Intravenous glutamine supplementation enhances renal de novo arginine synthesis in humans: a stable isotope study1–4

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ABSTRACT

Background: Arginine plays a role in many different pathways in multiple cell types. Consequently, a shortage of arginine, caused by pathologic conditions such as cancer or injury, has the potential to disturb many cellular and organ functions. Glutamine is the ultimate source for de novo synthesis of arginine in humans via the intestinal-renal axis. Therefore, we hypothesized that parenteral glutamine supplementation may stimulate the interorgan pathway of arginine production.

Objectives: The objectives were to quantify arginine production from its precursor glutamine and to establish the contribution of the kidneys to de novo synthesis of arginine in patients receiving intravenous supplementation of glutamine dipeptide during major abdominal surgery.

Design: Whole-body and renal metabolism of glutamine, citrulline, and arginine was assessed by stable isotope techniques in 7 patients receiving a perioperative supplement of intravenous alanyl-glutamine (0.5 g · kg⁻¹ · d⁻¹).

Results: Plasma glutamine, citrulline, and arginine concentrations increased significantly in patients receiving intravenous glutamine dipeptide. At whole-body level, 91% of total citrulline turnover was derived from glutamine, whereas 49% of whole-body citrulline turnover was used for de novo synthesis of arginine. The kidneys were responsible for 75% of whole-body arginine production from citrulline.

Conclusions: Glutamine and citrulline are important sources for de novo arginine synthesis. The kidneys are the main production site for endogenous arginine. After comparison of these results with previous similar studies, our data suggest that an intravenous glutamine supplement doubles renal arginine production from citrulline. This trial was registered at www.trialregister.nl as NTR2914.


INTRODUCTION

Arginine is a conditionally essential amino acid. This means the normal endogenous arginine production meets the need of the adult human body, but in pathophysiologic conditions, de novo arginine synthesis becomes inadequate. Without adequate supplementation of arginine or its precursors, arginine availability may decrease to concentrations jeopardizing normal biological responses (1, 2). Arginine is a molecule of particular interest because it plays a role in many different pathways in multiple cell types. Arginine is an important regulator of protein synthesis and proteolysis, and it serves as the precursor for nitric oxide, creatine, agmatine, polyamines, proline, and glutamate (3).

Studies in animals and humans showed that arginine becomes an essential amino acid in several pathologic conditions, such as after traumatic surgical injury and during cancer, critical illness, and infections (4, 5). This arginine deficiency may lead to immunosuppression, impaired recovery, disturbed inflammatory response, and diminished vascular function (2, 4). Unfortunately, arginine supplementation showed controversial effects in critically ill patients (6). Yet, it was also found that glutamine administration increases plasma concentrations of arginine (7–10). Metabolic studies showed that glutamine is an important precursor for the synthesis of citrulline in the intestines and arginine by the kidneys (glutamine-citrulline-arginine intestinal-renal axis) (11–13) and also has been quantified by using a stable isotope method (12–16).

Moreover, glutamine supplementation has positive effects on clinical outcome in several diseases (17–20). It has been suggested that a major part of glutamine’s effects can be attributed to the formation of arginine from the administered glutamine (21).

However, data on the clearly defined net contribution of glutamine to the synthesis of citrulline and arginine when extra glutamine is administered are lacking. When translating research, the development of optimal nutritional strategies relies on distinct insights into the effect of a supplemental component on metabolic pathways. Especially now that a recently published randomized trial has shown controversial effects of high-dose glutamine supplementation (22), in-depth metabolic data on the effects of glutamine supplementation are desired more than ever. We hypothesized that a parenteral supplement of glutamine, provided as a dipeptide, stimulates citrulline formation and enhances de novo arginine synthesis in the kidneys in humans in the post-absorptive state. We designed this stable isotope study in glutamine-supplemented patients undergoing abdominal surgery to investigate the effects of extra glutamine on whole-body and renal metabolism of glutamine, citrulline, and arginine.

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SUBJECTS AND METHODS

Patients

Whole-body and renal amino acid kinetics were quantified in 7 patients during abdominal surgery. Patients with parenchymal liver disease, renal failure, inborn metabolic disease, type 1 diabetes, recent weight loss, cachexia, or other indications of metabolic disorders were excluded from the study. After a baseline blood sample was drawn along with a blood sample for routine preoperative laboratory tests, a primed continuous intravenous infusion of 0.5 g alanyl-glutamine · kg⁻¹ · d⁻¹ (Dipeptiven; Fresenius Kabi) was administered 1 d before surgery. The dosage of 0.5 g alanyl-glutamine · kg⁻¹ · d⁻¹ has been proven to be safe and is recommended by the manufacturer. Oral intake was allowed only until 12 h before surgery, except for water. Written informed consent was obtained from all patients. The study protocol was approved by the Medical Ethics Committees of the VU University Medical Center, Amsterdam, Netherlands, and was registered at www.trialregister.nl in the Netherlands trial register (NTR2914).

