

# Renal metabolism of amino acids: its role in interorgan amino acid exchange<sup>1-3</sup>

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## ABSTRACT

The kidneys play a role in the synthesis and interorgan exchange of several amino acids. The quantitative importance of renal amino acid metabolism in the body is not, however, clear. We review here the role of the kidney in the interorgan exchange of amino acids, with emphasis on quantitative aspects. We reviewed relevant literature by using a computerized literature search (PubMed) and checking relevant references from the identified articles. Our own data are discussed in the context of the literature. The kidney takes up glutamine and metabolizes it to ammonia. This process is sensitive to pH and serves to maintain acid-base homeostasis and to excrete nitrogen. In this way, the metabolism of renal glutamine and ammonia is complementary to hepatic urea synthesis. Citrulline, derived from intestinal glutamine breakdown, is converted to arginine by the kidney. Renal phenylalanine uptake is followed by stoichiometric tyrosine release, and glycine uptake is accompanied by serine release. Certain administered oligopeptides (eg, glutamine dipeptides) are converted by the kidneys to their constituent components before they can be used in metabolic processes. The kidneys play an important role in the interorgan exchange of amino acids. Quantitatively, for several important amino acids, the kidneys are as important as the gut in intermediary metabolism. The kidneys may be crucial "mediators" of the beneficial effects of specialized, disease-specific feeding solutions such as those enriched in glutamine dipeptides. *Am J Clin Nutr* 2004;79:185-97.

**KEY WORDS** Kidney, amino acids, urea, ammonia, glutamine, citrulline, arginine, phenylalanine, tyrosine, glycine, serine, asymmetrical dimethylarginine, homocysteine, dipeptides, nutrition, gut, liver, interorgan nitrogen exchange, acidosis, renal failure

## INTRODUCTION

The kidneys fulfill a wide range of functions, among which are the maintenance of acid-base equilibrium and electrolyte and fluid balances, the regulation of hematopoiesis, and the excretion of waste products. A vast amount of literature has been published on the urinary excretion of waste nitrogen in the form of urea, creatinine, and ammonia. In addition to its role in waste nitrogen disposal, urinary excretion of ammonia is a means of excreting excess protons (1), which are mainly those derived from dietary protein intake (2). Several publications,

however, provided 2 lines of evidence (3-10) that the kidneys also play a very important role in the exchange of nitrogenous metabolites between organs. These data suggest that specialized, disease-specific nutrition, enriched in arginine, glutamine dipeptides, or a combination of the 2, may in fact require metabolic handling by the kidney to achieve its beneficial effects.

The first line of evidence has emerged from research on exogenous administration of glutamine-containing dipeptides as a supplement to parenteral nutrition (11-13). Sustained interest in this area arises from the knowledge that glutamine-enriched nutrition may be beneficial during critical illness (11, 12, 14). The instability of glutamine in parenteral nutrition solutions (15, 16) can be overcome by providing glutamine as a dipeptide, for example, as glycyl dipeptide (11) or alanyl dipeptide (12). Some of these dipeptides are predominantly hydrolyzed in the kidney after intravenous infusion (15, 17). As a consequence, the kidney reverses from the function of glutamine uptake from the bloodstream to that of glutamine release during the administration of, for instance, glycyl-glutamine (18), which is accompanied by an increase in the plasma concentration of the composing amino acids, notably glutamine (16). We feel that this indicates that the beneficial effects of glutamine dipeptide supplementation require the kidney as a metabolic mediator.

Another line of evidence arose from observations by Houdijk et al (14, 19, 20). In rats fed a glutamine-enriched enteral diet, they observed an increase in arterial citrulline and arginine concentrations concomitant with increased renal citrulline uptake and arginine release (20). Such data would be compatible with the known conversion of glutamine to citrulline in the gut, which is followed by the conversion of citrulline

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to arginine in the kidney (*see* below). This led those investigators to suggest that the beneficial effects of glutamine supplementation (14, 21) might in fact be mediated by increased arginine production in the kidney in rats (20), as well as in humans (14). Given these facts, we felt it would be appropriate to review the role of the kidney in interorgan nitrogen exchange under normal physiologic circumstances, with particular emphasis on amino acid metabolism. Information on the role of an organ in interorgan amino acid exchange can be obtained by measuring arteriovenous differences and plasma flow (22–25) as well as the net exchange or flux across that organ (23, 26). Such measurements provide highly relevant qualitative and quantitative information on integrative amino acid physiology.

This article is not intended as an exhaustive review of the effects of acidosis, chronic renal failure, or the inherited disorders of metabolism. For a comprehensive review of these subjects, the reader is referred to relevant literature (eg, 27, 28). The key issues of the present review are, first, the question of the qualitative role of the kidneys in the metabolism of various amino acids—ie, is there renal uptake or release?—and, second, the question of the quantitative importance of renal amino acid uptake or release compared with whole-body metabolism and daily amino acid requirements.

The renal metabolism of 2 amino acids—glutamine and arginine, which currently receive considerable attention as conditionally essential amino acids—will be reviewed first. The conversion of phenylalanine to tyrosine and of glycine to serine will be addressed next. Then, attention will be paid to branched-chain amino acid metabolism. Finally, the remaining amino acids and the scarce literature on the effects of renal failure will be discussed (the latter only briefly).

## GLUTAMINE

Glutamine is a nonessential amino acid (29–31). Among the amino acids used for protein synthesis, it is the most abundant free amino acid in the body (32, 33). Glutamine turnover probably is  $\approx 70$  g/d in humans under normal postabsorptive circumstances (34–36). Approximately 30% of this glutamine flux is derived from protein breakdown, and the remainder is synthesized *de novo* (36). Glutamine can be synthesized in several organs (29). From a quantitative point of view, the most important site of glutamine synthesis probably is skeletal muscle (10, 24, 32, 37, 38). In addition, the brain (4, 39–44), adipose tissue (45), the heart (46), and the lungs (47–51) have been shown to release glutamine under certain physiologic and pathologic conditions. As for the contribution of the lungs to glutamine synthesis, it should be stressed that, because the blood flow across the lungs is high, the arteriovenous differences will be small and difficult to measure even if the flux across the lungs is substantial (45). This makes the measurements less reliable and may explain the discrepancies among the results obtained by several authors with regard to the role of the lungs in glutamine exchange (47–52). The role of the liver in glutamine synthesis and breakdown is variable and depends, among other factors, on acid-base homeostasis; under physiologic conditions, the glutamine balance across the liver is close to zero (25, 53). It has been postulated that, under conditions of increased demand, endogenous glutamine supplies can become a limiting factor for protein synthesis and other metabolic processes; for this reason, glutamine is some-

times referred to as a conditionally essential amino acid (16, 32).

