Interorgan Amino Acid Transport and its Regulation[1,2]

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ABSTRACT Interorgan amino acid transport is a highly active and regulated process that provides amino acids to all tissues of the body, both for protein synthesis and to enable amino acids to be used for specific metabolic functions. It is also an important component of plasma amino acid homeostasis. Net movement of amino acids depends on the physiological and nutritional state. For example, in the fed state the dominant flux is from the intestine to the other tissues. In starvation the dominant flux is from muscle to the liver and kidney. A number of general principles underlie many amino acid fluxes: *i*) The body does not have a store for amino acids. This means that dietary amino acids, in excess of those required for protein synthesis, are rapidly catabolized; *ii*) Amino acid catabolism must occur in a manner that does not elevate blood ammonia. Thus, extrasplanchnic amino acid metabolism often involves an innocuous means of transporting nitrogen to the liver; *iii*) Because most amino acids are glucogenic, there will be a considerable flux of amino acids to the gluconeogenic organs when there is a need to produce glucose. In addition to these bulk flows, fluxes of many specific amino acids underlie specific organ function. These include intestinal oxidation of enteral amino acids, the intestinal/renal axis for arginine production, the brain uptake of neurotransmitter precursors and renal glutamine metabolism. There is no single means of regulating amino acid fluxes; rather, such varied mechanisms as substrate supply, enzyme activity, transporter activity and competitive inhibition of transport are all found. J. Nutr. 133: 2068S–2072S, 2003.

KEY WORDS: • *arteriovenous differences* • *intestinal metabolism* • *perivenous and periportal hepatocytes* • *glutamine* • *arginine*

Interorgan amino acid transport is a highly active and regulated process that provides amino acids both for protein synthesis and to enable amino acids to be used for specific functions. The principal vehicles for this transport are the free amino acids themselves. The total concentration of the free amino acids in plasma is ~2.5 mM, with glutamine being the most abundant. However, distribution via proteins and peptides should also be considered. Peptides have been proposed to be an important vehicle for interorgan amino acid traffic. Certainly, some peptides that arise physiologically (such as the C-peptide of proinsulin or peptide products of angiotensinogen proteolysis) are very rapidly catabolized, most likely in the kidney. Some peptides also arise extracellularly during collagen turnover. Nevertheless, the idea that plasma contains a large pool of peptides that play a major role in interorgan traffic (1) is controversial and has not been well supported experimentally. There certainly is some significant contribution of plasma proteins to interorgan amino acid traffic. For example, the liver of a healthy adult human produces and secretes some 20 g/d of albumin and a similar quantity is catabolized in peripheral tissues, principally by fibroblasts (2). Thus, ~20 g of amino acids are made available each day, to peripheral tissues, as a result of albumin catabolism.

Amino acid uptake or output across an organ is determined by measuring their arteriovenous difference, i.e., the concentration of amino acids in the blood (or plasma) in the vessel entering the organ and in the efferent vessel. The difference between the arterial and venous concentrations (the A-V[4] difference) is a semiquantitative measure of amino acid metabolism across a given organ. When multiplied by the rate of blood (or plasma) flow across the organ, a quantitative measure is obtained. However, it is important to realize that the A-V difference is often a rather imprecise measurement as it is usually a small difference between two large numbers, each with an appreciable measurement error. For example, if the concentration of an amino acid in arterial plasma is 100 μM and in venous plasma is 90 μM, the true A-V difference is 10 μM. However if the coefficient of variation for the measurement of this amino acid is 3%, then the measured A-V difference may range from ~4 μM to ~16 μM. The imprecision inherent in such a measurement has an important consequence. We can only measure, with confidence, the major interorgan fluxes; many small, though physiologically important, fluxes certainly escape detection.

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[4] Abbreviation used: A-V, arteriovenous

A number of general principles underlie many amino acid fluxes:

i) The body does not have a store of amino acids in the same way that triacylaglycerol is a store of fatty acids. The body's proteins are synthesized for their functional properties, not by a need to store extra amino acids. This means that dietary amino acids, in excess of those required for protein synthesis, are rapidly catabolized.
ii) Amino acid catabolism must occur in a manner that does not elevate blood ammonia. Much amino acid catabolism occurs in the liver, which contains the urea cycle, and in the intestine, which can immediately provide its nitrogenous products to the liver via the hepatic portal vein. Amino acid metabolism in extrasplanchnic tissues often involves a means of transporting nitrogen to the liver in a manner that does not increase the blood ammonia concentration.
iii) Because most amino acids are glucogenic, there will be a considerable flux of amino acids to the gluconeogenic organs (liver and kidney) when there is a need for gluconeogenesis (e.g., starvation, sepsis, major trauma).