Study design

The study design consisted of a 2.5-h tracer infusion and blood sampling during open abdominal surgery, enabling steady-state isotopeomer calculations. The metabolic study was conducted during major abdominal surgery to quantify renal citrulline and arginine turnover. The tracer infusion and the blood sampling were performed during the first exploratory phase of the laparotomy, before invasive surgical intervention of abdominal or retroperitoneal anatomical structures. During the study period, an antecebulal artery catheter was used for tracer infusion. This catheter was already in place for alanyl-glutamine infusion and clinical purposes. Blood was sampled from a radial artery catheter, installed according to a standard perioperative protocol. In all patients, anesthesia and epidural analgesia were applied according to a standard protocol.

Stable isotope tracers

The tracers [2-¹⁵N]glutamine, [5-¹³C,²H₄]citrulline, and [¹⁵N₂]arginine were purchased from Cambridge Isotope Laboratories. The Department of Clinical Pharmacy at the Erasmus Medical Center in Rotterdam, Netherlands, prepared sterile and pyrogen-free stock solutions of the tracers. The glutamine tracer was prepared the day before surgery because of the limited stability of glutamine in solution (72 h). The stock solutions were diluted with a physiologic saline solution just before the start of the tracer infusion.

Tracer infusion, blood sampling, and renal blood flow measurement

After baseline sampling, a primed, continuous intravenous infusion of the stable isotope tracers was administered and continued for 2.5 h. Tracer infusion was controlled by a calibrated, volume-controlled pump (Graseby 3000; Graseby Medical Ltd).

Blood samples were drawn at 30, 60, 90, 120, and 150 min after the beginning of the tracer infusion. After ~120 min, at isotopic steady state, blood was drawn from both the radial artery catheter and the renal vein by direct puncture simultaneously to study renal metabolism. The renal vein was sampled before organ clamping or transection.

Blood was collected in heparinized vacuum tubes (Vacutainer; Becton Dickinson) and placed on ice. First, the hematocrit of the blood samples was measured. Blood was centrifuged (10 min, 1910 × g, 4°C), and 50 µL of the supernatant was deproteinized with 20 mg dry sulfosalicylic acid within 1 h after sampling. After mixing, samples were stored at −80°C until analysis.

To quantify renal amino acid fluxes, we determined renal blood flow with a color Doppler ultrasound (Aloka Prosound SSD 5000; Aloka Co Ltd) as described previously (12); time-averaged mean velocity of the bloodstream and cross-sectional area of the right renal artery were measured during the explorative phase of the operation. Blood flow was calculated by multiplying the cross-sectional area with the velocity of the bloodstream. Plasma flow was calculated by the following equation: plasma flow = blood flow/(1 − hematocrit). Total renal flow was estimated by multiplying plasma flow by 2. Mean renal plasma flow was used to calculate amino acid fluxes across the kidneys.

Mass spectrometric analysis

Plasma enrichments of the infused tracers and the tracer products were measured by liquid chromatography/mass spectrometry (23). Briefly, 20 µL hydrochloric acid, 20 µL internal standard, and 200 µL cation exchange solution [0.1 mg/mL AG 50W-X8 resin (Bio-Rad), 200–400 mesh] were added to 50 µL deproteinized plasma. After mixing and centrifugation, the supernatant was removed. The resin was washed with 1 mL water, and the amino acids were extracted twice with 500 µL methanol with 0.5 mmol/L tridecafluoroheptanoic acid and 20 µL of 6 mol/L ammonia, respectively. The eluate was dried in a SpeedVac (Genevac Ltd) and redissolved in 80 µL acetonitrile, 20 µL of 1 mol/L sodium carbonate, 200 µL water, and 140 µL pyridine-ethanol (4:1). Derivatives of the amino acids were prepared by adding ethyl chloroformate and incubating for 5 min. After 2 extractions with ethyl acetate, the combined solutions of both the first (400 µL) and second (400 µL) extractions were evaporated under a gentle stream of nitrogen at room temperature until it was almost dry and redissolved in 100 µL 20% methanol. Analyses were performed with liquid chromatography/mass spectrometry (Velas Pro; Thermo Fisher) by injecting 10 µL sample extract on a 2.1- to 100-mm, 1.7-µm Waters Acquity BEH C18 column. Elution was performed at a stable temperature of 40°C by using mobile phases consisting of 0.5 mmol/L tridecafluoroheptanoic acid and 1 mL/L formic acid (phase A) and methanol with 0.5 mmol/L tridecafluoroheptanoic acid and 1 mL/L formic acid (phase B). Ion abundance was monitored in full scan by using the zoom-scan modus for glutamine (m/z 245–260), citrulline (m/z 274–290), ornithine (m/z 303–320), and arginine (m/z 273–290).

