

# Glutamate and Glutamine in Metabolism

## Glutamate, at the Interface between Amino Acid and Carbohydrate Metabolism<sup>1,2</sup>

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**ABSTRACT** The liver is the major site of gluconeogenesis, the major organ of amino acid catabolism and the only organ with a complete urea cycle. These metabolic capabilities are related, and these relationships are best exemplified by an examination of the disposal of the daily protein load. Adults, ingesting a typical Western diet, will consume ~100 g protein/d; the great bulk of this is metabolized by the liver. Although textbooks suggest that these amino acids are oxidized in the liver, total oxidation cannot occur within the confines of hepatic oxygen uptake and ATP homeostasis. Rather, most amino acids are oxidized only partially in the liver, with the bulk of their carbon skeleton being converted to glucose. The nitrogen is converted to urea and, to a lesser extent, to glutamine. The integration of the urea cycle with gluconeogenesis ensures that the bulk of the reducing power (NADH) required in the cytosol for gluconeogenesis can be provided by ancillary reactions of the urea cycle. Glutamate is at the center of these metabolic events for three reasons. First, through the well-described transdeamination system involving aminotransferases and glutamate dehydrogenase, glutamate plays a key catalytic role in the removal of  $\alpha$ -amino nitrogen from amino acids. Second, the "glutamate family" of amino acids (arginine, ornithine, proline, histidine and glutamine) require the conversion of these amino acids to glutamate for their metabolic disposal. Third, glutamate serves as substrate for the synthesis of *N*-acetylglutamate, an essential allosteric activator of carbamyl phosphate synthetase I, a key regulatory enzyme in the urea cycle. *J. Nutr.* 130: 988S–990S, 2000.

**KEY WORDS:** • *gluconeogenesis* • *urea synthesis* • *liver metabolism* • *dietary protein* • *glutamate* • *glutamine*

In considering relationships between glutamate and carbohydrate metabolism, this paper will focus exclusively on gluconeogenesis. However, for physiologic relevance, it is important not to consider glutamate alone, but glutamate in the context of the metabolism of all of the amino acids. Second, it is important not to consider gluconeogenesis alone, but other pathways with which it is integrated, in particular, the urea cycle. Therefore, this paper examines the metabolic disposal of the dietary protein load and considers the specific role of glutamate in this process under the following three headings: 1) the key role of glutamate and of glutamate dehydrogenase in transdeamination of amino acids; 2) the metabolism of the glutamate family of amino acids; and 3) the synthesis of *N*-acetylglutamate. The remarkable metabolic versatility of glutamate will also be discussed.

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### Metabolic disposal of dietary protein

An active, healthy adult male, eating a typical Western diet, will ingest ~100 g of protein per day. Assuming that the amino acid composition of this protein is similar to that in meats and that digestion is highly efficient, the body will be faced with disposing ~1000 mmol of amino acids per day. Such an adult will be in nitrogen balance so that the rates of protein synthesis and of protein degradation will be equal, over 24 h. Thus the 1000 mmol of amino acids must be oxidized. The liver is the major organ of amino acid metabolism; it is frequently stated that the liver is responsible for the oxidation of dietary amino acids. This statement is incorrect for two reasons. First, it ignores the fact that extrahepatic tissues (in particular, the intestine, muscle and kidney) are quantitatively important in the disposal of some specific amino acids. Often, however, amino acid metabolism in extrahepatic tissues produces other amino acids (e.g., renal glycine metabolism produces serine; intestinal glutamine metabolism produces alanine) that must be metabolized by the liver. Thus, the liver is faced with metabolizing ~900 mmol of amino acids per day. The second reason why it is incorrect to state that the liver is responsible for the oxidation of amino acids relates to the term "oxidation." The liver does, indeed, metabolize ~900 mmol of amino acids per day, but it does not oxidize them completely. Indeed, it cannot oxidize them within the confines of its oxygen consumption and energy requirements. These matters have been considered extensively by Jungas et al. (1992).

