum*.

Plasma and Urine Measurements. After four days on a standard rat chow and tap water, the rats had baseline collections of blood and 24-hr urine (Day 0). They were then fed the two protein diets supplemented or not supplemented with L-arginine. Blood and 24-hr urine specimens were also obtained at the end of the experimental period, 30 days later; biochemical measurements derived from these collections are referred to as experimental values. These procedures were performed as described previously (7). Briefly, rats placed in individual metabolic cages had free access to food and water. Urine volume was determined by weight. An aliquot was kept frozen at −80°C for determination of levels of urea, creatinine, and orotic acid. After baseline 24-hr urine collections were completed, rats were anesthetized with halothane (Ayerst Laboratories Inc., New York, NY) and blood (500 μl) was collected from the left femoral vein; plasma was separated and stored at −80°C for determination of urea and creatinine.

Experimental Protocol. After the first 24-hr urine and plasma collection was obtained, rats were separated into four different groups of six rats each. Each group of rats had free access to water and food during the study. Group 1 was fed a low-protein diet (12% casein, 0.504% L-arginine; ICN Biomedicals Inc., Cleveland, OH) and given tap water; Group 2 was fed a low-protein diet and given tap water supplemented with 1% L-arginine (Sigma Chemical Co., St. Louis, MO); Group 3 was fed a high-protein diet (40% casein, 1.68% L-arginine) and given tap water; and Group 4 was fed a high-protein diet and given tap water supplemented with 1% L-arginine.

Determinations of water and food intake were performed at baseline (Day 0) and at the end of the experimental period, 30 days later, by weighing containers filled with water and food before and after the 24-hr urine collections.

Total intake of L-arginine was calculated from the sum of the L-arginine ingested in the rat chow (0.504% in the low-protein diet, 1.68% in the high-protein diet) and the amount of L-arginine drunk in the water (0% for Group 1 and 3, 1% for Group 2 and 4). In rats drinking water containing 1% L-arginine, the total amount of water drunk was used to calculate the amount of L-arginine ingested.

Chemistries. Concentrations of urea and creatinine in plasma and urine were determined in a Multistat III Plus machine (Laboratory Instruments Inc., Boston, MA) using reagents from Fisher Scientific (St. Louis, MO).

Orotic Acid Determinations. These measurements were performed only in the urine of rats fed the high-protein diet (Group 3 and 4). They were done in the laboratory of Dr. Saul W. Brusilow (Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD), using a high-pressure liquid chromatography (HPLC) method (8).

Determination of Total Body Weight and Kidney Weight. Total body weight was determined 1 month apart, after the first and second 24-hr urine collections. After the second collection, animals were anesthetized with halothane, the aorta was clamped above the renal arteries, and the kidneys were perfused *in situ* with 50 ml PBS (pH 7.4, 37°C). At this time the kidneys were decapsulated *in situ,* severed from the hilus, and weighed.

Statistics. Values are expressed as mean ± SEM. Intragroup comparisons were performed by paired *t* test. Intergroup comparisons were performed by one way analysis of variance (ANOVA) with Bonferroni's correction. Differences were considered significant when $P < 0.05$.

Results

Baseline values reported are given for the combined groups of rats, and as a result do not differ among groups. Body weight increased during the experimental period (approximately 100 g per rat/30 days) and was similar in the four groups of rats. The final values of body weights were not influenced by L-arginine administration (Table I). Rats fed a low-protein diet (Group 1 and 2) gained less weight than

Table I. Body and Kidney Weights, and Food, Water and L-Arginine Intake in the Four Groups of Rats