Calculations

All equations used for calculating the whole-body and organ metabolism of glutamine, citrulline, and arginine are described in Table 1. Isotope enrichments were expressed as mole percent excess, calculated as enrichment at steady state minus isotopic background measurements at baseline. Whole-body turnover of glutamine, citrulline, and arginine was calculated. Furthermore,
Renal arginine production from citrulline

\[ QCitrulline \]

Renal TNB (MPE); EArg M+1, plasma enrichment of arginine [M+1] (MPE); EArg M+5, plasma enrichment of arginine [M+5] (MPE); ECit M+1, plasma enrichment of glutamine infusion (glutamine to arginine; QGln-Cit, whole-body glutamine to citrulline conversion rate (TNB

Renal FE (%)

\[ NB = (|A| - |V|) \times F \]

Renal disposition

\[ FE = \text{Tracer NB}/(|A| \times EX \times F) \]

Renal production

\[ \text{TNB/EV} \]

Renal arginine M+5 output

\[ (|V| \times EY \times F) - (|A| \times EX \times F \times (1 - FE)) \]

Renal arginine production from citrulline

\[ Q\text{Citrulline}\rightarrow\text{Arginine} = \text{Arginine}_{M+5} \times \text{Output}/(|A_{Citrulline}} \times E_{A-Citrulline} \times F)) \times (|A_{Citrulline}} \times F) \]

Whole-body turnover

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

Whole-body endogenous glutamine flux

\[ Q_{\text{endogenous}} = \text{Total}Q_{\text{cit}} - Q_{\text{glu-exo}} \]

Whole-body conversion rate of glutamine to citrulline

\[ Q_{\text{Citrulline}} = E_{Citrulline} \times Q_{\text{Citrulline}} \times Q_{\text{Citrulline}} \]

Whole-body conversion rate of citrulline to arginine

\[ Q_{\text{Arginine}} = E_{\text{Arginine}} \times Q_{\text{Arginine}} \times Q_{\text{Arginine}} \]

Whole-body conversion rate of glutamine to arginine

\[ E_{\text{Arginine}} = E_{\text{Arginine}} - E_{\text{Arginine}} \]

Whole-body conversion rate of glutamine to citrulline

\[ E_{\text{Citrulline}} = E_{\text{Citrulline}} - E_{\text{Citrulline}} \]

Net renal influx

\[ NB = (|A| - |V|) \times F \]

Tracer NB

\[ (|V| \times EY \times F) - (|A| \times EX \times F \times (1 - FE)) \]

Renal FE (%)

\[ FE = \text{Tracer NB}/(|A| \times EX \times F) \]

Statistical analysis

Results are presented as means ± SEMs. Enrichment curves were fitted to determine a mean steady state value per individual by using Prism 5.0 for Windows (GraphPad Software Inc). Quantile-quantile plots of the data showed linearity, indicating that the distribution is consistent with the assumption of normality. The Student’s t test was used to determine significant differences in amino acid concentrations and isotopic enrichments between the plasma sample before the start of parenteral alanly-glutamine administration and during parenteral alanly-glutamine supplementation just before the tracer infusion. The 1-sample t test was used to test whether the values were significantly different from zero. SPSS 20.0 for Windows software (SPSS Inc) was used to perform statistical tests. P < 0.05 was considered to indicate a significant difference.

