Comparative Aspects of Tissue Glutamine and Proline Metabolism$^{1,2}$

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Abstract

The cellular metabolism of glutamine and proline are closely interrelated, because they can be interconverted with glutamate and ornithine via the mitochondrial pathway involving pyrroline-5-carboxylate (P5C). In adults, glutamine and proline are converted via P5C to citrulline in the gut, then citrulline is converted to arginine in the kidney. In neonates, arginine is a semiindispensable amino acid and is synthesized from proline completely in the gut; because of low P5C synthase activity, glutamine is not an important precursor for neonatal arginine synthesis. Thus, splanchnic metabolism of glutamine and proline is important, because both amino acids serve as key precursors for arginine synthesis with some developmental differences. Studies investigating splanchnic extraction demonstrate that about two-thirds of dietary glutamine and almost all dietary glutamate are extracted on first pass and the vast majority is oxidized in the gut. This capacity to extract glutamine and glutamate appears to be very large, so diets high in glutamine or glutamate probably have little impact on circulating concentrations and consequent potential toxicity. In contrast, it appears that very little proline is extracted by the gut and liver, at least in the neonate, which may result in hyperprolinemia and potential toxicity. Therefore, the upper limits of safe dietary intake for glutamine and proline, and other amino acids, appear to be substantially different depending on the extent of first-pass splanchnic extraction and irreversible catabolism. J. Nutr. 138: 2032S-2039S, 2008.

Introduction

Glutamine and proline metabolism are interconnected via glutamate and pyrroline-5-carboxylate (P5C), which is a nexus between the tricarboxylic acid and urea cycles. Because of these pathways, both amino acids can serve as important dietary precursors for arginine and urea synthesis. To understand the roles of these amino acids in ammonia disposal, interorgan metabolism needs to be understood, which can partly be explained by enzyme localization in various tissues. The key organs regulating these metabolic conversions are the small intestine, liver, and kidneys. Muscle is probably quantitatively the most important site of glutamine synthesis in the body, with additional net contributions by brain, adipose, heart, and lung (1,2). This release of glutamine from muscle is part of the glutamine shuttle, which removes ammonia from muscle for disposal by the gut and kidney. So glutamine is involved in 2 key mechanisms to rid the body of excess ammonia from amino acid catabolism: by shuttling ammonia to the gut and kidney for excretion and as a precursor to arginine and urea synthesis.

It is instructive to evaluate the range of whole body flux rates of glutamine, proline, and related amino acids in adult humans. We have summarized reported data on whole body flux rates in humans and pigs measured using i.v. constant infusions of labeled amino acids (Table 1). Notably in adults, glutamine has a flux rate several-fold higher than glutamate, which is in turn higher than proline. It is also important to note that under anabolic or catabolic conditions, such as in rapidly growing neonates or catabolic burn patients, respective fluxes increase as expected. However, it is important to understand that these flux rates are measured using i.v. infusions of isotope with blood sampled as the central pool. Under these methodological conditions, only label that exchanges with the central plasma pool would be measured and interpreted as whole body flux. But it has been well established that this approximation of whole body flux severely underestimates the sum of intraorgan flux rates in the body, because not all metabolites readily exchange with the plasma pool. For example, it has been estimated that the hepatic arginine flux within the urea cycle is ~239 μmol·kg⁻¹·h⁻¹ (31),

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$^3$Abbreviations used: ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; CPS-I, carbamoyl phosphate synthetase; OAT, ornithine aminotransferase; OTC, ornithine transcarbamylase; P5C, pyrroline-5-carboxylate; P5CDH, pyrroline-5-carboxylate dehydrogenase; PDV, portal-drained viscera.

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amino acids. The primary site for the whole body arginine synthesis (2). Glutamine is used primarily for energy production but is also metabolized by enterocytes for conversion to other amino acids such as proline, arginine, ornithine, and citrulline and by crypt cells for DNA and protein syntheses. It has been demonstrated in pigs and humans that glutamine is the primary fuel for small intestinal cells, but the overall preference is for the gut (32-35). Glutamine is used primarily as a dietary precursor for whole body metabolism (i.e., the gut and liver) and the disproportionately high metabolism by these tissues cannot be ignored in measurements of whole body flux rates.