In many animals (54–60) and humans (61–63), glutamine is a preferred fuel for the gut as well as for several other rapidly dividing tissues, such as those of the immune system (37, 64–68). It is difficult to measure directly the consumption of glutamine by the human gut, but extrapolations from studies in dogs (69) suggest that the amount is  $\approx 10$  g/d (34). This is in keeping with reported differences in arteriovenous concentrations across the gut in humans (61, 70), assuming portal blood flow to be  $\approx 1$  L/min (71). The human immune system probably consumes  $\geq 10$  g glutamine/d (34, 72). Intestinal glutamine consumption appears to be clinically relevant, because impaired intestinal barrier function, bacterial translocation, and gut mucosal atrophy are prevented by glutamine supplementation during total parenteral nutrition (11, 63) and during experimental critical illness (73, 74) in rats. In addition, sepsis and endotoxemia lead to diminished intestinal glutamine consumption (70). Furthermore, mucosal atrophy has been associated with decreased glutamine concentrations in intestinal mucosa (75), which may explain how glutamine supplementation plays a role in maintaining the integrity of the intestinal barrier (11, 12, 76–80).

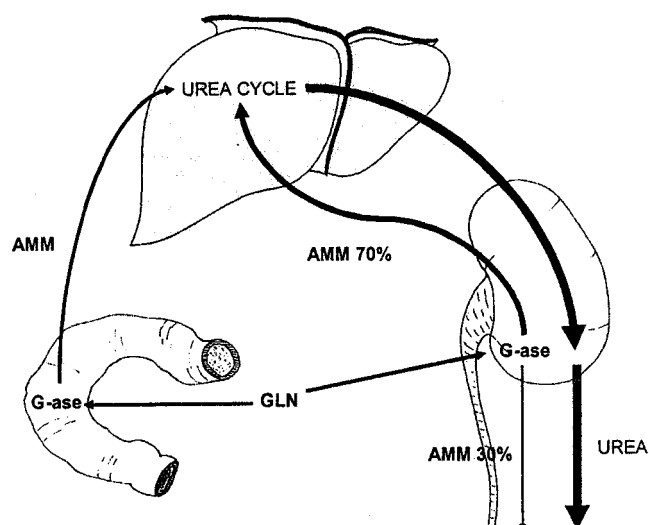
In addition to glutamine's role as a fuel for the gut, Robinson and Robinson (81) suggested that this conditionally essential amino acid plays a role in determining the intrinsic life span of specific proteins. According to those authors, spontaneous deamidation of glutamine would lead to a loss of protein structure and thus would serve as a natural protein clock.

Apart from these roles (32, 33, 37), glutamine plays a crucial role as a nontoxic carrier of nitrogen between organs (32, 80). Thus, in the normal fasted state, the kidney takes up glutamine from blood (3–7, 10, 27, 28, 82–86), although the kidney has been shown to release glutamine under certain circumstances in some species (45, 87, 88). Renal uptake of glutamine in humans ranges between 7 and 10 g/d, an amount that equals 10–15% of whole-body glutamine flux (28, 34, 69, 82). After being taken up, glutamine is metabolized primarily by the intramitochondrial phosphate-dependent enzyme glutaminase (EC 3.5.1.2); only  $\approx 10\%$  is metabolized by membrane-bound  $\gamma$ -glutamyl transferase (EC 2.3.2.2) in the distal proximal tubule (89–94). This process (glutaminase activity) yields ammonia and glutamate (27, 95; **Figure 1**).

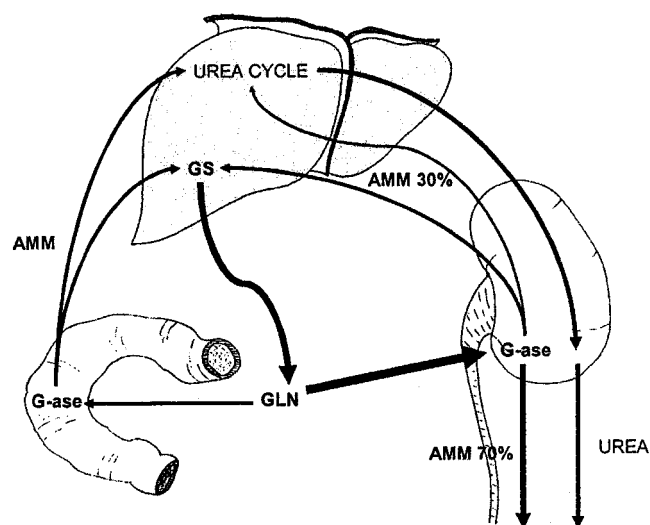
There are essentially 2 major types of glutaminase in the body, and both are located in the mitochondria (45). Hepatic glutaminase is inhibited by low pH, and its activity is dependent on the presence of ammonia (2, 45, 96, 97). In contrast, high ammonia and glutamate concentrations (29, 45) inhibit renal glutaminase. The ammonia generated in the glutaminase reaction can be either excreted in the urine or released back into the renal vein (10, 88, 98–102). In the physiologic situation,  $\approx 70\%$  of all ammonia generated in this reaction is released into the renal vein, and the remainder is excreted in the urine (88, 103–105). Thus, in the normal *in vivo* situation, the kidney is an organ that produces ammonia in the body (100, 106). High ammonia concentrations favor subsequent ammonia excretion in urine (4–7, 89, 107–109).

Glutamate derived from renal glutamine degradation in the glutaminase pathway can have  $\geq 3$  fates (103, 110). Glutamate can be released into the systemic circulation, transaminated to form alanine and  $\alpha$ -ketoglutarate (103), or further degraded in





**FIGURE 1.** Role of the kidney in interorgan glutamine (GLN) exchange under physiologic circumstances. GLN is taken up by the gut and kidney and metabolized by the glutaminase (G-ase) pathway to yield ammonia (AMM). AMM generated by the kidney is excreted in the urine or released into the renal vein. Renal AMM released into the circulation and AMM generated by the gut are metabolized in the liver in periportal hepatocytes to form urea, which is excreted in the urine.