It should also be appreciated that one of the functions of interorgan amino acid traffic is homeostatic, the maintenance of the relatively constant extracellular amino acid concentrations in which tissues are bathed. We know much too little about this aspect of amino acid metabolism, but it is a key component of cell nutrition. It may be anticipated that genetic knockouts, in which enzymes of amino acid metabolism or amino acid transporters are ablated in a tissue-specific manner, will provide much new information. Perfusion studies by Schimassek and Gerok (3) suggest that the liver may play a major role in determining the plasma concentrations of amino acids, except for the branched-chain amino acids.

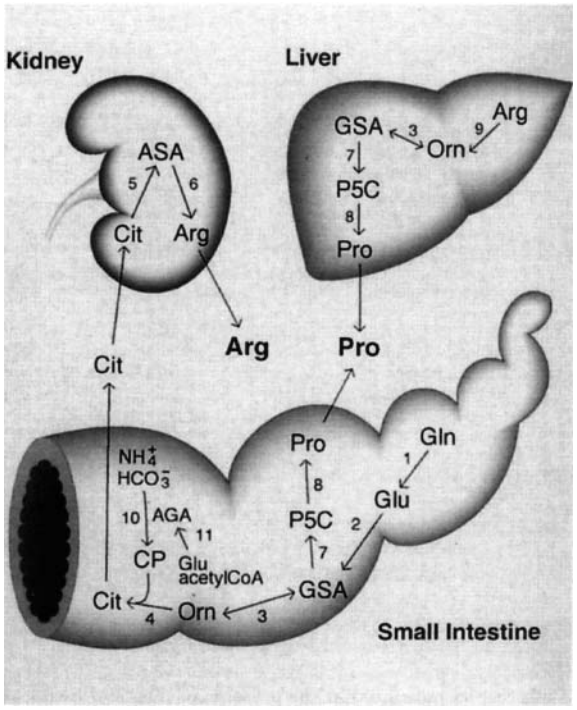

FIGURE 1 Primary pathways of arginine and proline synthesis. The numbered enzymes are: 1, glutaminase; 2, pyrroline-5-carboxylate synthase; 3, ornithine aminotransferase; 4, ornithine transcarbamoylase; 5, argininosuccinate synthetase; 6, argininosuccinate lyase; 7, spontaneous reaction; 8, pyrroline-5-carboxylate reductase; 9, arginase; 10, carbamoyl-phosphate synthetase; 11, N-acetylglutamate synthetase. Reproduced from reference (5), with permission from the Annual Review of Biochemistry, volume 44, © 1975 by Annual Reviews. www.annualreviews.org.

Amino acid fluxes across specific organs

The past decade has seen remarkable advances in our knowledge of intestinal amino acid fluxes, particularly in neonatal animals. It has been known for years that glutamine is a major fuel for the intestine and that nitrogenous products derived from glutamine metabolism are released into the portal vein. These include alanine and proline, which are metabolized by the liver, as well as citrulline. The citrulline is taken up by the kidney (**Fig. 1**) and converted to arginine (4,5). An interesting species difference in citrulline synthesis is evident in strict carnivores, such as cats, who have lost the ability to synthesize citrulline in their intestines, due to low activities of ornithine aminotransferase and of pyrroline-5-carboxylate synthase (6). As a consequence, these animals have an absolute requirement for dietary arginine. Indeed, new work with neonatal animals has shown that many dietary amino acids are extensively metabolized in the intestine. Dietary glutamate is virtually quantitatively metabolized in the intestine. In addition, as much as one-third of the dietary supply of a number of amino acids, including essential amino acids such as threonine, leucine, lysine and phenylalanine are metabolized within intestinal cells (7). It is also apparent that there are important developmental differences between adult and neonatal animals with regard to intestinal amino acid fluxes. This is best exemplified by citrulline and arginine. The intestinal citrulline release observed in adult animals does not occur in their neonates. In neonatal piglets, the entire pathway for the de novo synthesis of arginine is present in enterocytes and arginine, not citrulline, is released into the portal blood (8). It will be of the greatest interest to know whether this is also true of other species, including humans.

