Glutamine Metabolism and Function in Relation to Proline Synthesis and the Safety of Glutamine and Proline Supplementation

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Abstract

At normal intakes, dietary glutamine and glutamate are metabolized by the small intestine and essentially all glutamine within the body is synthesized de novo through the action of glutamine synthetase. The major sites of net glutamine synthesis are skeletal muscle, lung, and adipose tissue and, under some conditions, the liver. In addition to the small intestine, where glutamine is the major respiratory fuel, other sites of net glutamine utilization include the cells of the immune system, the kidneys, and the liver. The intestine expresses pyrroline 5-carboxylate (P5C) synthase, which means that proline is an end product of intestinal glutamine catabolism. Proline can also be synthesized from ornithine and the exact contribution of the 2 pathways is not certain. Infusion of proline i.v. to increase circulating concentrations is associated with increased proline oxidation and decreased proline synthesis. In contrast, conditions of proline insufficiency, after feeding low-proline diets or in response to high rates of proline catabolism in burn patients, do not result in increased proline synthesis. Glutamine supplementation is widespread and up to 0.57–0.75 g/kg/d is well tolerated. Similarly, the only study of proline supplementation, in which patients with gyrate atrophy were given 488 mg/kg/d, reported no deleterious side effects. In the absence of controlled trials, it is currently not possible to estimate a safe upper limit for either of these 2 amino acids. J. Nutr. 138: 2003S–2007S, 2008.

Introduction

Glutamine, the amide of glutamic acid, is the most abundant free α-amino acid in the body with a body pool of ~80g, >95% of which is held within skeletal muscle cells. Similarly, glutamine represents some 20% of the free α-amino acids in plasma and the plasma pool is turning over very rapidly, in the order of 80 g/d in a healthy individual (1–3). Glutamine plays an important role as a substrate for a number of amidotransferases that are responsible for the synthesis of purines, pyrimidines, NAD, glucosamine, and asparagine (Fig. 1) (4). The bulk of glutamine metabolism in the body, however, involves hydrolysis to glutamate and ammonia via the action of glutaminase (Fig. 2). The glutamate can then be further metabolized to glutathione, proline, ornithine, and arginine or undergo catabolism to yield either CO₂ or glucose (via hepatic and renal gluconeogenesis), with the nitrogen being excreted either as urea or ammonia (4).

Interorgan flux of glutamine

Although glutamine and glutamate comprise some 10–20% of dietary protein, both of these amino acids undergo extensive metabolism in the enterocytes of the small intestine. Thus, at normal levels of intake, there is no net absorption of glutamine or glutamate and therefore the extensive body glutamine pool is synthesized de novo (5). The only enzyme capable of glutamine synthesis in the mammalian body is glutamine synthetase (Fig. 2; EC 6.3.1.2), which is expressed in most tissues (6). The major site of net glutamine synthesis and release is skeletal muscle, although both adipose tissue and the lungs have been reported to show net glutamine release (Fig. 3) (3,4). The liver has both the capacity for glutamine synthetase and glutamine utilization with the enzymes compartmentalized in different cell populations (7). In healthy postabsorptive conditions, the liver probably shows a small net glutamine production, but this can change with physiological and pathological conditions and the liver serves to fine-tune plasma glutamine homeostasis. Under healthy conditions, the major site of glutamine utilization is the absorptive columnar epithelium of the small intestine where glutamine serves as the major respiratory fuel of enterocytes (8). The end products of intestinal glutamine metabolism are CO₂, NH₃, alanine, lactate, citrulline, and proline.