There is also a substantial degree of tissue-specific and interorgan exchange involved in the metabolism of glutamine, glutamate, proline, and arginine. The key organs involved include the small intestine, liver, and kidney. In the small intestine, glutamine and proline can be converted to PSC as enterocyte for conversion to other amino acids such as proline, arginine, ornithine, and citrulline and by crypt cells for DNA and protein syntheses. It has been demonstrated in pigs and humans that glutamine is the primary fuel for small intestinal cells, but the overall preference is for enteral, as opposed to arterial supply of glutamine (12,36). Indeed, clinical studies have shown that parenteral administration of glutamine-dipeptides is more effective than enterally administered glutamine-dipeptides in reducing morbidity of intensive care unit patients (37). In fact, the original work by Windmueller and Spaeth (35) suggested that enteral glutamine is probably more important than glutamine as a dietary fuel for intestinal tissues and this has been confirmed more recently (3,12,13). Both of these amino acids can also be converted to proline and urea cycle amino acids in the small intestine. Proline can also be converted to urea cycle amino acids and, to a more limited extent, to glutamate and glutamine in the gut. In the liver, glutamine is delivered to perportal cells where most of it is catabolized due to an abundance of glutaminase, whereas there is net glutamine synthesis via glutamine synthase in perivenous cells; the net balance across the whole organ is usually 0 (38-40). In addition, the liver is the primary site for urea synthesis due to very high arginase activity and is also a site for proline catabolism. Finally, the kidneys are involved in ammonia disposal via the glutamine nitrogen shuttle from muscle as well as via its role as the primary site of whole body arginine synthesis (2). Glutamine is also one of the most important gluconeogenic amino acids in the kidney representing 50-70% of gluconeogenesis from all amino acids.

### TABLE 1 Intravenous flux rates in healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th>Infants</th>
<th>Young pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>234-393</td>
<td>423-781</td>
<td>334</td>
</tr>
<tr>
<td>Glutamate</td>
<td>83-131</td>
<td>1287-1900</td>
<td>1190</td>
</tr>
<tr>
<td>Proline</td>
<td>67-128</td>
<td>1250</td>
<td>106-292</td>
</tr>
<tr>
<td>Arginine</td>
<td>56-88</td>
<td>1250</td>
<td>106-292</td>
</tr>
</tbody>
</table>

1. Data from references (6-30).
2. Data from references (5-19).
3. Data from reference (14).
4. Data from reference (12).
5. Data from reference (15).
6. Data from reference (11).
7. Data from references (16-21).
8. Data from references (22-25).
9. Data from references (20,21).
10. Data from reference (28).
11. Data from references (23,29,30).

Whereas the whole body flux is estimated at only 56-86 μmol·kg⁻¹·h⁻¹ (Table 1). Therefore, these i.v. flux estimates do not represent the true whole body metabolism of these amino acids and caution is warranted when extrapolating these data. Most importantly, i.v. infusion of isotope bypasses splanchnic metabolism (i.e., the gut and liver) and the disproportionately high metabolism by these tissues cannot be ignored in measurements of whole body flux rates.

**Role of proline and glutamine in arginine synthesis**

Overall, both glutamine and proline have key roles as dietary precursors for arginine synthesis. Arginine is a semi-essential amino acid involved primarily in urea synthesis but also key in nitric oxide, polyamine, and creatine syntheses. The roles of proline and glutamine in arginine metabolism change depending on stage of development. In fact, the changes in these metabolic pathways can be predicted by the relative enzyme activities in the various tissues involved.

Glutamine and glutamate can be converted to proline and the urea cycle amino acids via PSC, but this conversion occurs only in the gut, because PSC synthase activity is localized primarily to this tissue (41) (Figs. 1 and 2). The small intestine also has appreciable activities of PSC dehydrogenase (PSCDH) for proline synthesis as well as high activities of ornithine aminotransferase (OAT), carbamoyl phosphate synthetase I (CPS-I), and ornithine transcarbamoylase (OTC), which results in a net synthesis of citrulline (42). Because intestinal argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) activities are low, citrulline is released into the portal circulation and almost no arginine is synthesized in the gut. The intestine also has an appreciable level of arginase activity, which results in arginine to citrulline conversion while generating urea, a pathway once thought to occur only in the liver (43). Proline oxidase and OAT allow proline to also serve as an important dietary precursor for citrulline synthesis in the gut.

