Arginine synthesis from enteral glutamine in healthy adults in the fed state

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Tomlinson C, Rafii M, Ball RO, Pencharz P. Arginine synthesis from enteral glutamine in healthy adults in the fed state. Am J Physiol Endocrinol Metab 301: E267–E273, 2011. First published May 3, 2011; doi:10.1152/ajpendo.00006.2011.—Recent studies have documented transfer of labeled nitrogen from [2-15N]glutamine to citrulline and arginine in fasting human adults. Conversely, in neonates and piglets we have shown no synthesis of arginine from [2-15N]glutamine, and others have shown in mice that glutamine is a nitrogen, but not a carbon donor, for arginine synthesis. Therefore, we performed a multitracer study to determine whether glutamine is a nitrogen and/or carbon donor for arginine in healthy adult men. Two glutamine tracers, 2-15N and 1-13C, were given enterally to five healthy men fed a standardized milkshake diet. There was no difference in plasma enrichments between the two glutamine tracers. 1-13C isotopomers of citrulline and arginine were synthesized from [1-13C]glutamine. Three isotopomers each of citrulline and arginine were synthesized from the [2-15N]glutamine tracer: 2-15N, 5-15N, and 2,5-15N2. Significantly greater enrichment was found of both [5-15N]arginine (0.75%) and citrulline (3.98%) compared with [2-15N]arginine (0.44%) and [2-15N]citrulline (2.62%), indicating the amino NH2 from glutamine is not a carbon donor, for arginine synthesis. Hence, studies using [2-15N]glutamine will overestimate arginine synthesis rates.

THE PROCESS OF ARGININE SYNTHESIS from dietary precursors is complex, involving at least eight enzymes directly and the intracellular and interorgan transfer of substrates. The seminal studies of Windmueller and Spaeth using mass balance techniques were the first to investigate this issue and suggested synthesis of citrulline from enteral glutamine (25). That glutamine is a dietary precursor for arginine has largely been accepted by the scientific community (10); yet, until recently, there have been few stable-isotope studies investigating the issue in vivo. Furthermore, our group has shown in human neonates (20) and piglets (2, 7) that only proline acts as a dietary precursor for arginine with no synthesis from enteral glutamate.

Using 15N-labeled glutamine, the Deutz group has demonstrated incorporation of nitrogen from [2-15N]glutamine into arginine and citrulline in mice (3, 4) and in adult humans (11, 12, 23). Although these studies have significantly advanced knowledge on arginine synthesis in mammals, the use of [2-15N]glutamine combined with the mass spectrometry methods used and the complexity of arginine synthesis, makes the documented transfer of one nitrogen atom to citrulline and arginine difficult to interpret, given the potential isotopomers that may be synthesized from this tracer.

The first step in arginine synthesis from glutamine is via glutaminase to produce glutamate and ammonia, followed by synthesis of pyrroline-5-carboxylate (P5C). No pathway has been described in which conversion of glutamine to ornithine (and subsequently to arginine) may occur without conversion first to glutamate. To the basic 5-carbon chain of P5C is added an amino group. This comes from the α-amino N of glutamate to form ornithine via the enzyme ornithine aminotransferase. To ornithine is added a carbamoyl group from carbamoyl phosphate (CO2 from bicarbonate, NH3 from ammonia released during deamination of amino acids, e.g., glutamine to glutamate) to form citrulline. The final NH3 group is derived from aspartate via argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). The source of the amino group for aspartate synthesis is the amino N of glutamate via the enzyme aspartate aminotransferase. These pathways are outlined in Fig. 1, and it is clear that the α-amino N of glutamine may be incorporated into any of the four nitrogen atoms of arginine.

This complexity was recently confirmed by Marini et al. (15) using three different glutamine tracers 2-15N, 5-15N and D5 (1H5). In that multitracer study in adult mice, the authors demonstrated that enteral glutamine was a poor carbon donor for arginine and citrulline and contributed nitrogen almost entirely as [15N-ureido]citrulline with little label appearance at the 2- or 5-nitrogen atoms of citrulline. The authors concluded that glutamine was a nitrogen donor for arginine synthesis but not a carbon donor. The contradictory results from these two groups [Ligthart-Melis et al. (11, 12) and Marini et al. (15)] have resulted in considerable discussion in this Journal recently (14, 16).