**FIGURE 2.** Role of the kidney in interorgan glutamine (GLN) exchange during acidosis (see also Figure 1). Urea synthesis is diminished during acidosis. Ammonia (AMM) coming from the gut or kidneys and escaping periportal urea synthesis is subsequently scavenged in the perivenous hepatocytes in the glutamine synthetase (GS) pathway to form GLN. GLN is released back into the circulation and subsequently undergoes degradation by glutaminase (G-ase) in the gut and kidney. During acidosis, more AMM is generated in the kidney, and a greater fraction is excreted in the urine.

the glutamate dehydrogenase (EC 1.4.1.2) pathway, yielding  $\alpha$ -ketoglutarate and a second ammonia moiety (102). Then  $\alpha$ -ketoglutarate can be metabolized in the Krebs cycle (102, 111). If glutamate were an end product of renal glutamine metabolism, one would expect glutamate to be released into the renal vein. Observations in normal rats and in humans provided contradictory data on this subject. Houdijk et al (20) observed a slight renal glutamate uptake in rats. We (5, 7) and others (82) found no glutamate uptake or even a release of limited amounts of glutamate ( $\approx 10\%$  of glutamine uptake) into the systemic circulation (6). These findings probably should be interpreted as evidence for further degradation of glutamate to  $\alpha$ -ketoglutarate, because urinary excretion of glutamate represents considerably  $<1\%$  of the amount of glutamine taken up by the kidney (6, 112).

At this point, it is important to consider the changes that take place during acidosis (26, 88, 89, 102, 103, 113–115), because these changes forcibly illustrate the role of the kidney in interorgan metabolite exchange (Figure 2). Acidosis (acute or chronic) induces an increase in the renal uptake and a breakdown of glutamine (83, 104, 105, 108, 116, 117). This increase leads to enhanced ammoniogenesis mediated by an increased activity of the glutaminase enzyme (1). In addition, the normal physiologic ratio of renal venous ammonia release to urinary excretion is reversed. Hence, during acidosis, 70% of all ammonia generated in the kidney is excreted in the urine, and the remainder is released back into the renal vein (102, 103). As a consequence, the kidney becomes an important organ of ammonia disposal in this situation. Conversely, during alkalosis, the kidney reduces ammonia excretion, and thus it reduces the disposal of ammonia and proton (1, 99, 118, 119).

It was shown that the total amount of nitrogen excreted in the urine as urea plus ammonia did not change significantly during acidosis (104, 120). In this situation, urinary ammonia excretion is enhanced (108), whereas urea excretion diminishes

(120), and there is change in the total urea nitrogen excretion (104, 120). The fact that nitrogen disposal remains unchanged is probably attributable to diminished urea synthesis in the liver in this situation (15). Acidosis leads to the consumption and subsequent decreased hepatic availability of bicarbonate, which is a crucial precursor of urea synthesis (2, 96). There may also be a direct influence of pH on the uptake of precursor amino acids for urea synthesis (121). In this context it is important to consider urea synthesis as a bicarbonate-removing and pH-regulating function (2, 96, 121), although the actual significance of that possibility has been a subject of discussion (122, 123).

The consequence of this reduction in urea synthesis during acidosis is that less ammonia is scavenged in hepatic urea synthesis. Hepatic metabolism of glutamine, ammonia, and urea is zoned (53): the urea-synthesizing hepatocytes are located predominantly in the periportal area (53, 124). These hepatocytes also contain glutaminase, which, as already mentioned, is stimulated by ammonia (45, 53, 97, 117). The perivenous or pericentral hepatocytes contain the enzyme glutamine synthetase (EC 6.3.1.2), which synthesizes glutamine from glutamate and ammonia (53). Thus, any ammonia escaping detoxification in the urea cycle will be trapped downstream in the perivenous hepatocytes in the glutamine synthetase reaction (53, 96). The net effect of this "enzymatic zonation" of the liver is that, during acidosis, less urea but more glutamine is exported from the liver (15, 117). In this particular situation, the liver can become a net exporter of glutamine (104, 116, 117, 120), and glutamine in turn will function as the nontoxic carrier of nitrogen to the kidney. Renal breakdown of glutamine will then liberate the ammonia, which can be excreted into the urine (Figures 1 and 2). The abovementioned enzymatic zonation may also help explain the observation that the

intrahepatic pathway of a metabolite determines its metabolism in persons with cirrhosis (125).

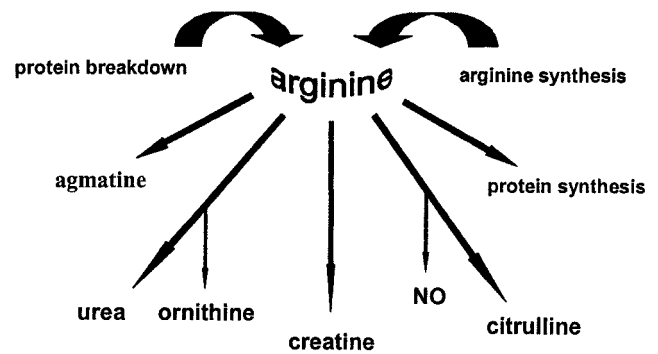
The changes in renal and hepatic glutamine and ammonia handling during acidosis illustrate the complex interorgan interactions that take place between the kidneys and the liver. This underpins the crucial role the kidneys play not only in the excretion of toxic metabolites and the regulation of the acid-base balance (55, 99, 101–103) but also in the provision to other organs of precursors for vital biochemical reactions. This is even more clearly illustrated by the interactions in citrulline and arginine metabolism (*see below*).

In conclusion, the kidney takes up glutamine and metabolizes it to ammonia. This process is sensitive to pH and serves to maintain acid-base homeostasis and to excrete nitrogen. Renal ammonia excretion is complementary to hepatic urea synthesis, and it increases when hepatic capacity of urea synthesis decreases, as during acidosis. Thus, renal glutamine uptake (10 g/d) is quantitatively as important as utilization by the gut or immune system, and it represents 10–15% of daily whole-body glutamine turnover (70 g/d). From a clinical point of view, it is important to point out that administration of glutamine dipeptides as a supplement to parenteral nutrition may abrogate net renal glutamine consumption (17). During the administration to healthy subjects (17, 126) of glycyl-glutamine in quantities slightly greater than those used in patients (11, 12), net renal glutamine uptake of  $\approx 10$  g/d is reversed to glutamine release of 7 g/d (11, 12, 17, 126). This change in renal glutamine handling accounted for 80% of the glycyl-glutamine administered and would be equivalent to 25% of whole-body glutamine turnover, or all the glutamine needed by the gut or immune system. This probably implies that the renal handling of glutamine dipeptides liberates the glutamine from these dipeptides. Subsequently, part of this glutamine is used within the kidney, which reduces or stops the need for glutamine uptake from the blood. The remainder of the glutamine liberated from the dipeptides is released back into the bloodstream.