Portal citrulline is not taken up by the liver and enters post-hepatic circulation, where the kidney takes up arterial citrulline and converts it to arginine via abundant activities of ASS and ASL (39,44). Low renal arginase activity allows the bulk of synthesized arginine to be released into circulation for use by the whole body. This lack of hepatic metabolism of citrulline is in contrast to portal arginine, which is transported into the liver, where near-units saturated activities of arginase catabolizes arginine completely. The net synthesis of citrulline by the gut provides an effective strategy to bypass hepatic metabolism and allow renal conversion to arginine, which is then available to whole body tissues. Ironically, dietary arginine is also converted to citrulline in the gut, only to be reconverted back to arginine in the kidney (43). It is also notable that arginine becomes semi-essential, or co-indispensable with proline, in situations of gut bypass or intestinal injury, because de novo synthesis of arginine depends on intestinal conversion of glutamine and glutamate to PSC as well as for de novo synthesis of citrulline (45-47).

Therefore, in the adult, proline, glutamine, and glutamate are effective dietary precursors for whole body arginine synthesis but only when converted to citrulline in the gut (40). Regulation of

**FIGURE 1 Metabolic pathways of glutamine, proline, and related amino acids.**

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occurs by releasing excess arginine to the portal circulation for catabolism by the liver. Indeed, in situations where high protein (i.e., high arginine) diets are fed, excess arginine downregulates CPS-I and OTC and is not converted to citrulline (48). The intact arginine is released to the portal vein for disposal by the liver. In contrast, low-protein (i.e., low-arginine) diets upregulate CPS-I and OTC and citrulline synthesis so more arginine can be synthesized in the kidney for whole body needs (43). Therefore, to provide more arginine to the adult, the dietary strategy should be based on feeding dietary precursors, not arginine. However, when the gut is bypassed, as in total parenteral nutrition feeding, then arginine (or citrulline) needs to be fed to meet arginine requirements.

Role of proline and glutamine in arginine synthesis in the neonate

In contrast to the healthy adult, neonates of several species have been shown to require some dietary arginine, because de novo synthesis is not sufficient to meet whole body requirements (22,49–51). This requirement is particularly evident in light of the observation that mammary milk in several species (i.e., primate, ruminant, pig, rat, llama, and elephant) is abundant in glutamine, glutamate, and proline but low in arginine (52). Probably because so much glutamate and glutamine are available, negligible conversion of proline to glutamate and glutamine (42), P5CDH activity in the neonatal gut also translates to Propositions that arginine is synthesized and released by the neonatal gut. Furthermore, the neonatal gut, which allows arginine of gut origin to be available for whole body metabolism. Furthermore, the neonatal kidney has lower activities of ASS and ASL so the conversion of citrulline to arginine is not as important in preweaning neonates. These enzyme activity patterns in the suckling neonate seem to change to adult patterns sometime postweaning, at least in pigs (42).

The importance of proline as the sole precursor for arginine synthesis and its dependence on gut metabolism has been demonstrated using clinical metabolic outcomes such as hyperammonemia in piglets infused with arginine-free diets (51). In i.v. fed piglets, severe hyperammonemia developed within hours of feeding an arginine-free diet, regardless of whether proline was provided (Table 2). However, when piglets are fed i.g., provision of proline undergoes first-pass metabolism by the intestine, which ameliorates the moderate hyperammonemia that results from the arginine-free diet. The synthesis of arginine in neonates is dependent on an intact gut and an adequate supply of proline. These findings have been confirmed by kinetic studies where 3H-proline infusion via either the gastric or portal route was used to ameliorate the moderate hyperammonemia that results from the arginine-free diet (51). Indeed, only ~5% proline flux ends up in glutamate and glutamine across the gut in piglets (22).

Indeed, only ~5% proline flux ends up in glutamate and glutamine across the gut in piglets (22). The net synthesis of arginine is facilitated by the higher activities of ASS and ASL and a near absence of arginase in the gut of the neonate, unlike the adult (42,53). In addition, the neonatal liver does not readily take up portal arginine, which allows arginine of gut origin to be available for whole body metabolism. Furthermore, the neonatal kidney has lower activities of ASS and ASL so the conversion of citrulline to arginine is not as important in preweaning neonates. These enzyme activity patterns in the suckling neonate seem to change to adult patterns sometime postweaning, at least in pigs (42).