In light of our neonatal studies (5), in which we showed no arginine synthesis from glutamate and recognizing the controversy in the literature, we sought to add to the published work in humans by performing a study using 2 isotopomers of glutamine, 1-13C and 2-15N, combined with tandem mass spectrometry to define the separate contribution of the carbon skeleton and α-amino N from glutamine to the synthesis of citrulline and arginine.

METHODS

Subjects. Five healthy men were recruited for the study. Their characteristics are summarized in Table 1. At the time of the study, they were in good health, as determined by medical history and simple physical exam, and were taking no medications. As described below, the study took place on 3 separate days at least 1 wk apart. To ensure uniformity and to habituate to a standardized diet, prior to each study...
day the subjects were asked to eat only a milkshake-based diet (Scandishake; Axcan Scandipharm, Birmingham AL) for 48 h. When reconstituted, this diet provides 2,400 kcal/day and 52 g/day protein. No other food was allowed, but the subjects could drink water ad libitum. Subjects provided informed consent and were given remuneration for taking part. The study was approved by the Research Ethics Board at the Hospital for Sick Children.

Study protocol. On each day of the study, the subjects were admitted to the Clinical Investigation Unit at the Hospital for Sick Children. The studies lasted 8 h. To simulate normal physiology, this study was carried out in the fed state. For the duration of the study the subjects drank hourly amounts of milkshake providing them with an energy intake of 150 kcal/h and a protein intake of 3.25 g/h. In addition, the subjects could drink water ad libitum but no other food or drink for the duration of the study. For convenience of blood sampling, an intravenous line was inserted in the antecubital fossa of each subject.

At 5 h into the study, an oral isotope “infusion” was commenced. The isotopes were drunk by the subjects from medicine containers rinsed with water afterward to ensure the entire dose was given. A constant infusion was simulated by giving the subjects a prime dose of the labeled amino acids followed by half-hourly amounts for 2.5 h. (6). Three blood samples were collected at baseline, and four blood samples were collected at plateau enrichments at 90, 120, 150, and 180 min after starting the isotope infusion. The study protocol is outlined in Fig. 2.

Blood samples were kept on ice until they were centrifuged at 4,000 rpm for 10 min to obtain plasma. Samples were then stored at −20°C until analysis.

Stable isotope tracers. Three isotopes were used in this study: [guanidino-15N2]arginine, [1-13C]glutamine, and [2-15N]glutamine, given in random order. Given the complexity of analyses and the expectation that there would be multiple isotopomers of arginine and related amino acids formed from the nitrogen-labeled glutamine, the three isotopes were given on separate days with a 1-wk washout in between studies. The background enrichments did not change over the study period (data not shown). The labeled amino acid tracers were obtained from Cambridge Isotope Laboratories (Andover, MA). The [guanidino-15N2]arginine tracer had an enrichment of 99.4% and D-isotopomer content of 1.2%. The D-amino acid content necessitated the use of chiral chromatography (19). The [2-15N]glutamine tracer had isotopic enrichment >99.5% and undetectable D-isotopomer and

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
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<tbody>
<tr>
<td>Mean</td>
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<tr>
<td>------</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Height, m</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
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</table>

Physical characteristics of 5 male volunteers.

Fig. 1. Outline of pathways by which the carbon skeleton of glutamine and 2-15N from glutamine can be incorporated into arginine. Note particularly the various transamination reactions.

Fig. 2. The study was performed on three separate days at least a week apart, the only difference being the isotopes administered.
the [1,13C]glutamine tracer had isotopic enrichment >99.9% and undetectable n-isotopomer.

The glutamine tracers were administered with a priming dose of 18 μmol/kg followed by 30-min bolus doses of 15.5 μmol·kg⁻¹·h⁻¹ to simulate a constant infusion. These doses were the same as those used by Lichtart-Melis et al. (12). The prime and infusion doses for the [guanido-15N₂]arginine were 5.9 μmol/kg and 5.9 μmol·kg⁻¹·h⁻¹, as described by Castillo et al. (9).