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In the absorptive state, dietary glutamate and glutamine are the major sources of fuel for the enterocytes, but once dietary supplies have been exhausted, these cells rely on circulating glutamine. Other sites of net glutamine utilization in the body include the kidney, where glutamine is the major substrate for renal ammoniagenesis involved in acid-base balance, with the carbon skeleton being recovered through renal gluconeogenesis, and the cells of the immune system where glutamine is the major respiratory fuel. Although quantitatively these patterns can change with the pathological and physiological state, with the exception of the liver, the direction of net production or utilization is not changed. In hypercatabolic states, there is a large increase in glutamine utilization by the immune cells and the kidney together with a net uptake by the liver for acute phase protein synthesis and increased gluconeogenesis. This is accompanied by increased net glutamine synthesis in skeletal muscle, probably arising from increased muscle proteolysis, together with a decrease in the utilization of glutamine by the small intestine. Similarly, the presence of a tumor often results in an increased demand on the body glutamine pool, because many tumors exhibit very high rates of glutamine utilization, using it as their primary respiratory fuel. Two other conditions that result in extensive repartitioning of glutamine flux are pregnancy and lactation. During pregnancy, the developing fetus utilizes large amounts of glutamine derived from both the maternal circulation and local synthesis within the placenta. Similarly, during lactation, the mammary gland removes glutamine that, to a certain degree, is exported directly into the milk. Both of these conditions are accompanied by increased food intake and intestinal growth and thus intestinal glutamine utilization also increases. The substrates for such increased glutamine turnover are presumably dietary amino acids, although there is some evidence of increased muscle protein breakdown in some species at peak lactation.

Glutamine as a signaling molecule
In addition to the above roles of glutamine as an important transporter of carbon, nitrogen, and energy between tissues, it has recently been recognized that glutamine can play an important role as a signaling molecule. For example, glutamine can downregulate the level of glutamine synthetase protein in a variety of cell types in culture. This occurs with no change in the abundance of the glutamine synthetase mRNA and is due to glutamine increasing the rate of glutamine synthetase protein degradation. In C2C12 muscle cells, we have shown that this effect occurs at physiological concentrations of glutamine and can be mimicked by the nonmetabolizable glutamine analogue, diazonorleucine, indicating a direct effect of glutamine. Glutamine is also required at a number of steps in the growth, differentiation, and metabolism of 3T3L1 adipocytes, but the exact mechanisms and biological importance are not known. Exogenous glutamine has long been known to be required for most mammalian cells in culture and this has usually been explained by the biosynthetic role of glutamine or that glutamine is being used as a fuel. Rhoads et al. showed that the glutamine stimulation of intestinal epithelial cell-6 proliferation could not be explained by such mechanisms but involved stimulation of mitogen-activated kinase and activation of the extracellular signal-regulated kinase and c-jun N-terminal kinase pathways. Similarly, many of the other signaling roles of glutamine involve different mechanisms. For example, the glutamine suppression of apoptosis in intestinal cells involves pyrimidine synthesis, whereas the maintenance of intestinal tight junctions requires glutathione production. In Caco2 cells, glutamine stimulates expression of argininosuccinate synthetase by the glucosamine-driven O-glycosylation of the transcription factor Sp1. Conversely, glutamine suppresses expression of GADD153 in human breast cells and this involves a change in the
stability of mRNA (19). In immunocytes (12), glutamine is known to inhibit apoptosis, increase respiratory burst and nitric oxide synthesis, and enhance cell death repair, with the latter involving upregulation of heat shock protein expression, again via a glucosamine-dependent mechanism. Glutamine has also been reported to increase insulin secretion in pancreatic β cells, enhance collagen and extracellular matrix formation in fibroblasts, and activate myosin heavy chain in myocytes (12). As is apparent from this brief outline of the signaling effects of glutamine, the phenomenon exists in a variety of cell types, acts on a wide variety of pathways, and involves a number of different intracellular signaling pathways. Some of these effects require glutamine metabolism to a specific metabolite, but others are apparently due to a direct action of glutamine.