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### TABLE 2

<table>
<thead>
<tr>
<th>Plasma ammonia concentration changes</th>
<th>Test Diet</th>
<th>i.g.</th>
<th>i.v. (IV) fed piglets given diets deficient in arginine (–Arg/+Pro), arginine and proline (–Arg+/+Pro), or proline (–Pro/+Arg) for 8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td>Arg/+Pro</td>
<td>76</td>
<td>68</td>
</tr>
<tr>
<td>At baseline</td>
<td>Arg/Pro</td>
<td>74</td>
<td>51</td>
</tr>
<tr>
<td>At baseline</td>
<td>Pro/+Arg</td>
<td>51</td>
<td>64</td>
</tr>
<tr>
<td>At cessation</td>
<td>Arg/+Pro</td>
<td>81</td>
<td>344</td>
</tr>
<tr>
<td>At cessation</td>
<td>Arg/Pro</td>
<td>177</td>
<td>323</td>
</tr>
<tr>
<td>At cessation</td>
<td>Pro/+Arg</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>Change per hour</td>
<td>Arg/+Pro</td>
<td>51</td>
<td>84</td>
</tr>
<tr>
<td>Change per hour</td>
<td>Arg/Pro</td>
<td>31</td>
<td>74</td>
</tr>
<tr>
<td>Change per hour</td>
<td>Pro/+Arg</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Adapted with permission from Brunton et al. (51).

Diet infusion stopped at ~6 h due to severe hyperammonemia.
specifically quantify first-pass small intestinal metabolism (22, 23). In these studies, proline conversion to ornithine was negligible when gut metabolism was bypassed (i.e. intraportal infusion), so proline is only an effective precursor if gut metabolism is included.

The multi-organ system of arginine metabolism in adults is reduced to a gut-only system in neonates, where glutamine and glutamate are not available as precursors for arginine synthesis. So in suckling neonates, dietary arginine and some de novo synthesis from proline is essential to meet whole body requirements. It has been estimated based on the kinetics of whole body flux that one-half of the neonate’s arginine needs are met by gut first-pass de novo synthesis and this does not change when arginine is deficient or in excess (22). Therefore, to provide more arginine to neonates, arginine should be fed directly and some proline can be included but only if delivered enterally.

Splanchnic metabolism of glutamine and glutamate

A key determinant of whole body metabolism of glutamine, proline, and related amino acids is the extent of splanchnic tissue metabolism. As clearly demonstrated by recent research, the role of glutamine, glutamate, and proline as precursors for arginine synthesis is largely dictated by the different roles of the splanchnic organs and stage of development. However, it has become evident in recent years that splanchnic amino acid metabolism is dominated by the gastrointestinal tissues more so than the liver. Although gastrointestinal tissues represent only 4-6% of body mass, these tissues disproportionately account for 20-35% of whole body energy expenditure and protein turnover (56). The anatomical position of the splanchnic tissues also emphasizes that first-pass metabolism of amino acids needs to be considered, because it determines whole body systemic exposure to dietary doses of these amino acids. Not only do these tissues filter dietary excesses reaching the general circulation, they also preferentially utilize dietary amino acids, as opposed to arterial amino acids, for protein synthesis and conversions (12,36,57-59). So the fate of dietary excesses of proline, glutamine, and glutamate largely depends on splanchnic metabolism, especially first-pass metabolism by the gut.

Glutamine splanchnic extraction

Arguably, the most important amino acid associated with gut metabolism is glutamine. Its role as a fuel for small intestinal enterocytes has been investigated extensively since the seminal work of Windmueller and Spaeth (32-35) in rats. In adult humans, a substantial body of literature also has been generated comparing glutamine kinetics during parenteral vs. oral stable isotopic tracer infusions. A series of human studies involving such infusions observed that much of the label disappears when infused orally compared with i.v. (3,5-7,60). Thus, the calculated whole body flux rates during oral infusion were consistently higher than those using parenteral infusion. These differences in whole body flux can be ascribed to first-pass splanchnic metabolism of the oral tracer, because all components of flux were identical, such as protein synthesis, breakdown, and dietary inputs.