The solutions of the tracers were prepared and dispensed by the pharmacy at The Hospital for Sick Children, in sterile water and stored at 4°C until use. The concentration of each amino acid solution was arginine 15 mg/ml and glutamine 10 mg/ml. Solutions were confirmed to be sterile and pyrogen free before use, and the concentrations were confirmed by HPLC.

The labeled tracers were used to calculate flux rates for enterally administered amino acids. The appearance of the glutamine tracers into glutamate, ornithine, citrulline, and arginine was assessed by tandem mass spectrometry as described below.

**Analysis of enrichment and chiral separation.** Plasma samples were thawed and 25 μl was deproteinized by the addition of 500 μl of methanol, vortexed for 15 s, and then centrifuged at 13,000 rpm for 15 min. The supernatant was dried under N₂ and reconstituted in 0.1% methanol, vortexed for 15 s, and then centrifugation at 13,000 rpm for 15 min. The injection volume was 20 μl, with an overall analysis time (injection to injection) of 35 min. Isotope analysis was carried out using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) operated in positive electrospray ionization mode. This was coupled to an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). All aspects of the system operation were controlled using The Analyst NT v. 1.4.1 software (Applied Biosystems/MDS Sciex). Maximum sensitivity for L- and D-arginine, citrulline, ornithine, glutamate, and glutamine was achieved by measuring product ions multiple reaction monitoring from the fragmentation of the protonated [M+H]⁺ molecules of each amino acid. Only the enrichments of the L-isofoms of the amino acids were analyzed and are presented for this study.

The [M+H]⁺ precursor (parent) ion for each of the unlabeled amino acids was (m/z) 175.1 for arginine, 176.1 for citrulline, 133.1 for ornithine, 147.1 for glutamine, and 148.1 for glutamate). The signal for the most abundant product (daughter) ions (m/z 70.1 for arginine, citrulline, ornithine and 84.1 for both glutamate and glutamine) were also optimized.

The labeled [15N₂-guanidino]arginine has a parent ion with m/z 177.1 and a daughter ion m/z 70.1 and was used to calculate arginine flux. The [1,13C]glutamine tracer was used to calculate glutamine flux. The [2,15N]glutamine tracer was identified using the parent/daughter pair of 148/84, and the [2-15N]glutamate was identified using the ion pair of 148/88.

There is no known pathway whereby the label of [1-13C]glutamine can be incorporated into the carbon skeleton of glutamate, ornithine, citrulline, and arginine other than in the I-C position. Therefore, the appearance of the [1,13C]glutamine tracer in these amino acids was determined by measuring enrichment of the precursor/product m/z ion pair of 176/70 for arginine, 177/70 for citrulline, 134/70 for ornithine, and 149/84 for glutamate.

It was anticipated that there would be considerable transfer of label from [2-15N]glutamine to the product amino acids in the 2 position, the 5 position, and in combination. In addition, it was possible that the ureido N of citrulline and one or both guanidino N of arginine would be labeled. These potential isotopomers with their parent and daughter ion pairs are summarized in Table 2. From this table it can be seen that some isotopomers of citrulline and arginine cannot be distinguished, as it was not possible to differentiate [5-15N]citrulline from [ureido-15N]citrulline or the equivalent isotopomers of arginine.

The appearance of the label in [2-15N]glutamate (149/85) was also measured. The potential for newly synthesized isotopomers of glutamine 5-15N (148/84) and 2,5-15N₂ (149/85) was also assessed.

**Data analysis.** Enzyme enrichment was expressed as molecule percent excess (MPE), calculated as enrichment at plateau minus the background measurement at baseline.

Turnover of the enteral amino acid tracer was calculated using the equation

\[ Q = i(E/E_i) - 1 \]

where \( Q \) is the turnover of the amino acid through the free amino acid pool, \( i \) is the rate of infusion of the tracer, \( E_i \) is the enrichment of the tracer in the infusion, and \( E_p \) is the MPE of the tracer in plasma at plateau (21).