		Group 1 (low protein + tap water, $n = 6$)	Group 2 (low protein + L-arginine, $n = 6$)	Group 3 (high protein + tap water, $n = 6$)	Group 4 (high protein + L-arginine, $n = 6$)
Body weight (g)	Baseline	95 ± 1	95 ± 1	95 ± 1	95 ± 1
	Experimental	199 ± 2[a]	194 ± 4[a]	205 ± 4[a]	211 ± 5[a]
Weight gain (g/30 days)		102 ± 3	99 ± 3	113 ± 4	114 ± 7
Kidney weight (g)		1.41 ± 0.04	1.36 ± 0.04	1.76 ± 0.04[b,c]	1.65 ± 0.03[b,c,d]
Kidney/body weight × 100		0.708 ± 0.02	0.701 ± 0.02	0.859 ± 0.02[b,c]	0.785 ± 0.03[b,c,d]
$Water_{intake}$ (ml/24 hr)	Baseline	26 ± 1	26 ± 1	26 ± 1	26 ± 1
	Experimental	15 ± 1[a]	34 ± 5[a,c,d]	16 ± 2[a]	55 ± 8[a,c,d]
$Food_{intake}$ (g/24 hr)	Baseline	22 ± 1	22 ± 1	22 ± 1	22 ± 1
	Experimental	30 ± 5	29 ± 3	23 ± 5	22 ± 4
$L\text{-}arg_{intake}$ (mg/24 hr)	Baseline	339 ± 20	339 ± 20	339 ± 20	339 ± 20
	Experimental	160 ± 27[a]	530 ± 59[a,c]	387 ± 86	928 ± 109[a,d]

Note. Values are mean ± SEM. $L\text{-}arg_{intake}$, total intake of L-arginine.
[a] $P < 0.05$ vs baseline values.
[b] $P < 0.05$ vs values for Group 2 (low protein + L-arginine).
[c] $P < 0.05$ vs values for Group 1 (low protein + tap water).
[d] $P < 0.05$ vs values for Group 3 (high protein + tap water).

rats fed a high-protein diet (Group 3 and 4). However, these differences did not reach statistical significance.

Rats fed a high-protein diet (Group 3 and 4) had significantly greater kidney weights than rats fed a low-protein diet given (Group 2) or not given (Group 1) L-arginine. Rats fed a high-protein diet and given tap water supplemented with L-arginine (Group 4) had significantly lesser kidney weights ($P < 0.01$) than rats fed a high-protein diet given tap water (Group 3) (Table II).

The ratio of kidney weight to body weight was significantly greater in rats fed a high-protein diet and given tap water (Group 3) than in rats fed a low protein diet and given or not given L-arginine (Group 1 and 2). In rats fed a high-protein diet and given L-arginine in the drinking water (Group 4), the ratio of kidney weight to body weight was significantly less than in rats fed a high-protein diet and given tap water, but significantly greater than in the two groups of rats fed a low-protein diet.

After 30 days, the total water intake was significantly greater in the groups of rats given L-arginine (Group 2 and 4) than in the rats given tap water and fed either a low- (Group 1) or a high- (Group 3) protein diet. Rats fed a high-protein diet and given L-arginine had the greatest total water intake.

After 30 days the total food intake in each group of rats was not significantly different than at baseline and was not different among the four groups of rats.

Due to differences in water intake and the different protein content in the diets, the net intake of L-arginine by Day 30 was significantly different among the groups of rats studied. Total oral intake of L-arginine was not significantly different among the four groups of rats at baseline. Rats given tap water supplemented with L-arginine (Group 2 and 4) had significantly greater intake of L-arginine than their respective controls (Group 1 and 3). Rats of Group 4 had the greatest total intake of L-arginine.

Rats fed a low-protein diet and given tap water had

Table II. Plasma and Urine Determinations of Urea and Creatinine in the Four Groups of Rats

		Group 1 (low protein + tap water, $n = 6$)	Group 2 (low protein + L-arginine, $n = 6$)	Group 3 (high protein + tap water, $n = 6$)	Group 4 (high protein + L-arginine, $n = 6$)
$Plasma_{Urea}$ (mg/dL)	Baseline	20.7 ± 0.1	20.7 ± 0.1	20.7 ± 0.1	20.7 ± 0.1
	Experimental	14.0 ± 1.3[a]	21.0 ± 1.5[b]	28.7 ± 1.5[a]	27.5 ± 1.3[a]
$Urine_{Urea}$ (mg/24 hr)	Baseline	55 ± 3	55 ± 3	55 ± 3	55 ± 3
	Experimental	62 ± 11	139 ± 15[a,b]	484 ± 50[a]	535 ± 81[a]
$Plasma_{Cr}$ (mg/dL)	Baseline	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01
	Experimental	0.50 ± 0.03	0.48 ± 0.02	0.40 ± 0.01	0.47 ± 0.03
$Urine_{Cr}$ (mg/24 hr)	Baseline	1.71 ± 0.1	1.71 ± 0.1	1.71 ± 0.1	1.71 ± 0.1
	Experimental	4.78 ± 0.51[a]	4.07 ± 0.40[a]	5.61 ± 0.41[a]	4.72 ± 0.70[a]