The liver is the major organ of amino acid disposal. It is the only organ with the enzymatic armamentarium to metabolize all of the amino acids, although its capacity to metabolize the branched chain amino acids is limited. It is the only organ with a urea cycle. It is hardly surprising, therefore, that much of the dietary complement of amino acids is metabolized within the liver. However, it seems that the carbon skeletons of these amino acids are not completely oxidized in the liver, even in the fed state, but are largely converted to glucose (9). Oxidation of these amino acids within the liver would produce much more ATP than the liver could actually use. During starvation, hepatic gluconeogenesis plays a crucial role in providing glucose for the brain and other obligatory glucose-using organs. Amino acids, arising primarily from muscle proteolysis are the principal substrates for this process. There is a remarkable concordance between the pattern of amino acids released from human skeletal muscle and those taken up by the splanchnic organs, during starvation (**Fig. 2**).

Hepatic glutamine metabolism provides us with a striking example of the limitations of the simple A-V difference technique in revealing important aspects of amino acid fluxes. The liver contains both glutaminase and glutamine synthetase. They are found in different parts of the hepatic acinus: glutaminase is located in the periportal hepatocytes and glutamine synthetase is restricted to the perivenous hepatocytes (**Fig. 3**). Both glutaminase and glutamine synthetase may be simultaneously active. This means that the mass balance of glutamine across the liver only reveals the net result of these two processes.

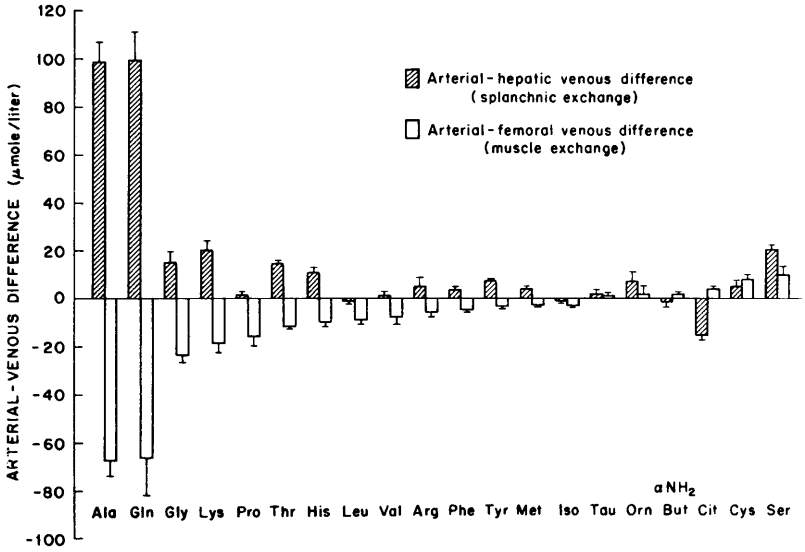

FIGURE 2 Net splanchnic and peripheral exchange of amino acids in normal postabsorptive humans. Reproduced from reference (10), with permission from Annual Reviews of Biochemistry, volume 44 © 1975 by Annual Reviews. www.annualreviews.org.

Indeed it is possible that there may be a net mass balance of zero but that there may be considerable flux through both of these enzymes (11). The combination of mass balance and isotopic methodology is required to quantify these two components of hepatic glutamine flux.

The kidney plays a major role in the interorgan metabolism of citrulline, arginine, glycine, serine and glutamine (4). As mentioned previously, circulating citrulline is converted to arginine, which is released. The enzymes that accomplish this, argininosuccinate synthetase and argininosuccinate lyase, are found in the cells of the proximal tubule (4). The kidney takes up glycine and releases serine. Rather more serine is released then can be accounted for by glycine uptake so that serine synthesis from glycolytic intermediates must also be invoked (4). Renal glutamine uptake is critical for acid-base homeostasis as it is the precursor for urinary ammonia production. The excretion of ammonia in the urine facilitates the elimination, from the body, of strong metabolic acids, such as sulphuric acid that arises during the catabolism of methionine and cysteine or the ketoacids that arise in uncontrolled type-1 diabetes mellitus. Renal glutamine extraction displays very considerable physiological variation. Quite small uptakes in normal individuals can change to massive uptakes during severe metabolic acidosis (12).