The conversion rates of glutamine to arginine were calculated by the formula

\[ Q_{\text{Gln-Arg}} = E_{\text{Arg}}/E_{\text{Gln}} \times Q_{\text{Arg}} \]

Similarly, the synthesis rate of arginine from citrulline was calculated by the formula

\[ Q_{\text{Cit-Arg}} = E_{\text{Arg}}/E_{\text{Cit}} \times Q_{\text{Arg}} \]

The fractional conversions of glutamine to ornithine and citrulline were calculated by the formula

\[ \text{fractional conversion}_{\text{Gln-product}} = E_{\text{product}}/E_{\text{Gln}} \]

Statistical analysis. Data are expressed as means ± SD. Enrichment of amino acids above baseline was analyzed using Student’s t-test. Significance was assumed if \( P < 0.05 \). Statistical analysis was performed using SAS v. 9.1 (SAS Institute, Cary, NC).

**RESULTS**

All subjects completed the study, and there were no adverse events or protocol violations.

Enrichment was measured in arginine and both glutamine tracers in plasma (Table 3). There was no statistical difference in enrichment between the two glutamine tracers. The turnover

<table>
<thead>
<tr>
<th>Potential Isomer</th>
<th>Parent m/z</th>
<th>Daughter m/z</th>
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<tbody>
<tr>
<td>Ornithine</td>
<td>133</td>
<td>70</td>
</tr>
<tr>
<td>2-15N</td>
<td>134</td>
<td>71</td>
</tr>
<tr>
<td>5-15N</td>
<td>134</td>
<td>70</td>
</tr>
<tr>
<td>2,5-15N₂</td>
<td>135</td>
<td>71</td>
</tr>
<tr>
<td>Citrulline</td>
<td>176</td>
<td>70</td>
</tr>
<tr>
<td>2-15N</td>
<td>177</td>
<td>71</td>
</tr>
<tr>
<td>5-15N</td>
<td>177</td>
<td>70</td>
</tr>
<tr>
<td>2,5-15N₂</td>
<td>178</td>
<td>71</td>
</tr>
<tr>
<td>Ureido-15N</td>
<td>177</td>
<td>70</td>
</tr>
<tr>
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<td>178</td>
<td>71</td>
</tr>
<tr>
<td>5-15N[Ureido-15N]</td>
<td>178</td>
<td>70</td>
</tr>
<tr>
<td>2,5-15N₂[Ureido-15N]</td>
<td>179</td>
<td>71</td>
</tr>
<tr>
<td>Arginine</td>
<td>175</td>
<td>70</td>
</tr>
<tr>
<td>2-15N</td>
<td>176</td>
<td>71</td>
</tr>
<tr>
<td>5-15N</td>
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<td>70</td>
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<tr>
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<tr>
<td>Guanidino-15N</td>
<td>176</td>
<td>70</td>
</tr>
<tr>
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<td>177</td>
<td>71</td>
</tr>
<tr>
<td>5-15N[guanidino-15N]</td>
<td>177</td>
<td>70</td>
</tr>
<tr>
<td>2,5-15N₂[guanidino-15N]</td>
<td>178</td>
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<tr>
<td>Guanidino-15N</td>
<td>177</td>
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<tr>
<td>2-15N [gguanidino-15N₂]</td>
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<td>71</td>
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<tr>
<td>5-15N [gguanidino-15N₂]</td>
<td>178</td>
<td>70</td>
</tr>
<tr>
<td>2,5-15N₂[gguanidino-15N₂]</td>
<td>179</td>
<td>71</td>
</tr>
</tbody>
</table>
of the \(^{15}\text{N}\)-labeled tracer was 909 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) and the \(^{13}\text{C}\) tracer was 767 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). The measured turnover for the arginine tracer was 93 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\).

There was measurable enrichment of \(M+1\) isotopomers of arginine, citrulline, ornithine, and glutamate when the \([1\text{-}{^{15}\text{C}}]\)glutamine isotope was administered (Table 4). The \(M+1\) isotopomers were detected in all subjects, and the enrichment of each was significantly increased over background \((P < 0.05)\). By use of these values, the estimated synthesis rate of arginine from \([1\text{-}{^{11}\text{C}}]\)glutamine was 8.1 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). This is <1% of glutamine turnover and 8.7% of arginine turnover.