We have summarized splanchnic glutamine extraction data in studies using isotope tracers (Table 3). In general, about two-thirds of glutamine delivered orally is extracted on first pass and does not reach the peripheral circulation. In adult human studies that measured the fate of this label, the vast majority of this extracted glutamine (77-93%) was oxidized. Recent studies, employing portal-drained viscera (PDV) tracer balance in adults undergoing abdominal surgery, have further demonstrated that the majority of this splanchnic extraction is due to intestinal extraction (36,40). These results are similar to PDV balance experiments conducted in young pigs (12) and in situ small intestinal experiments in adult rats (33,34) and support the observation that the net glutamine balance across the liver is essentially 0 (38-40). In animal studies, the nonoxidative products of glutamine extracted by the gut are alanine, proline, citrulline, ornithine, and arginine (12,33). It is notable that 15-33% of arterial glutamine is extracted by the PDV in adult humans (36), rats (33), and young pigs (12). Quantitatively, this translates into a substantial amount of glutamine metabolized, because the total flux of glutamine delivered to the gut by artery is several-fold higher than that typically fed (58) and of this arterially extracted glutamine, ~57-70% is oxidized (12,32,34).

Although the vast majority of enteral glutamine is extracted by splanchnic organs, increasing the amount of enteral glutamine does lead to increased plasma concentrations of glutamine and glutamate. In a study by Dechelotte et al. (61), jejunal infusions in adult humans of 1 to 6 times the typical dietary intake led to increasing glutamine concentrations in plasma from 598 to 2773 μmol/L; in addition, glutamate concentrations increased from 50 to 177 μmol/L over the same range of infusions. However, these infusions were over 90 min, so the sudden increase in glutamine and glutamate concentrations might be a transient effect that disappears over chronic intakes, especially considering the high arterial extraction capacity of the gut in the postabsorptive state. Indeed, in healthy adults given a 5- to 8-g glutamine bolus (0.1 g/kg), plasma glutamine concentrations doubled by 30 min (~800-1500 μmol/L) and returned to baseline values within 2 h, demonstrating the rapid removal of glutamine (62). Of the many studies on glutamine supplementation to date, no problems of toxicity have been observed (63). Whether or not these increased plasma concentrations of glutamate are of concern requires more information on glutamate splanchnic metabolism.

Glutamate splanchnic extraction

Although glutamine is often considered the most important amino acid for the gut, the extraction and oxidation of glutamate by the intestine is more extensive than that for glutamine. Indeed, it has been proposed that glutamate may be the most important fuel for intestinal metabolism (56). The nearly complete extrac-

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Studies on the splanchnic extraction of glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction, %</td>
<td>Oxidized, %</td>
</tr>
<tr>
<td>Enteral</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Adult humans</td>
</tr>
<tr>
<td>53-74</td>
<td>Adult humans</td>
</tr>
<tr>
<td>58-64</td>
<td>Adult humans</td>
</tr>
<tr>
<td>66-76</td>
<td>Adult humans</td>
</tr>
<tr>
<td>62</td>
<td>Adult humans</td>
</tr>
<tr>
<td>46-53</td>
<td>Preterm infants</td>
</tr>
<tr>
<td>44</td>
<td>Adult abdominal surgery patients</td>
</tr>
<tr>
<td>67</td>
<td>60</td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Adult abdominal surgery patients</td>
</tr>
<tr>
<td>25-33</td>
<td>57</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
</tr>
</tbody>
</table>

1 IG-IV refers to studies comparing oral and i.v. infusions of tracers; PDV refers to studies employing portal vein sampling; % oxidized refers to percentage of extracted glutamine that was oxidized.
tion of glutamate by splanchnic tissues in adult humans, by the PDV in young pigs, and by the small intestine of adult rats (Table 4) supports the more important role of glutamate as a fuel for the gut. In addition, 81–86% of this extracted glutamate was oxidized in human subjects, which, when combined with the increased extraction rate, makes glutamate a more important oxidative fuel, at least on first pass. Interestingly, however, negligible amounts of arterial glutamate are extracted by the gut. In either the fed or postabsorptive state, when the arterial supply of amino acids is the only source available, glutamine is the most important fuel source for the gut. In the postabsorptive state, the preferred oxidative fuel by the gut over glucose (12,35).