Because the bulk of the body's protein is in the form of muscle protein, it is apparent that this tissue will play an important role in interorgan amino acid metabolism. Certainly, there is an uptake of plasma amino acids for protein synthesis when protein is being acreted and a release when the muscle is catabolic. However, muscle's role is more complex than this. Skeletal muscle is a major organ for the catabolism of the branched-chain amino acids. Much of these are released as branched-chain keto acids in the rat, but it is apparent that, in humans, there is a substantial metabolism of these within muscle (9). During starvation, when there is release of amino acids from muscle, it is evident that amino acids are not released in the proportions in which they are found in muscle protein; rather, glutamine and alanine account for ~50% of the amino acids released (Fig. 2). The alanine arises via the glucose-alanine cycle where the carbon skeleton is derived from pyruvate and the amino group from the branched-chain amino acids, via transamination. The glutamine arises from the intramuscle metabolism of glutamate, aspartate, asparagine, valine and isoleucine (13). There is also a remarkable depletion of glutamine (the most abundant free amino acid in human skeletal muscle) in catabolic illnesses (14).

The brain does not play an important quantitative role in the interorgan flux of amino acids. However, the uptake of

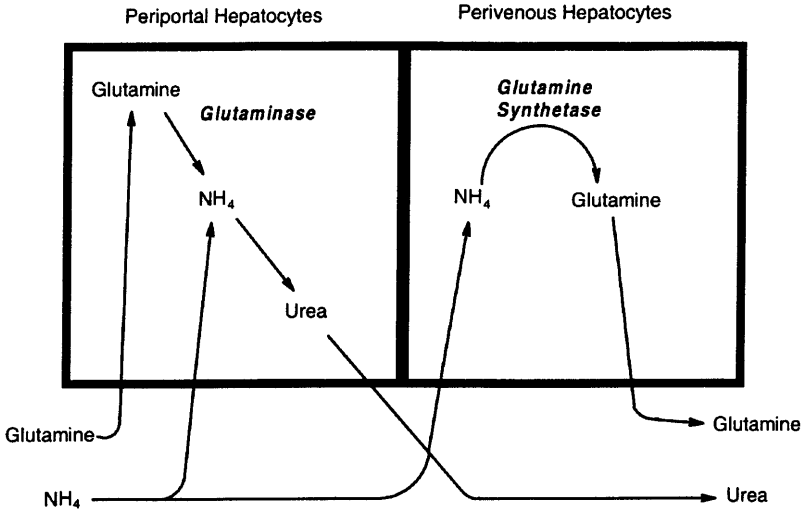

FIGURE 3 Hepatic zonation of glutamine metabolism. Both glutaminase, in periportal hepatocytes, and glutamine synthetase in perivenous hepatocytes may be simultaneously active.

small quantities of neurotransmitter precursors, such as tyrosine and tryptophane is of paramount importance. There is also evidence that human adipose tissue takes up glutamate and releases glutamine and alanine in quantities sufficient to make a significant contribution to the whole body economy of these amino acids (15).

Regulation of interorgan amino acid transport

There appears to be no single mechanism that predominates in the regulation of interorgan amino acid transport. Rather, a variety of different mechanisms, (such as substrate supply, concentration of competitive inhibitors, transporter activity, enzyme activity and hormonal action) play regulatory roles.

It is clear the renal arginine synthesis is determined by citrulline supply. This has been demonstrated directly in rats where the kidney promptly responds to citrulline infusion by increasing the uptake of this amino acid and increasing renal arginine production (16). This is also of importance in short-bowel syndrome. In such situations, decreased intestinal production of citrulline has been demonstrated in rats (5) as well as decreased circulating citrulline concentrations in humans (17).