### ARGININE AND CITRULLINE

Arginine is an essential amino acid for some mammals, such as cats, as well as for growing children (29, 127). In most adult mammals, it is considered to be a semiessential or conditionally indispensable amino acid (128–131). This means that, under normal circumstances, it can be synthesized in sufficient amounts in the body to maintain growth and equilibrium (29, 132, 133). Normal daily intake of arginine is  $\approx 5$ –6 g (30, 134), whereas whole-body arginine flux ranges between 15 and 20 g/d (129, 135, 136).

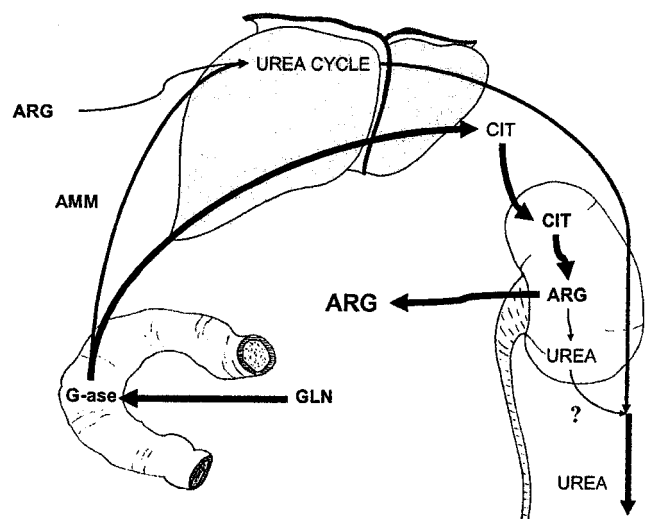
Apart from being an essential component of proteins, arginine plays a key role in several other metabolic pathways (18, 30; **Figure 3**). It is a precursor in the synthesis of the polyamines putrescine, spermine, and spermidine (133, 137). These compounds are among those (reviewed in 138) that are important to the growth and differentiation of intestinal mucosal cells (137). In addition, arginine is a precursor of nitric oxide (NO; 135, 139), a molecule that currently receives considerable attention in view of its widespread effects, especially in the cardiovascular system (140). Arginine is a precursor for urea synthesis in the liver (129) and the kidney (141, 142), and as such it plays an important role as a waste nitrogen carrier in the



**FIGURE 3.** Metabolic pathways involved in arginine metabolism. The metabolic pathways described do not have to be present in one organ, one region of an organ, or one compartment of the cell. The figure merely describes the enzymatic pathways involved in arginine metabolism. NO, nitric oxide. Adapted from references 18, 129, and 137.

urea cycle. Moreover, arginine is a precursor in the renal synthesis of creatine (30, 134, 143), which is an important constituent of skeletal muscle (18). Finally, arginine appears to be converted by the enzyme arginine decarboxylase (EC 4.1.1.19) to agmatine, a metabolite that has been suggested to play a role in cell signaling, proliferation, and the regulation of NO synthesis, through the NO synthase (EC 1.14.13.39) pathway (18). Arginine has been proposed to have immunotrophic effects, it causes the release of pituitary growth hormone and prolactin (30, 137) and glucagon (134), and, of all amino acids, it has the strongest insulinogenic activity (133).

The synthesis of arginine is probably regulated in a more complex way than has been assumed until recently. Arginine is released into the renal vein after being synthesized from citrulline taken up from the bloodstream (23, 143, 144; **Figure 4**). Quantitatively speaking, the human kidneys take up  $\approx 1.5$  g

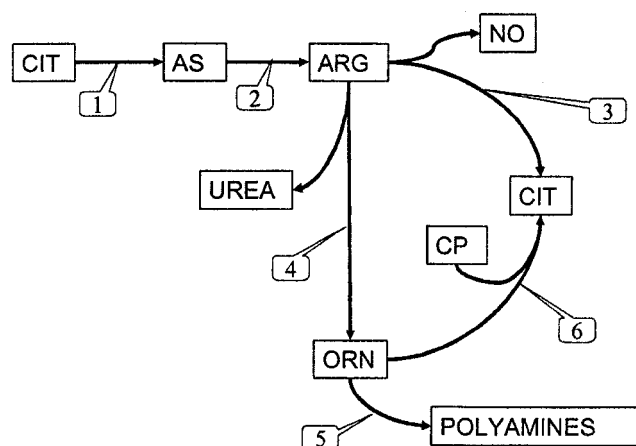


**FIGURE 4.** Role of the kidney in interorgan citrulline (CIT) and arginine (ARG) exchange in the normal situation. Glutamine (GLN) is metabolized in the gut, yielding CIT and ammonia (AMM) as nitrogenous end products. AMM is metabolized to urea, whereas CIT passes through the liver without significant uptake. CIT is then taken up by the kidney and converted to ARG; the latter is released back into the circulation. The question mark in the figure refers to the fact that it is not currently known (and is doubtful) whether urea synthesis in the kidney actually contributes to urea excretion in the urine. G-ase, glutaminase.

citrulline/d from the blood (82). The amount of arginine released back into the bloodstream has been reported to be between 2 g/d (28, 82) and 4 g/d (129) in humans. The uptake of citrulline appears to be regulated by circulating citrulline concentrations (144). Citrulline, in turn, is a nonessential (132), nonprotein amino acid and a nitrogenous product in the small-intestine metabolism of glutamine (59, 60, 127, 135, 145, 146). The liver does not take up citrulline (142), and, hence, any citrulline synthesized by the bowel reaches the systemic circulation (127). Most of the citrulline synthesized by the gut is subsequently taken up by the kidneys (7, 147, 148). The importance of this pathway is illustrated by the fact that arginine becomes a dietary essential amino acid when intestinal citrulline synthesis is inhibited (146), for example, after intestinal resection (130) and in animals with low rates of intestinal citrulline synthesis (eg, cats; 144).

