ABSTRACT The kidney plays a major role in arginine metabolism in 3 principal ways: arginine synthesis, creatine synthesis, and arginine reabsorption. Appreciable quantities of arginine are synthesized in the kidney from citrulline produced by the intestine. The renal enzymes of arginine synthesis, argininosuccinate synthetase and argininosuccinate lyase, occur in the cells of the proximal tubule. The rate of arginine synthesis depends on citrulline delivery and does not appear to be regulated by dietary arginine availability. Renal arginine synthesis in humans produces ~2 g arginine/d, which may be compared to an intake, from a Western diet, of ~4 to 5 g/d. Spontaneous, nonenzymatic breakdown of creatine and creatine phosphate to creatinine causes the excretion of 1 to 2 g creatinine/d and requires the replacement of an equivalent amount of creatine from the diet and by endogenous synthesis. The first enzyme of creatine biosynthesis, L-arginine:glycine amidinotransferase, occurs in the kidney and produces guanidinoacetate, which is released into the renal vein. The renal output of guanidinoacetate, however, is rather low, and we propose that the entire pathway of creatine synthesis may also occur in the liver. Renal arginine reabsorption salvages ~3 g arginine/d. At the apical membrane of proximal tubular cells, arginine shares a transporter with lysine, ornithine, and cystine. Defects in this heteromeric transporter cause cystinuria, which is also characterized by urinary loss of arginine, lysine, and ornithine. Arginine is transported out of the proximal tubular cells at the basolateral membrane by another heteromeric transporter, which also transports lysine and ornithine. Defects in this transporter cause lysinuric protein intolerance. J. Nutr. 134: 2791S–2795S, 2004.

KEY WORDS: • arginine synthesis • citrulline • kidney • creatine synthesis • arginine reabsorption

This review article is concerned with quantitatively major aspects of renal arginine metabolism. Adults consuming a Western diet ingest ~4 to 5 g arginine/d. The 3 processes we discuss are renal arginine synthesis, creatine synthesis, and the renal reabsorption of arginine. Each of these processes involves the production, utilization, or salvage of 2 to 3 g arginine/d and therefore plays a major role in arginine homeostasis.

Renal arginine synthesis

Windmueller and Spaeth (1) were the first to demonstrate a continuous release of citrulline from the small intestine. They showed that this citrulline is an end product of intestinal glutamine metabolism. The great bulk, ~85%, of intestinal citrulline release can be accounted for by its uptake by the kidneys and conversion to arginine, which is then released into the renal vein. Such a pathway gives physiological significance to the occurrence of argininosuccinate synthetase (EC

6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) in the kidneys, in the absence of a complete urea cycle. A second key finding regarding this intestinal–renal axis for arginine synthesis was the discovery, by Wakabayashi and Jones (2), of a novel enzyme in the intestinal mucosa, pyrroline-5-carboxylate synthase (EC number not assigned), that converts glutamate to pyrroline-5-carboxylate. Figure 1 illustrates the intestinal–renal axis for arginine synthesis (3). It should be appreciated that the occurrence of this axis is dependent on species and on developmental stage. For example, in cats the small intestine does not produce citrulline, and therefore the kidney in vivo does not produce arginine (4). The fact that cats have survived without the ability to synthesize arginine may be attributed to the customary high protein intake of carnivores. In neonatal mice and pigs, the small intestine does not release citrulline but rather produces arginine (5,6).