The brain uptake of tyrosine and tryptophane affords an interesting example of physiological competitive inhibition. The blood-brain barrier contains a transporter responsible for the uptake of the large neutral amino acids (principally tyrosine, tryptophane, leucine, isoleucine, valine and methionine) into the brain. The affinity of this transporter for these amino acids is close to their plasma concentrations so that they compete with each other for uptake (18). For example, there is clear evidence that the elevated concentrations of branched chain amino acids seen in diabetes inhibits tyrosine uptake by the brain (19).

Enzyme activity (whether via expression or activation) is a common determinant of amino acid fluxes. This is best exemplified by the developmental switch in the pattern of intestinal amino acid production. The switch from arginine production in neonatal pigs to citrulline production in older piglets is brought about by the loss of argininosuccinate synthase and argininosuccinate lyase from intestinal cells (8). Another example is provided by renal glutamine metabolism. The increased renal glutamine metabolism that occurs in metabolic acidosis is brought about by increased glutaminase activation and expression (11), whereas the delivery of glutamine to renal cells, by filtration, remains relatively constant.

Finally, transporter function may also determine amino acid fluxes. The loss of massive amounts of glutamine from skeletal muscle during catabolic illness has been attributed to altered activity of the amino acid transport system N (20). It is also evident that increased hepatic amino acid transport facilitates the increased removal of amino acids in animals fed high protein diets (21).

Many of these control mechanisms in amino acid fluxes are, themselves, regulated by hormones that play profound roles, both physiologically and in pathological conditions. Both insulin and glucagon can bring about hypoaminoacidemia, though for different reasons. Insulin inhibits the flux of amino acids from muscle to plasma by inhibiting proteolysis (22) and activating cellular uptake of amino acids. It is now apparent that insulin rapidly promotes the recruitment of system A transporters to the plasma membrane from an endosomal pool (23). Glucagon activates system A in the liver (24) as well as conversion of amino acids to glucose (9). Glucorticoids stimulate proteolysis in muscle and, hence, amino acid release from this tissue (25). In pathological situations, the proinflammatory cytokines (interleukin-1 and tumor necrosis factor-α) increase the hepatic uptake of amino acids; this effect is synergistic with that of glucagon (26).

Impact of increased intake of specific amino acids on interorgan fluxes

There have been very few studies of the effects of increased intakes of amino acids on interorgan fluxes. Much of the information is indirect so that only inferences can be drawn from it. There is good evidence on the effects of citrulline ingestion from studies on patients with lysinuric protein intolerance. Such patients have a defect in the absorption of cationic amino acids such as lysine and arginine. There is also, apparently, a similar defect in renal reabsorption so that considerable quantities of these amino acids are found in the urine. The loss of arginine is accompanied by hyperammonemia. Such patients have been treated with 2.5–8.5 g/d of citrulline for many years. This is well tolerated. Citrulline administration increased plasma arginine levels and normalized orotic acid excretion. Protein intolerence lessened such that patients increased their protein intake (27) The inference to be made from this work is that exogenous citrulline may be converted, in substantial amounts, to arginine, either in the kidney or in other cells that contain both argininosuccinate synthase and lyase.

Increased ingestion of methionine or cysteine will result in increased production of sulphuric acid which must be excreted. This necessitates increased production of urinary ammonia. It may be inferred, that the increased ammonium excretion involves increased utilization of glutamine by the kidney. Lemann and Relman (28) gave 13 g DL-methionine daily, for 5 d, to human volunteers. This resulted in a metabolic acidosis, and a large increase in urinary net acid excretion and in ammonium excretion.

In summary, interorgan amino acid transport is a major metabolic process by which amino acids are distributed to the different tissues so as to accomplish their physiological functions. Some large-scale fluxes involve a number of different amino acids, as in the uptake of many dietary amino acids by tissues after a meal and the uptake of a mixture of amino acids, for gluconeogenesis, during starvation. There are also fluxes involving very specific uptake of individual amino acids, as in the renal uptake of glutamine for acid-base balance and the brain uptake of tryptophane for neurotransmitter synthesis. Interorgan amino acid metabolism is a regulated process, which uses a variety of control mechanisms. This includes regulation at the level of amino acid transport, enzyme activity, gene expression, competitive inhibition and hormone action. The relative constancy of the plasma levels of most amino acids, in the face of varied amino acid fluxes, suggests that homeostatic mechanisms may be at play (29). Interorgan amino acid metabolism would, certainly, play a major role in amino acid homeostasis.

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