There has been ongoing concern over glutamate's toxicity given the amino acid's role as a neurotransmitter and as implicated in the “Chinese Restaurant Syndrome” in the form of monosodium glutamate (65). Of the consistent observation that dietary glutamate is prevented from reaching the peripheral circulation by virtue of its nearly complete splanchnic extraction, we would expect glutamate toxicity to occur at very high levels of intake. In adult humans, blood levels of glutamate do not increase even with gavage doses of ~30 mg/kg body weight; indeed, only a slight increase in plasma glutamate was observed with a monosodium glutamate bolus of ~150 mg/kg body weight. Infants are also able to metabolize similar amounts given in infant formula (66) and a recent report in premature infants showed that an oral glutamate dose of 3-fold the normal intake was completely extracted by first-pass splanchnic metabolism (67).

A recent study in young pigs fed glutamate at a level 4 times the normal intake for 4 h showed that PDV glutamate utilization increased with increasing enteral glutamate such that the percent utilized did not change over the 4-fold range of intakes (15). However, with a 3-fold increase in dietary glutamate infusion, PDV extraction decreased from 97 to 88% and oxidation rates decreased from 49 to 33%; indeed, more glutamate was converted to glutamine and ornithine (but not citrulline, arginine, or proline) and arterial glutamate concentrations increased from 175 to 505 μM/L. With respect to toxicity, even with 400% of normal intake and a 3-fold rise in arterial concentrations, glutamate concentrations in brain and hypothalamus did not change, supporting the nontoxicity of glutamate at high concentrations (65). In addition, a study by Chung and Baker (68) showed that increasing dietary glutamate from 1.0 to 9.8% of diet had no effects on growth or feed efficiency over 3 wk. If dietary glutamine is prevented from reaching the peripheral circulation, unlike glutamate. But arterial glutamate appears to be oxidized at a similar rate (i.e. 80%) as enteral glutamate, so it must be extracted by the liver or transaminated to glutamine for uptake by the gut (13).

Although it has been suggested that glutamine and glutamate are interchangeable as substrates for intestinal metabolism (71), it should be noted that more dietary glutamate is extracted on first pass, whereas on second pass, glutamine continues to be extracted from the arterial circulation, unlike glutamate. But arterial glutamate appears to be oxidized at a similar rate (i.e. 80%) as enteral glutamate, so it must be extracted by the liver or transaminated to glutamine for uptake by the gut (13). Despite these metabolic differences, either amino acid can support intestinal structure and function in the absence of the other (71,72).

Splanchnic metabolism of proline

There are very few published reports on splanchnic extraction of proline and, to our knowledge, there is no information in humans. Recent studies on intestinal metabolism of proline in young pigs provide the only evidence available. In young pigs (5–6 wk old, postweaning), the PDV mass extraction of proline has been estimated at 9% (fed hourly for 7 h) (63), 12% (fed bolus with cumulative balance over 8 h) (73), 38% (fed hourly for 6 h) (58), 57% (fed continuously via duodenum for 6 h) (74), and 57% (fed continuously via duodenum for 24 h) (75). Although these data are wide-ranging due to the types of feeding regimes, it appears that much less proline is extracted on first pass (i.e. 9–57%) compared with glutamine (108–129%, or some net synthesis by PDV) or glutamine (71–99%) in these same studies. In younger 10-d-old piglets (i.e. preweaning), proline concentrations in plasma did not change when identical diets were infused enterally, intraperitoneally, or i.v. for 7 d, suggesting minimal extraction of proline by splanchnic organs (76). Indeed, in a series of isotope studies conducted in these piglets to investigate gut conversion of proline to arginine, proline extraction by the small intestine was determined by comparing fluxes from oral and intraportal infusions of labeled proline (22,23). Over a range of arginine intakes, small intestinal extraction was negligible during low and high arginine intakes and at most 14% during a moderate intake (Fig. 4). Indeed, even when arginine is deficient (i.e. 0.20 g·kg⁻¹·d⁻¹), extra proline is not extracted at a higher rate by the gut to synthesize arginine. Moreover, using intraportal and i.v. flux estimates, hepatic extraction of proline was also negligible (24).

If dietary proline is not extracted to an appreciable extent by the splanchnic tissues, then presumably plasma proline concentrations would increase with even small changes in dietary proline.