Glutamine and proline synthesis
In the first work, to our knowledge, to include a comprehensive description of intestinal glutamine metabolism, Windmüller and Spaeth (8) reported that ~7% of glutamine carbon metabolized within the rat small intestine was used for proline synthesis. Numerous studies have confirmed net proline synthesis by the intestine in rats and other species, including humans (20), and this is due to the presence of the key enzymes required, particularly pyrroline 5-carboxylate (P5C) synthase (EC number not assigned) (Fig. 4). Within the enterocyte, glutamine is degraded to glutamate, which then undergoes transformation to glutamate-γ-semialdehyde via P5C synthase. Glutamate-γ-semialdehyde spontaneously yields P5C that is then reduced to proline. Proline is also formed in the body from arginine and ornithine through the action of ornithine aminotransferase (EC 2.6.1.13) to glutamate-γ-semialdehyde and then via PSC reductase (EC 1.5.1.2) to proline (Fig. 4). The expression of PSC synthase is restricted to the intestine, however, and therefore this organ is the only site in the body where glutamine (glutamate) is a precursor of proline synthesis. It is generally accepted that the majority of proline synthesis in the body occurs via the glutamate/P5C synthase pathway and, hence, in the intestine (21). Such conclusions are based on work in either rats and piglets or from studies of cells in vitro, and because the ornithine aminotransferase pathway is present in many cells in the body, the relative contribution of the 2 pathways to total proline synthesis is not truly known. Proline is degraded by proline oxidase (EC number not assigned) to P5C and thus to glutamate-γ-semialdehyde, which can yield ornithine via ornithine aminotransferase or glutamate by PSC dehydrogenase (EC 1.5.99.8).

Regulation of proline synthesis
Proline has been shown to be essential for piglets and chickens (21,22), but it is not traditionally considered essential for humans. However, the original studies of Womack and Rose (23) did indicate a potential benefit (as assessed by nitrogen balance) for dietary proline under conditions where arginine was limiting in the diet. Furthermore, proline makes up 25% of collagen and therefore is in demand during times of growth and wound repair. It is currently not known how much body proline is derived from the diet, or from which substrates or in which tissue endogenous synthesis occurs.

Ball et al. (21,22,24) used the piglet model to determine the importance of intestinal proline synthesis and found that intestinal lumen glutamate was the preferred substrate over arterially delivered glutamate. Similarly, they reported that the conversion of ornithine to proline in the piglet was dependent on the gut (21). They also found that feeding proline-deficient diets resulted in decreased plasma proline concentrations, indicating that the piglet cannot increase proline synthesis enough to maintain plasma levels (22). Similar conclusions were drawn from the work with labeled glutamate where the authors calculated that proline synthesis from glutamate in the intestine could account for ~40% of the proline accumulated in the piglet carcass (24).

Because both PSC reductase and PSC synthase are subject to inhibition by proline in cells in vitro, and PSC synthase is also inhibited by ornithine, a simple feedback inhibition mechanism can be proposed for proline synthesis (25,26). High levels of proline would inhibit both pathways, whereas high ornithine would simply stop the flow of glutamate into the PSC pool and allow for ornithine to provide for proline synthesis. This hypothesis was tested in vivo by Young et al. (27), who found that i.v. proline infusion (20–40 mg·kg⁻¹·d⁻¹) increased both the concentration of circulating proline and the rate of proline oxidation and these were accompanied by decreased proline synthesis (Fig. 5). In contrast, when they fed a proline-deficient diet for either 7 d (28) or 4 wk (29), or a proline-deficient diet

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<th>[plasma proline]</th>
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**FIGURE 5** Plasma proline metabolism in human subjects. Arrows indicate the direction of change (white arrows decreased, black arrows increased) observed in plasma proline concentration, rate of proline synthesis, and rate of proline oxidation as assessed using [1-¹³C] or [5,5-³H] proline in healthy subjects and patients with severe burns. The 2- to 6-h infusion represents infusions of 20–40 mg·kg⁻¹·h⁻¹, the deficient diets were lacking proline, or lacking proline and the precursors (asparagine, aspartate, glutamate, and serine), and were fed for 1 or 4 wk. Proline synthesis did not differ from controls in burn patients, but rates of synthesis tended to decrease and hence are marked (?). Adapted from (27,28,29,30).
that was also deficient in proline precursors (arginine, aspartate, glutamate, and serine) for 4 wk, plasma proline concentrations and proline oxidation rates decreased, but this was accompanied by decreased, not increased, proline synthesis rates. Furthermore, plasma proline concentrations were decreased in burn patients (30), but although this was accompanied by increased rates of proline oxidation, there was no evidence of increased proline synthesis; indeed, the results tended to indicate a decrease in proline synthesis. Thus, although increased circulating proline concentrations do result in lower rates of de novo proline synthesis, decreased circulating proline concentrations are also associated with decreased proline synthesis rates. These results, together with the evidence of proline requirements in piglets (quo vide), could mean that endogenous proline synthesis is insufficient to maintain proline reserves in the face of long-term dietary proline deficiency. Such studies of plasma proline kinetics do not allow identification of the site of proline synthesis or of the substrates used. Given the wide distribution of the ornithine pathway of proline synthesis and the importance of proline in tissue growth and repair, it is possible that a considerable amount of proline is synthesized and utilized locally within a tissue and thus never exchanges directly with the peripheral circulation.