The intrarenal site of renal arginine synthesis has been investigated. Studies on microdissected rat tubules (7) indicate that argininosuccinate synthetase and argininosuccinate lyase are enriched primarily in the proximal convoluted tubule, with lesser activities in the proximal straight tubule. Gradient fractionation of isolated rat tubules in our own laboratory (8) indicated that this process occurs primarily in the proximal convoluted tubule. This localization is important because amino acid reabsorption primarily occurs in the proximal tubules (9). Although it has not been formally proven that citrulline delivery to the kidney for arginine synthesis occurs...
The regulation of renal arginine synthesis has also been investigated. Our own work with rats (10) quantified renal arginine production by means of simultaneous measurement of arteriovenous difference and renal blood flow. We showed (11) that renal arginine synthesis is independent of the arginine protein content of the diet. In humans, Young's group (12) showed that arginine synthesis de novo is unaffected by the consumption of an arginine-free diet. These observations indicate that renal arginine synthesis is not primarily regulated by arginine status. They are also consistent with reports from our own laboratory (13) and Young's laboratory (14) that the primary regulation of arginine metabolism occurs at the level of its catabolism. We showed that in rats (Table 1), arginine synthesis is critically dependent on the delivery of citrulline to the kidney (10). Thus, it appears that the kidney has a substantial excess capacity for the conversion of citrulline to arginine (Fig. 2) but is limited in vivo by the rate of delivery of citrulline. This suggests a master–slave relation between the intestine and the kidney in which the synthesis of citrulline by the intestine is the crucial regulatory event. Such a view is confirmed by experiments in rats in which intestinal citrulline synthesis is inhibited (15) or in which it is severely limited after resection of the small intestine (3). In both of these situations, arginine becomes an essential amino acid in rats.

The quantitative importance of renal arginine synthesis in humans was demonstrated by Tizianello et al. (16), who measured the net flux of amino acids across the kidney in normal subjects and in those with chronic renal insufficiency. In normal subjects, renal arginine synthesis amounted to $\sim 1.75$ g/d. Curiously, in the patients with chronic renal insufficiency, even though the glomerular filtration rate was reduced to 13% of normal, the kidneys still produced $\sim 0.7$ g arginine/d. The reason for this is probably related to the elevation in plasma citrulline concentration seen in chronic renal insufficiency, from 29 to 67 $\mu$mol/L. Thus, the increased concentration of citrulline somewhat offsets the decreased filtration.

The kidney also exhibits arginase activity, from the mitochondrial isozyme, arginase II (EC 3.4.3.1) (7). This enzyme is found principally in the medulla. It has been proposed that it plays a role in the urinary concentrating mechanism by delivering urea to the countercurrent system. The amount of urea produced in this way, however, is very small.

**Creatine synthesis**

Creatine and creatine phosphate play major roles in the energy metabolism of tissues, such as muscle and brain. Both of these compounds are subject to spontaneous, nonenzymatic degradation, which produces creatinine at a rate that corresponds to $\sim 1.7\%$ of the total creatine pool per day (17). Adult humans excrete 1 to 2 g creatinine/d; the variability is primarily dependent on muscle mass, because this tissue contains the great bulk of the body’s creatine and creatine phosphate stores. Therefore, to maintain creatine status, humans need to replace 1 to 2 g creatine/d. Part of this may come from nonvegetarian dietary sources; the remainder is synthesized de novo. The

![FIGURE 1](https://academic.oup.com/jn/article-abstract/134/10/2791S/4688502)

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Arterial plasma concentration $\mu$mol/L</th>
<th>Renal flux$^2$ nmol $\cdot$ min$^{-1}$ $\cdot$ 100 g body wt$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citrulline</td>
<td>Arginine</td>
</tr>
<tr>
<td>Infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>62 ± 8</td>
<td>175 ± 26</td>
</tr>
<tr>
<td>Citrulline</td>
<td>242 ± 38$^*$</td>
<td>244 ± 35$^*$</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 5 rats/group; $^*$ different from saline infusion, $P < 0.05$. Data are from Dhanakoti et al. (10).
2 Positive values indicate uptake; negative values indicate output.

![FIGURE 2](https://academic.oup.com/jn/article-abstract/134/10/2791S/4688502)
pathway of creatine synthesis is simple (Fig. 3), comprising only 2 reactions. In the first reaction, catalyzed by l-arginine:glycine amidinotransferase (AGAT; EC 2.1.4.1), arginine donates an amidino group to glycine to form guanidinoacetate and ornithine. In the second reaction, catalyzed by guanidinoacetate N-methyltransferase (GAMT; EC 2.1.1.2), guanidinoacetate is methylated by S-adenosylmethionine to form S-adenosylhomocysteine and creatine. Creatine is released by the liver and taken up by tissues, such as muscle, via a specific transporter (17). The tissue localization of AGAT and GAMT is of relevance. In rats, AGAT is found in the kidney, whereas GAMT is absent from the kidney and is enriched in the liver (17). This has led to the proposed mechanism of an interorgan metabolism whereby guanidinoacetate, produced in the kidney, is released into the bloodstream, taken up by the liver, and methylated to creatine.