Note. Values are mean ± SEM. $Plasma_{Cr}$, plasma creatinine; $Urine_{Cr}$, urine creatinine.
[a] $P < 0.05$ vs baseline values.
[b] $P < 0.05$ vs values for Group 1 (low protein + tap water).

significantly lower plasma values of urea at Day 30 than at Day 0. This effect was not observed in rats of Group 2, fed the same diet but given drinking water supplemented with L-arginine. In contrast, rats fed a high-protein diet (Group 3 and 4) had significantly greater plasma levels of urea at Day 30 than at Day 0.

Rats fed a low-protein diet and given tap water had significantly lower excretion of urea in the urine at Day 30 than at Day 0. In contrast, rats fed a high-protein diet had a significantly greater excretion of urea in the urine at Day 30 than at Day 0. Excretion of urea in the urine at Day 60 was significantly greater in rats fed a low-protein diet and given tap water supplemented with L-arginine (Group 2) than in rats fed the same diet without additional arginine (Group 1).

All groups of rats studied had significantly greater excretion of creatinine in the urine at Day 30 than at Day 0, but the final values did not differ significantly among the groups.

Excretion of orotic acid in the urine of rats fed a high-protein diet supplemented or not supplemented with L-arginine is shown in Figure 1. These values are standardized for urine excretion of creatinine in 24 hr. Rats fed a high-protein diet and given tap water (Group 3) had significantly greater excretion of orotic acid in the urine than rats fed a high-protein diet and given tap water supplemented with L-arginine (Group 4).

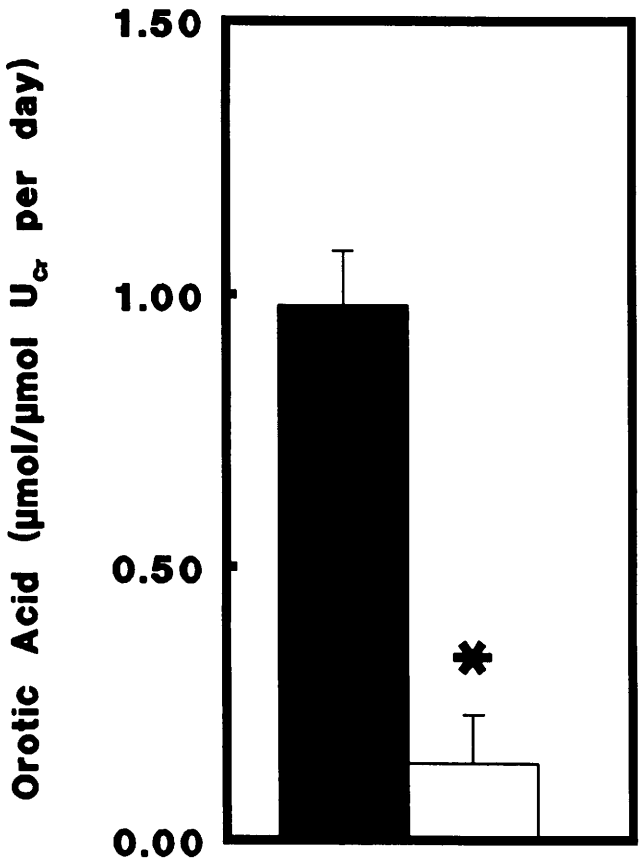

Figure 1. Excretion of orotic acid in the urine of rats fed a high-protein diet and given tap water alone (■) or tap water supplemented with 1% L-arginine (□). *$P < 0.0001$.