The amount of citrulline synthesized in the fasted state depends on the amount of intestinal tissue present (149). Whole-body citrulline flux has been estimated to be  $\approx 4$  g/d (135, 136). Studies in rats subjected to massive small-bowel resection showed that glutamine uptake by the residual bowel decreases (148, 150) concomitant with decreases in intestinal citrulline release (148). Thus, major loss of small-intestine length leads to diminished intestinal citrulline release, accompanied by decreased arterial citrulline concentrations in rats (130, 148) and humans (149, 151). Actually, the arterial concentration of citrulline was suggested to be an indicator of the likelihood for patients with short bowel syndrome to become independent of total parenteral nutrition (151). Although this correlation between small-intestine length and net citrulline release is well established (148, 149, 151), the consequences to arginine metabolism of the loss of intestinal length are less well known. Thus, we found that intestinal citrulline release in rats with short bowel syndrome diminished in proportion to the amount of small intestine resected (148). This led to decreased renal citrulline uptake and renal arginine release (3). However, it did not affect arterial arginine concentrations as was also observed by others (130), nor did it have any effect on whole-body arginine flux (3). Crenn et al (151) did, however, find decreased arterial arginine concentrations after small bowel resection in humans. Although it is generally believed that the kidney is the major site for de novo arginine synthesis in adult animals (23), the amounts of arginine synthesized are relatively small. Thus, Wu and Morris (18) estimated that endogenous arginine synthesis accounts for 5–15% of total body arginine flux (production), and the remainder is derived from protein catabolism (endogenous flux); this finding is comparable with data from our group (3).

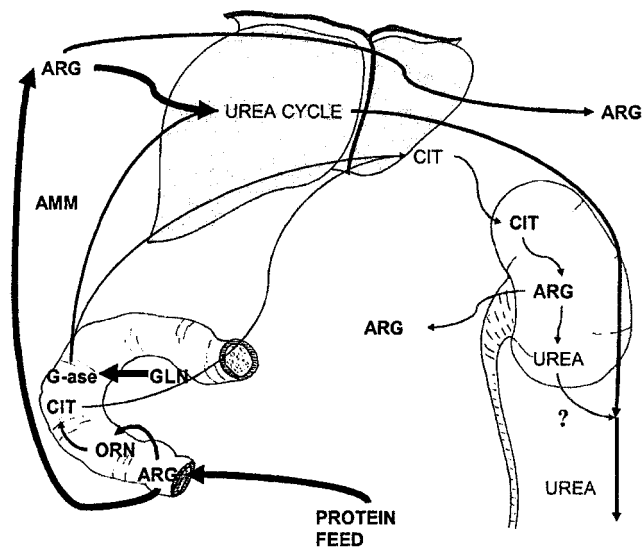
The figures for whole-body arginine appearance represent a measure of total plasma arginine flux and do not take into account the arginine that is formed in the liver. Arginine is synthesized mainly in the liver and kidney through a pathway involving argininosuccinate synthase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) (129; **Figure 5**). However, arginine synthesized in the liver does not reach the systemic circulation because of the high hepatic arginase (EC 3.5.3.1) content. Because the liver does not take up citrulline, it functions as an isolated compartment of arginine metabolism in the body. Thus, although flux through the urea cycle (and hence arginine synthesis) is several times greater ( $350 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) than total plasma arginine flux ( $\approx 75 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ),



**FIGURE 5.** Enzymes involved in arginine (ARG)-citrulline (CIT) metabolism. 1, argininosuccinate (AS) synthase; 2, AS lyase; 3, nitric oxide (NO) synthase; 4, arginase; 5, ornithine (ORN) decarboxylase; 6, ORN carbamoyl transferase. CP, carbamoylphosphate. Adapted from references 18, 129, and 137.

this arginine flux through the urea cycle will not be detected by assessment of whole-body kinetics (18, 131). The metabolic compartmentation of arginine is underlined by the fact that, after liver transplantation for argininosuccinate synthase deficiency, plasma citrulline concentrations remain high and plasma arginine concentrations remain low. Similarly, liver transplantation for ornithine carbamoyl transferase (EC 2.1.3.3) deficiency does not alter the low citrulline and arginine concentrations in plasma (129; **Figure 5**). Thus, the liver does not release significant amounts of arginine, and, in the basal state, only 5–15% of urea is derived from plasma arginine (18, 136).

The regulation of arginine synthesis is even more complex if the effects of dietary intake are taken into account (137). Thus, Cynober et al (129) suggested that prolonged administration of high-protein diets (rich in arginine) leads to an adaptation of the intestinal enzymatic machinery that results in the conversion of less arginine to citrulline in the intestine during the process of absorption. Basically, this adaptation represents a down-regulation of intestinal ornithine carbamoyl transferase (**Figure 5**) and *N*-acetylglutamate synthetase (EC 2.3.1.1; 129, 137). As a result, any arginine administered through the enteral route would be taken up as such and would gain access to the portal vein. In fact,  $\approx 60\%$  of arginine administered through the enteral route normally is absorbed intact and delivered to the portal blood (18, 145). The remainder is metabolized to ornithine (38%), citrulline, proline, carbon dioxide, or urea and released into the portal vein (129). Because arginine, unlike citrulline, is taken up by the liver and metabolized to urea, the effect of its administration through the enteral route would be that, with a high-protein diet, it would be scavenged by the liver (137; **Figure 6**). On the other hand, prolonged administration of low-protein diets (low in arginine content) leads to up-regulation of intestinal ornithine carbamoyl transferase and *N*-acetylglutamate synthetase, which results in the conversion of more arginine to citrulline (129). The net effect is that, after a period of low-protein diets, a greater proportion of arginine administered through the enteral route will be broken down to citrulline in the intestine and will then gain access to the portal



**FIGURE 6.** Role of the kidney in interorgan citrulline (CIT) and arginine (ARG) exchange during prolonged feeding of high-protein, high-ARG diets (see Figure 4). ARG derived from protein meals is either absorbed as such (high previous protein intake) or metabolized to ornithine (ORN) and CIT (low previous dietary protein intake) (see Arginine and Citrulline). CIT in turn passes through the liver without significant uptake, and conversion of CIT to ARG occurs in the kidneys. The question mark refers to the fact that it is currently not known (and is doubtful) whether urea synthesis in the kidney actually contributes to urea excretion in the urine. AMM, ammonia; G-ase, glutaminase; GLN, glutamine. Adapted from references 129 and 137.

vein than is the case after consumption of a diet with normal protein contents. Because citrulline, as pointed out previously, is not taken up by the liver, the effect of such an adaptation would be that, during low-protein feeding in comparison with normal protein feeding, more citrulline would get into the systemic circulation and be converted to arginine in the kidney in a situation of prolonged low protein intake (arginine-sparing effect). Moreover, arginine itself stimulates the synthesis of hepatic *N*-acetylglutamate, an obligatory allosteric activator of carbamoylphosphate synthetase (EC 6.3.4.16) and one of the crucial enzymes in the urea cycle (129, 137). Thus, arginine breakdown in the gut during the process of absorption would keep urea synthesis low during low protein intake (137).