The quantitative aspects of creatine synthesis are of importance. If 1.5 g creatine/d were to be synthesized, 2.6 g arginine/d could be required. Visek (18) suggests that this rate of creatine synthesis could require the arginine contained in 50 g protein/d. To gain insight into this question, we measured arteriovenous differences across the kidneys of rats and of humans for guanidinoacetate and ornithine. In all cases, there was a negative arteriovenous difference for guanidinoacetate, indicating its release into the renal venous plasma. In all cases, there was a positive arteriovenous difference for ornithine, indicating its loss to the urine. The renal production of guanidinoacetate, however, accounted for only some 15 to 20% of the creatinine lost. Thus, it appears that the proposed interorgan synthesis of creatine occurs in vivo in both rats and humans, but is not adequate to supply the bulk of the creatine required.

Where else could creatine arise? One obvious source is the diet, because the creatine content of meat is high. Creatine ingestion increases the body pool of creatine (19). We previously showed that dietary creatine ingestion downregulates renal AGAT (20) and decreases renal guanidinoacetate output. Vegetarians have no source of dietary creatine, so synthesis de novo is their only means of replacing creatine stores. Similarly, we fed rats a diet that contained no creatine.

We speculate that the liver can carry out the entire pathway of creatine synthesis. Although rat liver has not been shown to contain AGAT activity, immunofluorescence microscopy shows AGAT immunoreactivity in rat hepatocytes (21). A basic alignment search (22) of an expressed sequence tag database for human liver from the National Center for Biotechnology Information reveals the occurrence of a transcript identified as AGAT (AV648585 GLC Homo sapiens cDNA clone GLCLBA07). A recent paper by Xu et al. (23) reports high levels of expression of AGAT mRNA in human liver. It must, therefore, be considered that liver is capable of the entire pathway of creatine synthesis. A particular advantage of hepatic creatine synthesis is the occurrence in this tissue of all of the enzymes of the urea cycle. Thus, ornithine arising from AGAT need not be lost to catabolism but can be recycled to arginine. We envisage that this cycle of creatine synthesis occurs in parallel with the urea cycle so that arginine has 2 possible fates: to be converted to ornithine and urea by arginase I or to ornithine and guanidinoacetate by AGAT. We estimate that the rate of arginase I will exceed that of AGAT by a factor of ~30. We refer to this proposed shared pathway as the arginine bicycle (Fig. 4).

Reflux reabsorption of arginine

All of the free amino acids are filtered and reabsorbed in the kidney. Typically, ~<1% of the filtered load is lost in the urine. Quantitative data on arginine reabsorption in humans are provided by Tizianello’s work (16). At an average plasma arginine concentration of 90 μmol/L and a glomerular filtration rate of 190 L/d, it can be calculated that human kidneys filter, and reabsorb, ~18 mmol/d (3.1 g/d) of arginine.

Reabsorption of amino acids primarily occurs in the proximal tubule (9). Reabsorption involves transepithelial transport, requiring transporters on the apical and basolateral membranes of tubular cells. Typically, these apical and basolateral transporters are distinct and are also expressed in intestinal epithelial cells. Arginine reabsorption is currently of considerable interest for 2 reasons. First, certain clinical disorders are known to be caused by defects in either the apical or basolateral transporters. Second, there is new information concerning the molecular nature of these transporters.

Arginine transport from the tubular fluid, across the apical membrane, is brought about by a transporter, system b0,+AT, that also transports other cationic amino acids (lysine and ornithine) as well as cystine (24). Defects in the transporter cause cystinuria, which involves the urinary excretion of large quantities of arginine, lysine, and ornithine in addition to cystine (25). The transporter operates via an antipporter mechanism (24). Arginine (or lysine, ornithine, or cystine) is transported into the cell in exchange for neutral amino acids, such as leucine; high concentrations of these neutral amino acids are maintained by secondary active sodium-driven transport systems (Fig. 5). Molecular studies of the nature of this amino acid transporter show that it is heteromeric in nature, composed of a heavy subunit (rbAT) linked by means of a disulfide bond to a light subunit (b0,+AT) (24).