**Glutamine and proline supplementation**

Although neither glutamine nor proline are traditionally considered as essential in the human diet, they are required in increased amounts in some pathological conditions and thus are usually classified as conditionally essential. Glutamine supplementation, both enteral and parenteral, has been investigated in a number of settings, including clinical work and sports nutrition. In addition, glutamine supplementation is widely used by the general public and glutamine is readily available in many forms ranging from pure glutamine powder to glutamine-enriched drinks and energy bars. Although it is difficult to estimate how much glutamine is consumed by those buying such products, both clinical trials and other controlled studies have shown no deleterious effects with delivery of up to 0.57–0.75 g·kg⁻¹·d⁻¹ (40–55 g glutamine person⁻¹·d⁻¹) regardless of the mode of delivery (2,31–37). In fact, a common effect is that patients say that they feel healthier and report an improvement in mood (37). The efficacy and benefits of such supplementation are discussed by Roth (38), Wernerman (39), and Gleeson (40) in this supplement.

Proline, however, has received very little attention, with no published studies to our knowledge where the focus was proline supplementation directly. Proline supplements (up to 488 mg·kg⁻¹·d⁻¹) have been used to treat patients with gyrate atrophy (due to lack of ornithine aminotransferase) with no reports of any deleterious side effects (36,41). One point worth considering is that many patients with inborn errors of proline metabolism have extremely high plasma proline concentrations that do not appear to be detrimental (42).

Thus, currently it is not possible to set a safe upper limit for glutamine supplementation, because extremely high intakes are clearly well tolerated. The situation is more limited for proline, because without any data, it is impossible to even attempt to make any claims about the safety of proline supplementation at any level. Clearly, as proposed by Garlick in 2004 (36), there remains a need for extensive, well-controlled studies directed at the safety of supplementation with both of these amino acids before any definitive recommendations may be made.

Other articles in this supplement include references (43–50).

**Acknowledgment**

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**Literature Cited**


23. Womack M, Rose WC. The role of proline, hydroxyproline and serine (37) for 4 wk, plasma proline concentrations and proline oxidation rates decreased, but this was accompanied by decreased, not increased, proline synthesis rates. Furthermore, plasma proline concentrations were decreased in burn patients (30), but although this was accompanied by increased rates of proline oxidation, there was no evidence of increased proline synthesis; indeed, the results tended to indicate a decrease in proline synthesis. Thus, although increased circulating proline concentrations do result in lower rates of de novo proline synthesis, decreased circulating proline concentrations are also associated with decreased proline synthesis rates. These results, together with the evidence of proline requirements in piglets (quo vide), could mean that endogenous proline synthesis is insufficient to maintain proline reserves in the face of long-term dietary proline deficiency. Such studies of plasma proline kinetics do not allow identification of the site of proline synthesis or of the substrates used. Given the wide distribution of the ornithine pathway of proline synthesis and the importance of proline in tissue growth and repair, it is possible that a considerable amount of proline is synthesized and utilized locally within a tissue and thus never exchanges directly with the peripheral circulation.

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24. Murphy JM, Murch SJ, Ball RO. Proline is synthesized from glutamate during intragastric infusion but not during intravenous infusion in neonatal piglets. J Nutr. 1996;126:878–86.