Tracer transfer from \([2\text{-}{^{15}\text{N}}]\)glutamine was detected in \(M+1\) glutamate and both \(M+1\) and \(M+2\) isotopomers of ornithine, citrulline, and arginine. The enrichments of these isotopomers are shown in Table 5; all are significantly increased over background \((P < 0.05)\). No \(M+3\) isotopomer of citrulline or arginine or \(M+4\) isotopomer of arginine was detected (data not shown). As discussed in Methods, it was not possible, with current methodology, to distinguish \([\text{ureido-}{^{15}\text{N}}]\)citrulline and \([\text{guanidino-}{^{15}\text{N}2}]\)arginine from the respective \(5\text{-}{^{15}\text{N}}\) isotopomers of these amino acids. However, the ratio of \([2\text{-}{^{15}\text{N}}]\) to \([5\text{-}{^{15}\text{N}}]\)ornithine of 0.68 approximates that of \([2\text{-}{^{15}\text{N}}]\)- to \([5\text{-}{^{15}\text{N}}]\)citrulline (0.64) and \([2\text{-}{^{15}\text{N}}]\)- to \([5\text{-}{^{15}\text{N}}]\)arginine (0.61). Although these values are similar, it is possible that a small amount of \(M+1\) citrulline and arginine may be accounted for by incorporation of the label in the ureido and guanido groups.

In addition to the \([2\text{-}{^{15}\text{N}}]\)glutamine tracer, a further two synthesized isotopomers of glutamine were also demonstrated. There was significant enrichment of \([5\text{-}{^{15}\text{N}}]\)glutamine (mean MPE 0.34%, SD 0.05) and \([2,5\text{-}{^{15}\text{N}}2]\)glutamine (MPE 0.04%, SD 0.014). The \([5\text{-}{^{15}\text{N}}]\)glutamine would have been formed by glutamine synthetase from an unlabeled glutamate molecule and labeled ammonia released after conversion of \([2\text{-}{^{15}\text{N}}]\)glutamate to ketoglutarate. The dual-labeled glutamine arises from \([2\text{-}{^{15}\text{N}}]\)glutamate combining with labeled ammonia.

For ornithine, citrulline, and arginine, there was significantly greater enrichment of the \(5\text{-}{^{15}\text{N}}\) isotopomer compared with the \(2\text{-}{^{15}\text{N}}\) isotopomer (Table 5). This indicates that the amino \(N\) of glutamine (after deamination to glutamate) is transferred to these amino acids mostly by transamination rather than directly with the carbon chain. The enzyme which catalyzes this reaction is ornithine aminotransferase, with the reaction being bidirectional, from P5C to ornithine. Although there is no preference per se for glutamate to act as the nitrogen donor, it is the most abundant intracellular amino acid and so is most likely to donate the \(N\) for ornithine synthesis.

Table 6 compares the enrichments of glutamate, ornithine, citrulline, and arginine from the two glutamine tracers. There was significantly greater enrichment of \([2\text{-}{^{15}\text{N}}]\)ornithine, -citrulline, and -arginine of these amino acids compared with the \([1\text{-}{^{11}\text{C}}]\)isotopomers \((P < 0.05)\). The enrichment of \([2\text{-}{^{15}\text{N}}]\)glutamate approached significance \((P = 0.06)\) compared with \([1\text{-}{^{11}\text{C}}]\)glutamate.

An estimate of de novo arginine synthesis from citrulline can be obtained from the data by using the enrichment of citrulline from the glutamine tracers. Three rates can be estimated using \([1\text{-}{^{15}\text{N}}]\)citrulline to \([1\text{-}{^{11}\text{C}}]\)arginine, \([2\text{-}{^{15}\text{N}}]\)citrulline to \([2\text{-}{^{11}\text{N}}]\)arginine, and \([5\text{-}{^{15}\text{N}}]\)citrulline to \([5\text{-}{^{11}\text{N}}]\)arginine. The rates using enrichments from Tables 4 and 5 are 12.1 (±5.2), 17.9 (±11.58), and 18.1 (±3.1) \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\), respectively, with a mean of 16.0 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). These values are not significantly different and represent the whole body rate of conversion of citrulline to arginine, the majority occurring in the kidney, and as such should be the same for each isotopomer.