Arginine transport across the basolateral membrane is brought about by system y+L. Again, this transporter is heteromeric in nature, consisting of a heavy subunit, 4F2hc, and a light subunit, y+LAT1. This system transports arginine, ornithine, and lysine, but not cystine. It is thought that cystine

FIGURE 3 Proposed interorgan pathway for creatine synthesis. GAA, guanidinoacetate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

FIGURE 4 Proposed arginine bicycle pathway for urea and creatine synthesis in liver. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.
is reduced to cysteine in the highly reduced intracellular milieu and leaves the cell in this form.

A defect in the basolateral transporter (system y\textsuperscript{+}/L) causes lysinuric protein intolerance (25). This rare autosomal recessive disease is found primarily in Finland, but there are also clusters in Japan, Quebec, and southern Italy. Patients have low circulating concentrations of the cationic amino acids. Because system y\textsuperscript{+}/L is expressed in both the intestine and the kidney, the depletion of these amino acids is due both to impaired intestinal absorption and renal reabsorption. Patients suffer from bouts of hyperammonemia, caused by a deficiency of the amino acids of the urea cycle. Patients develop an aversion to protein-rich foods, which, together with the lysine deficiency, can produce protein malnutrition (26). Treatment with citrulline is beneficial. Absorption of citrulline, a neutral amino acid, does not involve the y\textsuperscript{+}/L transporter. Once absorbed, citrulline may be converted to arginine, increasing the plasma arginine concentration. The hyperammonemia is corrected and the aversion to protein-rich foods diminishes, which permits increased ingestion of dietary protein. Quite large intakes of citrulline (2.5–8.5 g/d) appear to be well tolerated for many years (26).

**Conclusion**

The kidney plays a quantitatively important role in arginine metabolism via 3 processes: arginine synthesis, creatine synthesis, and arginine reabsorption. However, the kidney may play a lesser role in creatine synthesis than was previously thought. Placing the entire pathway of creatine synthesis in the liver has the great advantage that ornithine can be recycled; creatine synthesis would not be a net consumer of arginine.

It is important to appreciate that the urea cycle is not capable of either the net synthesis or the net catabolism of arginine. Because it is a cycle, arginine removal is exactly balanced by arginine synthesis. In adult animals, net arginine synthesis is primarily achieved by the combined efforts of the intestine and the kidney. However, the kidney is not the only organ of arginine synthesis. Experiments with dogs show that renal arginine synthesis accounts for the greater part of whole-body arginine synthesis, but it is clear that ~40% of this occurs outside the kidney (27). The sources of this extrarenal arginine synthesis have not been completely identified, but they certainly include arginine regeneration from citrulline produced during NO synthesis in macrophages (28) and in endothelial cells (29). Such pathways may explain the occurrence of some enzymes of the urea cycle (argininosuccinate synthetase and argininosuccinate lyase) in tissues that lack a urea cycle.

There is also the issue of the metabolism of dietary citrulline. Because this amino acid is not found in protein (with some very minor exceptions), its importance as a dietary constituent is often ignored. However, some foods contain considerable quantities of free citrulline. For example, the fruit of the watermelon, Citrullus vulgaris, commonly contains ~1 g citrulline/kg. Citrulline seems to play a role in drought tolerance in plants, and under drought conditions quite high concentrations accumulate in watermelon leaves, where it may act as a potent scavenger of hydroxyl radicals (30). Dietary citrulline may replace dietary arginine in situations where exogenous arginine is required. Such nutritional equivalence of citrulline and arginine requires a pathway that can convert citrulline to arginine and that is not normally saturated with substrate. The renal pathway of arginine synthesis fulfills these criteria. However, it may not be responsible for the conversion of citrulline to arginine in patients with lysinuric protein intolerance, because the defect in the basolateral transporter would also interfere with the transport of newly synthesized arginine out of the renal proximal tubular cell. It is therefore very probable that the extrarenal pathways for the conversion of citrulline to arginine are important in the provision of arginine in these situations.

**LITERATURE CITED**