From this, it is clear that there is a crucial role for the interorgan exchange of nitrogenous compounds in the integrated metabolism of citrulline and arginine. An interesting aspect of this interorgan metabolic relation between the gut, the liver, and the kidneys was shown by Houdijk et al (20). They administered glutamine enterally to rats and observed a 30% increase over baseline concentrations of arterial citrulline and arginine, as well as a 40% increase in renal citrulline uptake and arginine release (20). They suggested that, in view of the proposed properties of arginine as a supplement (133, 137, 139, 152–154), the beneficial effects of glutamine administration may be partly explained by increased renal arginine production (20). Thus, these data might suggest that some of the postulated beneficial effects of glutamine supplementation (11, 77–80, 139, 155, 156) are mediated by intestinal conversion of glutamine to citrulline that is followed by renal conversion to arginine (14). In subsequent experiments, Houdijk et al (19) confirmed these results but did not find an increase in plasma

nitrate as a measure of NO production (140, 157), which suggested that NO might not be involved in the possible arginine-mediated beneficial effects of glutamine and which illustrated the complexity of the mechanisms involved (14, 139). It is interesting that evidence has been provided for the synthesis of arginine from glutamine in human macrophages (67).

Finally, concerning the relation between the renal production of arginine and urea synthesis (142), it was shown that arginine is metabolized to urea in the kidney by the enzyme arginase (142, 158–161). This enzyme is heterogeneously distributed along the kidney tubules, and its activity increases toward the renal medulla (141, 142, 159). This zonation or compartmentation of arginine synthesis (mainly in the cortex) (18) and breakdown (mainly in the medulla) (141) allows the kidney to both export arginine to the bloodstream and degrade it to urea (143). The latter process has been proposed to contribute to medullary recycling of urea, which in turn contributes to the counter-current urine concentration system in the kidney (141, 159). Renal arginase activity increases during protein deprivation (159), which was suggested to help maintain urine-concentrating ability (160). On the contrary, the amounts of urea produced in this way are small compared with both glomerular urea flow (2%; 141) and hepatic urea synthesis (143). In addition, this route of urea production was shown to be absent in animals with a greater urine-concentrating capacity than that of the rat (142). For this reason, it was suggested that the actual importance of the arginase pathway in the kidney may lie in the synthesis of ornithine rather than of urea (142). Ornithine would then be metabolized to polyamines by the enzyme ornithine decarboxylase (EC 4.1.1.17), and, in that particular case, ornithine would contribute to the maintenance of the integrity of cells exposed to unusual environmental conditions (142). These points illustrate quite clearly the incorrectness of the statement that urea production takes place only in the liver (10, 162), because the kidneys and the gut also contain several of the enzymes necessary for urea synthesis (129, 131). This implies that extrahepatic synthesis of urea from arginine or citrulline (via arginine) can occur in these organs. In general, it would be more correct to state that a full urea cycle exists only in the liver because this is the only organ that contains considerable activity of carbamoylphosphate synthetase (29, 130).

In conclusion, arginine biosynthesis in the kidney probably accounts for 10–20% of total plasma arginine flux. This relatively small amount of arginine synthesis may not seem important (18), and arginine degradation may be of equal relevance in controlling whole-body arginine homeostasis in humans (131). However, the 2–4 g arginine synthesized by the kidney may provide 35–75% of normal daily arginine intake (5.4 g; 30, 134), which is in keeping with the fact that arginine can be synthesized in the human body but not at a rate commensurate with the requirements for maximum growth (128, 132).

#### DIMETHYLARGININE AND HOMOCYSTEINE

Clinical and fundamental studies have pointed out the importance of the kidney in the metabolism and disposal of the endogenous arginine analogue asymmetrical dimethylarginine (ADMA) and its biological inactive stereoisomer symmetrical

dimethylarginine. ADMA is an endogenous inhibitor of NO synthase (163). Protein-bound arginine is methylated (164, 165) by endothelial cells to form ADMA. Elevated ADMA concentrations are observed during renal failure and have been shown to contribute to cardiovascular mortality in these patients (166). ADMA is converted to arginine and dimethylamine by the enzyme  $N^G, N^G$ -dimethylarginine dimethylaminohydrolase (EC 3.5.3.18) in the kidney (167) or in the liver, as it was shown recently by van Leeuwen's group (168). Although  $N^G, N^G$ -dimethylarginine dimethylaminohydrolase and its newly discovered isoform  $N^G, N^G$ -dimethylarginine dimethylaminohydrolase II are widespread throughout the human body (169), there are currently no data on the contribution of organs other than the kidney and the liver to ADMA metabolism. Approximately 4.5% of ADMA is excreted in the urine (170), and the remainder is metabolized to arginine, arginine-derived amino acids, and by-products. Urinary ADMA excretion is  $>10$  mg/d in humans (163). This would mean that a total of  $\approx 200$ – $240$  mg ADMA/d is metabolized. When data from rat studies (168, 170) are extrapolated to humans, theoretically, it could be calculated that renal ADMA breakdown approximates 100 mg/d, whereas hepatic ADMA breakdown approximates 130 mg/d. Thus the kidney and the liver together could account for  $\geq 95\%$  of total body ADMA breakdown. However, further research is needed to verify the exact contributions of the kidney, the liver, and other organs to whole-body ADMA turnover.

The primary source of methyl groups used in arginine methylation is *S*-adenosylmethionine, an intermediate in the metabolic pathway from methionine to homocysteine (171–174). In addition, homocysteine inhibits  $N^G, N^G$ -dimethylarginine dimethylaminohydrolase activity (175), and a close correlation is found between plasma homocysteine and ADMA concentrations (176). On the basis of these observations, it was proposed that ADMA is the mediator of the atherogenic effects of homocysteine (175, 176). Although homocysteine concentrations are known to be increased in chronic renal insufficiency (177), the observation that the rat kidney takes up and metabolizes homocysteine *in vitro* (178) and *in vivo* (179) could not be confirmed in humans (180, 181). This nonconfirmability could indicate that hyperhomocysteinemia during renal insufficiency is only indirectly mediated by the kidney—for example, through the inhibitory effects of uremic toxins on homocysteine-degrading enzymes (182). Although impaired renal metabolism can offer a satisfactory explanation for increased ADMA concentrations during chronic renal insufficiency, it cannot do the same for the increased homocysteine concentrations that are observed during chronic renal insufficiency. Moreover, the possible inhibitory effects of homocysteine on ADMA degradation blur the direct relation between renal function and ADMA metabolism in chronic renal insufficiency.