An obvious, but key fact about the oxidation of amino acids is that it will consume oxygen and produce ATP. The oxidation of the 900 mmol of amino acids by the liver would consume  $\sim 3.8$  mol  $O_2/d$  and would produce  $\sim 22$  mol ATP (Jungas et al. 1992). We now appreciate that the actual ATP yield will be somewhat lower than this theoretical value because of normal proton leakage across the mitochondrial membrane, either through the lipid bilayer or through uncoupling proteins (Rolfe and Brown 1997). However, the  $O_2$  consumption required for the complete oxidation of these 900 mmol of amino acids cannot change, because it is determined by the stoichiometry of the oxidation reactions. The liver consumes  $\sim 50$  mL  $O_2/min$  or  $\sim 3$  mol  $O_2/d$  (Hagenfeldt et al. 1980). Thus, even if no other substrate were consumed by the liver (i.e., no dietary fat or carbohydrate or alcohol were oxidized), it would not be possible for the liver to oxidize 900 mmol of amino acids per day to  $CO_2$  and water. Because it is certain that many other substrates are oxidized by the liver, the only viable conclusion is that liver amino acid metabolism, even in the prandial state, involves their conversion to glucose and ketones (Jungas et al. 1992). Even the conversion of these amino acids to glucose and urea is an oxygen-consuming process ( $\sim 1.4$  mol of  $O_2/d$ ). Gluconeogenesis is often referred to as an ATP-consuming process; this is true of substrates such as lactate when all three carbons of the precursor molecule are converted to glucose. But gluconeogenesis from a physiologic mixture of amino acids involves the necessary oxidation of some of the carbons to  $CO_2$  (with the consumption of oxygen and production of ATP) as well as the partial reduction of some of the carbons to glucose and the synthesis of urea. The net ATP balance is close to zero because the ATP produced by oxidative reactions is balanced by the ATP used in urea synthesis and in the gluconeogenic pathway. There is also close redox balance between the two processes so that the bulk of the NADH required in the cytosol for gluconeogenesis is provided by reactions ancillary to the urea cycle. Of course, glycogen, rather than glucose, may be one of the immediate products of this gluconeogenesis. It is now clear that fasting gluconeogenesis in humans continues for some time after a meal, with glycogen as the product (Shulman and Landau 1992). It is also known that, in experimental animals, synthesis of glycogen via the "indirect pathway" after a fast is increased if the meal is rich in protein (Rosetti et al. 1989).

### Key roles of glutamate in the metabolism of dietary protein

Glutamate is at the center of the disposal of the daily protein load for three reasons. First, there is the "glutamate family" of amino acids. These amino acids (glutamate, glutamine, proline, histidine, arginine and ornithine) comprise  $\sim 25\%$  of the dietary amino acid intake and will be disposed of via conversion to glutamate. Second, there is the key role of glutamate dehydrogenase together with the glutamate-linked aminotransferases in effecting the removal of  $\alpha$ -amino nitrogen from almost all of the amino acids via transdeamination. Third, there is the key role of glutamate in *N*-acetylglutamate synthesis, which ensures that the rate of urea synthesis is in accord with rates of amino acid deamination.

### The "glutamate family" of amino acids

These amino acids (glutamate, glutamine, proline, histidine, arginine and ornithine) have always been considered as a group because their metabolism converges on that of glutamate itself. However, it is now quite apparent that rather significant differences exist between them with respect to the

tissue and cellular location of their metabolic disposal. Dietary glutamate is metabolized to a great extent in the gastrointestinal tract. The more recent data are particularly persuasive in that they show quite clearly, in fed infant pigs, that almost no enteral [ $^{13}C_5$ ] glutamate appeared in portal venous blood (Reeds et al. 1996). Possible products of gastrointestinal metabolism are alanine, arginine, proline and glutathione, but the quantitation of these products remains to be clarified. The situation with glutamine is more complex. Stumvoll et al. (1998) argued that the kidney, rather than the liver, is the primary organ of glutamine metabolism (especially, gluconeogenesis from glutamine) in fasting humans. We have shown that the kidneys of rats fed a high protein diet extract substantial quantities of glutamine and have increased activities of renal glutaminase (Brosnan et al. 1978, Brosnan 1987). It is evident that this renal glutamine metabolism is related to the production of urinary ammonia, which is used to facilitate the excretion of metabolic acids (principally, sulfuric acid) that arise from the catabolism of the sulfur-containing amino acids, methionine and cysteine. Thus, administration of sodium bicarbonate, sufficient to neutralize these acids greatly reduces the renal uptake and metabolism of glutamine. Of course, the intestine is also a major consumer of glutamine (Windmueller and Spaeth 1980). Proline and histidine catabolism have not been studied extensively, but it is clear that they occur, primarily in the liver (Jungas et al. 1992).