The measured rate of glutamine to arginine synthesis can be seen to be dependent on which enriched isotopomer of arginine is used for the calculation (Table 7). The synthesis of \([2\text{-}{^{15}\text{N}}]\)arginine occurs at a rate of 24 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\), and of \([5\text{-}{^{15}\text{N}}]\)arginine at a rate of 42 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). These calculated rates are greater than 100% of the citrulline-to-arginine conversion rate of 16.0 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) calculated above and therefore must be overestimates. This is as a result of the extensive first-pass metabolism of glutamine by the splanchnic
organs and recycling of the nitrogen label. The rate of synthesis from the 1-13C tracer of 8.1 μmol·kg⁻¹·h⁻¹ indicates that dietary glutamine contributes the carbon skeleton for ~50% of newly synthesized arginine (i.e., 8.1 of 16.0 μmol·kg⁻¹·h⁻¹).

DISCUSSION

The current metabolic and biochemical dogma is that in adult mammals the carbon skeleton of glutamine/glutamate is the most significant dietary precursor for arginine (10). Our experiments using multiple tracers demonstrate conclusively that these amino acids provide only 50% of the carbon skeleton of newly synthesized arginine. The results of recent experiments using multiple tracers demonstrate conclusively that dietary glutamine provides only 50% of the carbon skeleton of newly synthesized arginine (i.e., 8.1 of 16.0 μmol·kg⁻¹·h⁻¹).

Table 7. Differing rates of arginine synthesis

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Isotomer of Arginine</th>
<th>Rate (μmol·kg⁻¹·h⁻¹)</th>
<th>% Arginine Synthesis from Citrulline</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2-15N]glutamine</td>
<td>2-15N</td>
<td>24 (±7.32)</td>
<td>152</td>
</tr>
<tr>
<td>[2-15N]glutamine</td>
<td>5-15N</td>
<td>42 (±18.4)</td>
<td>253</td>
</tr>
</tbody>
</table>

Rates are μmol·kg⁻¹·h⁻¹ (±SD). Arginine synthesis from citrulline was estimated as 15.8 μmol·kg⁻¹·h⁻¹.

First, the majority of transferred label appears at the 5 position of ornithine, citrulline, and arginine, indicating that the nitrogen is transferred by transamination via ornithine aminotransferase (Fig. 1). Importantly, and strengthening this conclusion, the proportion of 5-15N to 2-15N isotopomers of each of these amino acids is approximately the same. One would expect from this synthesis pathway.

The second observation is that the enrichment of citrulline, in both 2-15N and 5-15N isotopomers, exceeded that of glutamine. This can be explained by one of two phenomena, channeling of substrates or recycling of the nitrogen label. The results indicate that both of these are occurring. Discussed above was the evidence for N recycling given the greater enrichment of 2-15N isotopomers compared with the 1-13C isotopomers of arginine and citrulline. The evidence that there is channeling of substrates comes from the observation that the enrichment of citrulline is greater than that of ornithine [Table 7.4; 2.6% vs. 1.0%]; ornithine 1.04%. This observation was made for both isotopomers for the labeled glutamine study and also from our previous study using [15N]proline (Ref. 18). As each molecule of citrulline can only be derived from a molecule of ornithine, the enrichment of the product should be the same or slightly less, due to dilution of the label. This was not the case; therefore, the labeled mitochondrial ornithine was not in equilibrium with plasma ornithine, presumably because it was channeled to citrulline synthesis. With our current methodology it is not possible to distinguish potential labeling of [ureido-15N]citrulline iso-
pomers from \([2-^{15}N]citrulline\); however, because we saw no 
M\(^{+3}\)-labeled isotopomer of citrulline, this would indicate no 
or minimal labeling of citrulline in the ureido group and would 
therefore not interfere with the interpretation of these results.

Central to stable-isotope methodology is the concept that the 
metabolic pool at the site of sampling is in equilibrium with all 
other metabolic pools of interest. It has long been realized that 
this is of relevance in arginine metabolism, as arginine within 
the urea cycle in the hepatocyte does not fully equilibrate with 
plasma arginine tracers. This is confirmed by the observation 
that urea flux is significantly greater than arginine flux (1) and 
is due to the tight channeling of products between the urea 
cycle enzymes (17). The results of this study also confirm that 
there is significant channeling of precursors during arginine 
synthesis in the enterocyte, as in all subjects, regardless of 
tracer used, ornithine enrichment (precursor) is less than cit-
rulline enrichment (product); i.e., mitochondrial ornithine is 
not in equilibrium with plasma ornithine. Again, this means 
that equations and flux estimates based on these enrichments 
must be used with caution.