#### PHENYLALANINE AND TYROSINE

It has been assumed for a long time that the enzymatic conversion of phenylalanine to tyrosine by phenylalanine 4-hydroxylase (EC 1.14.16.1; 28) is an exclusive function of the liver (183). Although we (3, 7) and others (27, 28, 82) repeatedly found the uptake of phenylalanine (3, 7, 27) and the release of tyrosine (7, 28, 82) in the kidneys of rats (3, 7) and

humans (7, 27, 28, 82), the idea that substantial phenylalanine 4-hydroxylase activity occurs in the kidney as well as the liver never gained general acceptance. However, data from Lichter-Konecki et al (184) clearly showed phenylalanine 4-hydroxylase activity in the kidney. The substantial role of the kidney in whole-body phenylalanine hydroxylation in humans was also elucidated with the use of stable isotope techniques to assess the renal conversion of phenylalanine to tyrosine *in vivo* (185, 186).

Renal phenylalanine hydroxylation accounts for  $\approx 50\%$  of whole-body phenylalanine hydroxylation (185, 186). Measurements of whole-body phenylalanine flux (ie, turnover) with the use of stable isotopes indicate that it is  $\approx 10$  g/d (185, 187). Tyrosine flux is  $\approx 7$  g/d (185, 187). Because the human kidneys take up 0.5–1 g phenylalanine/d from the circulation and release  $\approx 1$  g tyrosine/d (28, 82, 185), it follows that the kidneys account for  $>15\%$  of whole-body tyrosine flux. It is interesting to mention that the minimum and recommended daily requirements for phenylalanine are 1.1 and 2.2 g, respectively (128). The classic experiments of Rose et al (132) showed that dietary tyrosine could compensate for one-half of the minimum phenylalanine requirements. This probably means that one-half of the minimal phenylalanine required (0.5 g) is normally converted to tyrosine. Hence, the kidneys alone would be capable of producing all the tyrosine needed by the body. Moreover, because splanchnic tyrosine extraction exceeds splanchnic tyrosine release by 2-fold (185, 186), the kidney is the major source for circulating tyrosine. The importance of renal phenylalanine hydroxylation is underlined by the fact that chronic renal failure leads to an impairment of whole-body phenylalanine hydroxylation (188–191). The consequent hypotyrosinemia is only partly compensated for by a decreased splanchnic tyrosine uptake (192), and thus low arterial tyrosine concentrations are consistently found in these patients (28, 189–193). Although the clinical effectiveness of tyrosine supplementation in this context needs further investigation (194, 195), it has been suggested that insufficient phenylalanine hydroxylation and resulting tyrosine deficiency contribute to net protein catabolism and muscle wasting in persons with chronic renal failure (196, 197) and that tyrosine therefore should be considered as a dietary essential amino acid under these conditions (188, 196, 197).

#### GLYCINE AND SERINE

The normal rat and dog kidney takes up glycine and releases serine (3, 5, 7, 23, 198, 199). Similar observations were made in humans (23, 28, 82, 108), and this was interpreted as evidence for conversion in the kidney of glycine to serine, which explains why serine is not essential in human nutrition (200). The conversion of 2 glycine molecules to 1 serine molecule yields 1 bicarbonate and 1 ammonia fraction, and this process normally contributes  $\approx 10\%$  to renal ammonia production (23). This conversion is mediated by a pathway involving glycine cleavage enzyme and serine hydroxymethyltransferase (EC 2.1.2.1; 28) in the proximal tubule (23, 198). This pathway probably also uses glycine in the kidney supplied from sources other than uptake from the bloodstream, which explains why the uptake of glycine and serine are not stoichiometric (3, 5, 7, 28, 199). A second pathway is the phosphorylated intermediate pathway involving the conversion of gluconeogenic precursors,



such as glutamine, glutamate, and aspartate, to phosphoserine and subsequently to serine (23). This last pathway offers an alternative explanation of why glycine uptake is only 30% of serine release in most studies.

Alternatively, some of the glycine might be derived from tubular breakdown of glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; 27) by  $\gamma$ -glutamyltranspeptidase (EC 2.3.2.2; 201) and its subsequent reabsorption (27, 28, 201). The kidney extracts 80% of glutathione from the plasma in a single pass (17). Subsequently, glutathione is hydrolyzed in the brush border of the proximal tubule (17). Similar mechanisms are probably also important in the metabolism and subsequent release of several other dipeptides (27, 180).

Whole-body glycine turnover in humans is  $\approx 35$  g/d (202). Only  $\approx 1.5$  g glycine/d is taken up by the human kidneys (28), and that step is followed by the release of  $\approx 4$  g serine/d (23, 82). From a quantitative point of view, it is important to realize that this amount equals the average daily dietary intake (23). Yet renal synthesis of serine accounts for only 5–7% of total body turnover of serine (199), because both serine and glycine are nonessential amino acids with a very high turnover rate.

### BRANCHED-CHAIN AMINO ACIDS

The role of the kidney in the metabolism of the 3 branched-chain amino acids—leucine, valine, and isoleucine—remains incompletely understood. We observed the release of branched-chain amino acids from the kidney in overnight-fasted normal rats (5) and rats who have chronic portocaval shunting (7). This finding is compatible with data reviewed by Silbernagl (27). Tizianello et al, however, found no significant uptake or release of valine and leucine in overnight-fasted humans (28) but did find a slight uptake of isoleucine in similar subjects (82). In response to a subsequent oral amino acid load, they found a consistent uptake of the branched-chain amino acids by the kidney (82). Apparently, there is an influence of species difference as well as of food intake. In this context, it is interesting to mention that Abumrad et al (203) found evidence that the dog kidney converts  $\alpha$ -ketoisocaproate, the keto-analogue of the essential amino acid leucine, back to leucine. This observation is in keeping with the knowledge that most essential amino acids, but not threonine and lysine, can be replaced by their  $\alpha$ -keto-analogue (134). Evidently, this area requires further research.