### TABLE 4  Studies on the splanchnic extraction of glutamate

<table>
<thead>
<tr>
<th>Extraction, %</th>
<th>Oxidized, %</th>
<th>Subjects</th>
<th>Reference</th>
<th>Notes¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enteral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Adult humans</td>
<td>(5)</td>
<td>IG-IV</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>Adult humans</td>
<td>(13)</td>
<td>IG-IV</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>Preterm infants</td>
<td>(14)</td>
<td>IG-IV</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Adult rat</td>
<td>(33) Small intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Young pigs</td>
<td>(64) PDV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>Young pigs</td>
<td>(12) PDV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>Young pigs</td>
<td>(15) PDV</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arterial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>Adult humans</td>
<td>(4)</td>
<td>IG-IV</td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>Adult humans</td>
<td>(5)</td>
<td>IG-IV</td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>Adult humans</td>
<td>(13)</td>
<td>IG-IV</td>
<td></td>
</tr>
</tbody>
</table>

¹ IG-IV refers to studies comparing oral and i.v. infusions of tracer; PDV refers to studies employing portal vein sampling; % oxidized refers to percentage of extracted glutamate that was oxidized.
Concentrations decreased by 65% after 4 wk (18). Despite this extended adaptation to the lack of dietary proline, the adult human still cannot seem to synthesize sufficient proline to maintain plasma concentrations.

Summary

Glutamine, glutamate, and proline are very important dietary precursors in adult mammals for de novo arginine synthesis. Net citrulline production occurs in the gut from all 3 precursors and the citrulline released largely escapes hepatic metabolism and is converted to arginine in the kidney. There are key developmental differences in arginine synthesis such that in the neonate, glutamine and glutamate are not effective dietary precursors and only proline can be converted to arginine, but only in the gut on first pass. Thus, splanchnic metabolism and stage of development are key factors that affect the whole body metabolism of glutamine, glutamate, and proline.

The majority (i.e. ~50–75%) of dietary glutamine and almost all of dietary glutamate (i.e. ~90–98%) are extracted on first pass by splanchnic tissues and most of this metabolism occurs in the small intestine. In addition, the vast majority (i.e. ~60–90%) of this extracted glutamine and glutamate is oxidized as a fuel by the small intestine. Notably, although less glutamine than glutamate is extracted on first pass, a substantial arterial extraction of glutamine (i.e. 15–30%) by the small intestine occurs, especially during the postabsorptive state. Unlike glutamine, negligible amounts of arterial glutamate appear to be extracted, but glutamate can be readily utilized by the liver for gluconeogenesis or transaminated to glutamine for use by the gut. Less is known about proline extraction by splanchnic tissues, but limited data in young pigs suggest very little proline is extracted and therefore may present considerable toxicity potential compared with glutamate and glutamine. The oxidative and synthetic capacities for proline metabolism seem limited, even in adult humans.

The net result is that the plasma concentration of glutamine and glutamate is not a sensitive indicator of dietary load and de novo synthesis can increase plasma concentrations dramatically for nondietary reasons. However, the plasma proline concentration is sensitive to dietary inputs given the body’s limited capacity to synthesize or oxidize proline. The lower extraction rate and oxidative limitations may lead to a higher toxicity potential for proline, which may be associated with neurological problems (77). Our survey of the existing evidence clearly warrants further study of the extent of splanchnic proline metabolism in adult humans.

In the context of establishing the safe upper limits for dietary amino acid intake, especially those like glutamine or glutamate, it is important to consider the extent of first-pass splanchnic metabolism. In circumstances where splanchnic tissue metabolism is bypassed, such as total parenteral nutrition, the safe upper limit may be considerably lower than when fed orally. The application of tracer kinetics approaches to address safe upper limits of amino acid intakes needs to include oral delivery of isotope to accommodate the massive splanchnic metabolism, which is often the most substantial component of whole body metabolism.

Other articles in this supplement include references (78–88).

Literature Cited


76. Bertolo RF, Chen CZ, Pencharz PB, Ball RO. Intestinal atrophy has a greater impact on nitrogen metabolism than liver by-pass in piglets fed identical diets via gastric, central venous or portal venous routes. J Nutr. 1999;129:1045-52.