Clearly, the choice of tracers is crucial for any stable-isotope 
study design; however, these results indicate that arginine 
metabolism presents particular challenges and researchers must 
contend with the effects of \(\delta\)-arginine and tracer recycling and 
channeling, as well as the formation of multiple isotopomers.

We have performed our studies in the fed state believing, 
given the preference of the enterocyte for luminal amino acids 
for arginine synthesis (12), that this is the most physiological 
state. Additionally, our piglet (22) and newborn studies (20) 
are also performed in the fed state. There are no studies in 
either humans or animals in the postabsorptive state.

Ligthart-Melis and colleagues (11–13) assessed synthesis of 
arginine from glutamine by using arginine, citrulline, and 
glutamine tracers. Their study was performed in the fasted state 
in obese patients with malignancy and during surgery, all of 
which may affect arginine metabolism (24). It is not possible, 
therefore, to quantitatively compare these studies with ours, 
and it would be interesting to repeat our study both in the fasted 
state and also using the intravenous route for tracer adminis-
tration. Those authors estimated that plasma glutamine 
contributed up to 64% of de novo synthesis of arginine from 
citrulline. In the study using the \([1-^{13}C]glutamine\) via the 
enteral route, we found glutamine contributed 56% to de novo 
arginine synthesis from citrulline. Given the preference of the 
Enterocyte for luminal amino acids for citrulline synthesis (12), 
it is anomalous that the estimate of glutamine contribution to 
citrulline synthesis is lower than that measured using the 
intravenous tracer. We suggest that the value of 64% measured 
by Ligthart-Melis and colleagues is too high, at least in part 
due to multiple labeling of the citrulline \(N\) atoms from the 
\([2-^{15}N]glutamine\). As discussed above, in the studies 
using the \(N\) tracer, the rates for arginine synthesis from 
glutamine are more than 100%, indicating recycling of the nitro-
gen label. It is clear that, while \(N\) labeled glutamine tracers can 
provide important qualitative information on arginine biosyn-
thesis, they should not be used to analyze the pathway quan-
titatively.

Marini et al. (15) recently demonstrated in mice that glu-
tamine is not a substantial source of the carbon skeleton for 
arginine synthesis, but, using a \([U-^{13}C]glutamine\) molecule, 
they did demonstrate transfer of the carbon label into the ureido 
group of citrulline. This is not surprising given the high rate of 
glutamine oxidation and the corresponding amount of labeled 
\(\mathrm{CO}_2\) that would be produced, which in turn may be incorpo-
rated into bicarbonate and carbamoyl phosphate and, hence, 
into citrulline. This effect was not seen in the carbon labeled 
study of this study due to use of only a \(1-^{13}C\) tracer and a lower 
per-kilogram infusion rate. The pattern of label transfer from 
the same \(2-^{15}N\) tracer is also different between Marini et al. 
(15) and the current study. Marini et al. (15) noted labeling of 
all three \(N\) atoms of citrulline in roughly equal proportions. 
However, we demonstrated 50% greater transfer to the 5 
position than the 2 position, with no demonstrable enrichment 
of the ureido \(N\) atom. The reason for these differences is not 
clear but may be due to different tracer dosing as well as 
terrierspecies differences.

**Conclusion**

The aim of this study was to determine the synthesis of 
arginine and citrulline from two differently labeled glutamine 
tracers. We have demonstrated that the rates of synthesis based 
on the \(N\)-labeled glutamine exceed the rate of citrulline-to-
arginine conversion due to the extensive glutamine oxidation 
and nitrogen recycling and, hence, overestimate arginine syn-
thesis. Therefore, \(N\)-labeled glutamine (and glutamate) 
tracers cannot be used to quantitatively investigate arginine and cit-
rulline metabolism. In addition, we have demonstrated that \(N\) 
from glutamine is used for either \(N\) of ornithine and the 
equivalent isotopomers of citrulline and arginine. However, 
using carbon-labeled glutamine, we have shown significant 
synthesis of arginine from the carbon skeleton of dietary 
glutamine and that this accounts for \(\sim 50\%\) of newly synthe-
sized arginine.

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**DISCLOSURES**

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