### REMAINING AMINO ACIDS

In various studies in our laboratory, we failed to observe a consistent pattern of renal exchange for most of the remaining amino acids: alanine, threonine, histidine, tryptophan, asparagine, aspartate, lysine, methionine, proline, ornithine, and cysteine (3–7, 180, 204). For example, a slight alanine release was observed in fasted rats (5) and humans (28, 82), whereas alanine flux did not differ significantly from zero in normal fasted pigs. During feeding, the kidney changed to the function of alanine uptake in pigs (204), whereas the initial alanine release during fasting in humans ceased on feeding (82). Differences between species may be related to the fact that some animals, such as pigs (72, 204–206) and sheep (26, 207), have relatively low arterial glutamine concentrations and high arterial alanine concentrations. This fact is consistent with the

observations of Pitts and Stone (208) on the effects of acidosis and circulating alanine concentrations on renal alanine metabolism.

From the scarce available data, it appears that, in fasted humans, the kidneys probably take up proline and release cysteine (82, 180), threonine (82), and perhaps lysine, aspartate, and ornithine (27, 28). For most of the other amino acids, either no data are available in humans, or the exchange across the kidney does not differ significantly from zero (28, 82).

### URINARY AMINO ACID EXCRETION AND RENAL FAILURE

Under physiologic circumstances, only minimal amounts of amino acids are excreted into human urine. In most mammals,  $\approx 99\%$  of filtered amino acids are reabsorbed in the proximal tubule (209), which spares  $\approx 70$  g amino acids/d in a 70-kg person (23). Fractional excretions of most amino acids are between 0.2% and 2.5% (27, 112, 210), although this proportion may increase in various pathologic conditions (211). Because urinary amino acid excretion is quantitatively negligible compared with amino acid flux across the kidney, it seems reasonable to disregard urinary excretion in studies of renal amino acid flux. A rare cause of profound renal amino acid loss, however, is Fanconi syndrome (212), a disorder of the proximal tubules that causes impaired reabsorption of filtrated molecules, including glucose, small proteins, and amino acids. The etiology of the syndrome is associated with tyrosinemia, Wilson disease, Lowe syndrome, and, most predominantly, cystinosis (213). Cystinosis is characterized by a defective cystine transport that causes the lysosomal accumulation of cystine (214, 215). The disorder generally becomes clinically manifest within 6 mo after birth and presents with failure to thrive, polyuria, or polydipsia or all 3 conditions (213). Despite generalized aminoaciduria with a 10-fold increase in urinary amino acid concentrations (216), patients with cystinosis have normal plasma amino acid concentrations (212). End-stage renal failure, which develops at a mean age of 9 y (214), determines the prognosis and often necessitates kidney transplantation at a young age.

The data available from the literature would suggest that chronic renal failure in humans does not significantly alter urinary amino acid excretion (28). However, chronic renal insufficiency in humans (23) and rats (142) induces alterations in whole-body and renal amino acid metabolism. Renal insufficiency leads to diminished renal citrulline uptake (28). In addition, the amount of citrulline converted to arginine in the kidney is reduced (131). The observation that whole-body citrulline turnover is increased during chronic renal insufficiency (217) indicates that the increased arterial citrulline concentrations observed under these conditions (27, 129, 137) are not exclusively caused by diminished renal uptake. It has been shown that whole-body phenylalanine hydroxylation is attenuated during chronic renal insufficiency (188, 189, 191), and organ flux measurements suggest that this attenuation is caused by decreased renal phenylalanine hydroxylation (28). Also during chronic renal insufficiency, the uptake of glutamine and the release of serine and tyrosine decrease by 60–80% (23, 28), the uptake of glycine stops (28), and ammonia production and secretion in the urine are greatly reduced (28, 109). The importance of the kidney in this context is also illustrated by the




fact that the half-life of dipeptides such as alanyl-glutamine, glutamine-glutamine, and glycine-tryptophan is increased in patients with chronic renal failure (17).

### SUMMARY, CONCLUSIONS, AND IMPLICATIONS

In summarizing these data, we are led to conclude that the role the kidneys play in the interorgan metabolism of amino acids is of both qualitative and quantitative importance. Glutamine metabolism is subject to an intense and coordinated regulation on an interorgan basis between the gut, the liver, and the kidney. On a per-gram basis, renal glutamine uptake equals intestinal consumption. There is also a prominent interorgan axis between the gut and the kidney with regard to citrulline-to-arginine conversion. Quantitatively, the kidney synthesizes  $\approx 2\text{--}4$  g arginine/d, which is only slightly less than the normal average daily dietary arginine intake. In addition, the kidneys seem to play an important quantitative and qualitative role in the conversions of phenylalanine to tyrosine and of glycine to serine.

Furthermore, certain oligopeptides (eg. glutathione and glutamine-containing dipeptides administered as nutritional supplements) are converted by the kidney to their constituent components before they can be used in metabolic processes. This step seems to be of major quantitative importance in the conversion of parenteral nutrition containing supplemented glutamine dipeptides to the constituent amino acids. Consequently, the kidneys may function as crucial "mediators" of the beneficial effects of glutamine dipeptide administration. Likewise, evidence suggests that some beneficial effects of supplemental glutamine administered to humans (11, 12, 77) are actually mediated by prior intestinal metabolism of glutamine to citrulline, which is followed by renal conversion of citrulline to arginine.

On the basis of the scarce literature, it is difficult to judge how chronic renal failure affects interorgan amino acid metabolism. Interpretation of the data on renal citrulline-to-arginine conversion is hampered by the fact that many of these patients are undergoing hemodialysis. This fact, of course, influences the plasma amino acid profile, as do the dietary measures that are generally imposed on this patient group. In view of the reduced metabolism of dipeptides, it may be wise to reduce the dose of glutamine dipeptides in patients with renal failure. Further research is needed to improve our understanding of the role of the kidneys in interorgan nitrogen exchange during chronic renal failure.

Finally, recent experimental animal data and human data from our study (218) suggest that important changes occur in the handling of amino acid and ammonia by the kidney after a meal. From these studies and from previous work by Tizianello et al (82), it appears that renal ammonia production increases considerably after a meal, which partly explains the occurrence of hyperammonemia after protein administration in patients with liver cirrhosis (218). Future research in this area should focus on the role of the kidney in nitrogen exchange under various pathophysiologic conditions. 

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