Considerable new information has appeared recently on the location of arginine catabolism. Some arginine catabolism occurs in the intestine (Windmueller and Spaeth 1976), but liver is the principal site. We now know that hepatic arginine catabolism is confined to the perivenous hepatocytes. This conclusion is founded on two pieces of evidence, i.e., the demonstration by Kuo et al. (1991) that hepatic ornithine aminotransferase is restricted to the same small population of hepatocytes proximal to the terminal hepatic vein in which glutamine synthetase is found, and our own demonstration that arginine catabolism occurs in the isolated, nonrecirculating, retrogradely perfused liver (O'Sullivan et al. 1998). By this means, the liver maintains spatial separation of the two major pathways of hepatic arginine metabolism because the urea cycle is restricted to the periportal hepatocytes.

### The role of glutamate in transamination and deamination

The crucial role of glutamate/ $\alpha$ -ketoglutarate as transamination partners is well known. We now know that all of the common amino acids, except for lysine, may be transaminated. Similarly, the key role of glutamate dehydrogenase, in conjunction with the transaminases, in the transdeamination of amino acids is well established. These enzymes are freely reversible so that they also afford a mechanism for the synthesis of the nonessential amino acids.

### *N*-Acetylglutamate synthesis

The rate of urea synthesis must be regulated sensitively with respect to the rate of amino acid deamination. Simple regulation by substrate concentration is too crude a mechanism for this key process. For example, a threefold increase in urea synthesis would require (at least) a threefold increase in ammonia concentration, assuming Michaelis-Menten kinetics. Homeostasis would be served very poorly by a situation in which one would require a threefold increase in the concentration of toxic ammonia to effect a threefold increase in its disposal. The regulation of the urea cycle is brought about, chronically, by an adjustment in the amount of urea cycle

enzymes as dietary protein varies (Schimke and Doyle 1970) and, acutely, at the level of carbamoylphosphate synthetase-I. As an obligatory activator, this enzyme requires *N*-acetylglutamate, which is synthesized within liver mitochondria from glutamate and acetyl-CoA. The concentration of *N*-acetylglutamate can change quite rapidly to facilitate increased flux through the urea cycle (Meijer et al. 1990). One way in which *N*-acetylglutamate levels are regulated is through arginine, which is known to activate *N*-acetylglutamate synthetase. We must also consider that *N*-acetylglutamate synthesis may be regulated via increased provision of glutamate as a result of activation of glutaminase. Hepatic glutaminase has the remarkable property of being activated by its own product, ammonia (Curthoys and Watford 1995). Feedback activation is bizarre, unstable and unsustainable. Perhaps the best everyday example of feedback activation is an explosion in which the detonation of a small quantity of an explosive provides sufficient heat to detonate the rest of the material. What could possibly be the function of such a metabolic control system? The properties of glutaminase, in isolation, do not provide an answer to this conundrum; an answer may possibly arise if one considers that an important function of hepatic glutaminase is to provide intramitochondrial glutamate for *N*-acetylglutamate synthesis. Thus, when the hepatic ammonia concentration tends to increase, it would activate glutaminase which, by providing glutamate to *N*-acetylglutamate synthetase, increases intramitochondrial concentrations of *N*-acetylglutamate; these in turn, by activating carbamoylphosphate synthetase I, will actually effect the removal of ammonia.

### *The metabolic versatility of glutamate*

In almost all cells, the intracellular concentration of glutamate is maintained at quite high concentrations compared with its concentration in extracellular fluids. Typically, intracellular concentrations of 2–5 mmol/L are common, compared with extracellular concentrations of ~0.05 mmol/L. Our own data show that glutamate is one of the most abundant amino acids in liver, kidney, skeletal muscle and brain (Brosnan et al. 1983). Such high concentrations point to the important roles glutamate plays in all tissues. In addition to its role as a key transamination partner, glutamate is also required for the synthesis of glutathione, a key component in our defenses against oxidative stresses. Glutamate is also involved in the

glutamate/aspartate shuttle, which effects the oxidation of cytoplasmically produced NADH in many cells. Finally, glutamate, by virtue of being readily convertible to  $\alpha$ -ketoglutarate by means of a variety of reversible transaminases, can serve an anaplerotic function for the Krebs' cycle. No other amino acid displays such remarkable metabolic versatility.

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