Discussion

In humans or experimental animals, the acute or chronic administration of a high-protein diet causes a significant increase in GFR and renal plasma flow (reviewed in 1). Although the precise mechanism of this response has proven elusive there is evidence that it may be considered as a renal functional compensatory mechanism to dispose of the increased load of metabolic waste generated by the organism during protein feeding (9). If the adaptive renal response is prolonged, as determined by hyperfiltration, the kidney undergoes compensatory hypertrophy. In essence, renal compensatory hypertrophy is seen in otherwise normal kidneys in the setting of increased oral (10) or parenteral (11) administration of protein. Renal hypertrophy also occurs when renal mass is decreased but protein intake is maintained constant, as after subtotal nephrectomy (12, 13) or in rats with experimental diabetes (14) or animals given chronic acid loads (15). Hyperfiltration is also seen as part of normal aging (16). Unfortunately, the events that result in hyperfiltration and compensatory hypertrophy may cause morphological changes characterized by glomerular sclerosis and tubular atrophy, loss of renal function, and even death from uremia (2).

Maneuvers that blunt or decrease hyperfiltration and/or renal hypertrophy (reviewed in 1) may be of benefit in preserving renal function. Since L-arginine supplementation in the drinking water ameliorates the progression of renal disease in rats with subtotal nephrectomy (13), at least in part by decreasing intraglomerular hypertension (17), and also prevents hyperfiltration and decreases proteinuria in rats with experimental diabetes (14), we designed this study to examine the potential effects of L-arginine administration on total body and kidney weight of rats fed a high-protein diet. We found that, after 30 days, the group of rats fed a high-protein diet and given tap water had kidney weights that were significantly greater than the weights of the kidneys in the other groups of rats studied. Of interest, rats fed the same high-protein diet but given tap water supplemented with L-arginine during a similar time period had a mean kidney weight that was significantly lower than the nonsupplemented rats. The difference in mean kidney weight between these two groups of rats fed a high-protein diet cannot be explained by differences in final body weight or in total food or caloric intake. The major difference between these two groups of rats was the addition to the drinking tap water of 1% L-arginine in one of the groups.

Although the mechanism(s) by which L-arginine supplementation ameliorated renal hypertrophy in rats

fed a high-protein diet may not be completely clarified by the present study, perhaps a clue is provided by the changes observed in the excretion of orotic acid in the urine. In the rat, a total or relative deficiency of L-arginine causes a significant increase in plasma and urine levels of orotic acid (4). L-Arginine deficiency shunts the nitrogen load from the urea cycle toward the orotic acid metabolic pathway building up the pyrimidine pool for RNA synthesis (3). In rats fed a high-protein diet and given tap water alone, a relative deficiency of L-arginine may increase the availability of orotic acid for RNA synthesis, thus promoting renal hypertrophy.

Rats of Group 4 ingested, on average, 541 mg/24 hr (approximately 3140 μmol/24 hr) more L-arginine than rats of Group 3. However, not all of the excess L-arginine intake could be accounted for by a proportional increase in urea production, since only 850 more μmol/24 hr of urea were excreted in the urine of rats of Group 4 than in the urine of rats of Group 3. The remaining difference (approximately 2290 μmol/24 hr) cannot be accounted for by differences in excretion of creatinine in the urine since rats of Group 4 excreted approximately 15% less creatinine in the urine than rats of Group 3 (a difference of approximately 8 μmol/24 hr). This unexpected finding, that is, greater plasma levels (by 17%) and lower urine excretion (by 15%) of creatinine in rats of Group 4 may suggest a greater muscle mass in these rats than in rats of Group 3. A more complete analysis of L-arginine balance is not possible since we did not examine the characteristics of the intestinal absorption of L-arginine in the setting of a high-protein diet supplemented or not supplemented with L-arginine in the drinking water.

In summary, we report the novel observation that L-arginine supplementation effectively ameliorates renal hypertrophy in rats fed a high-protein diet, a response that was associated with a significant reduction in urine excretion of orotic acid, which may lead to a decrease in the pyrimidine pool. We suggest that the amelioration of renal hypertrophy in rats fed a high-protein diet supplemented with L-arginine may be mediated, at least in part, by an increase in the supply of L-arginine for the disposal of nitrogen through the urea cycle, and to the lower production of orotic acid, a precursor of nucleic acid synthesis.

We thank Dr. Saul Brusilow (Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD) for his helpful comments and for the determination of orotic acid concentrations in the urine.

The authors are also grateful to Mr. James Havranek for assistance in the preparation of this manuscript.

This work was supported by NIH Grant DK-09976 and DK-40321.

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