RESULTS

Baseline characteristics of the patients are shown in Table 2. The patients received 0.5 g alanly-glutamine · kg⁻¹ · d⁻¹ for at least 15 h before the start of the tracer protocol. Tracer dosage for [5-¹³C, 2H₄]citrulline and [¹⁵N₂]arginine was comparable with that in other studies (11, 18, 21); [2-¹⁵N]glutamine tracer dosage was corrected for the simultaneous alanly-glutamine infusion to reach adequate enrichments (Table 3). We found significant enrichments of glutamine [M+1], citrulline [M+5] and citrulline [M+1], and arginine [M+2] and [M+5], which confirms the metabolic route from glutamine to citrulline and arginine. No significant tracer enrichments for ornithine were detected. Arterial plasma enrichments of the infused tracers and the tracer products were observed to be in steady state (Figure 1).

![Table 1](https://academic.oup.com/ajcn/article-abstract/100/5/1385/4576623/1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-body turnover</td>
<td>[ Q = i([E/E_p] - 1) ]</td>
</tr>
<tr>
<td>Whole-body endogenous glutamine flux</td>
<td>[ Q_{\text{endogenous}} = \text{Total}Q_{\text{cit}} - Q_{\text{glu-exo}} ]</td>
</tr>
<tr>
<td>Whole-body conversion rate of glutamine to citrulline</td>
<td>[ Q_{\text{Citrulline}} = E_{Citrulline} \times Q_{\text{Citrulline}} \times Q_{\text{Citrulline}} ]</td>
</tr>
<tr>
<td>Whole-body conversion rate of citrulline to arginine</td>
<td>[ Q_{\text{Arginine}} = E_{\text{Arginine}} \times Q_{\text{Arginine}} \times Q_{\text{Arginine}} ]</td>
</tr>
<tr>
<td>Whole-body conversion rate of glutamine to arginine</td>
<td>[ E_{\text{Arginine}} = E_{\text{Arginine}} - E_{\text{Arginine}} ]</td>
</tr>
<tr>
<td>Whole-body conversion rate of glutamine to citrulline</td>
<td>[ E_{\text{Citrulline}} = E_{\text{Citrulline}} - E_{\text{Citrulline}} ]</td>
</tr>
<tr>
<td>Net renal influx</td>
<td>[ NB = (</td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Values (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, F/M (n)</td>
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</tr>
<tr>
<td>Age (y)</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Amino acid concentration (μmol/L)</td>
<td>Glutamine 667 ± 30</td>
</tr>
<tr>
<td>Glutamine</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Arginine</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>Preoperative laboratory results</td>
<td>Creatinine 84 ± 9</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min)</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td>7 infrarenal abdominal aorta aneurysm repairs</td>
</tr>
<tr>
<td>Urine production during protocol (mL)</td>
<td>156 ± 40</td>
</tr>
<tr>
<td>Total fluid provided during protocol (mL)</td>
<td>1489 ± 199</td>
</tr>
<tr>
<td>Total renal plasma flow (mL · kg⁻¹ · min⁻¹)</td>
<td>5.58 ± 0.5</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEMs unless otherwise indicated.
² Before alanly-glutamine administration.
Amino acid concentrations increase with a supplement of glutamine

The plasma concentrations of glutamine, citrulline, and arginine significantly increased after the administration of intravenous 0.5 g alanyl-glutamine \( \cdot \) kg\(^{-1}\) \cdot d\(^{-1}\) compared with baseline (Figure 2).

Whole-body amino acid turnover

Whole-body plasma turnover of glutamine, citrulline, and arginine was 423 ± 29, 9.5 ± 1.0, and 30.4 ± 2.2 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), respectively. Whole-body endogenous glutamine flux was 327 ± 29 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\). Estimated whole-body citrulline production from plasma glutamine was 8.1 ± 0.9 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), representing 91% of the total citrulline turnover. Forty-nine percent of total citrulline turnover was used for de novo arginine synthesis at the whole-body level, which was 4.5 ± 0.6 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\). Whole-body arginine \([M+1]\) production derived from glutamine \([M+1]\) was 6.33 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\). Because this is more than 100% of the citrulline-to-arginine conversion rate, this is probably an overestimate caused by nitrogen recycling or channeling, as discussed later.

Renal metabolism

To determine organ-specific amino acid handling, we used the enrichment of simultaneously taken arterial and venous samples. Arterial and venous plasma enrichments and concentrations are summarized in Table 4. Renal net balance of citrulline showed an uptake of 4.1 ± 1.2 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), and this was accompanied by a net release of arginine of 4.0 ± 0.7 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), illustrating the role of the kidney in arginine metabolism (Figure 3). Eighty-five percent of the net renal citrulline \([M+5]\) uptake was used for net arginine \([M+5]\) production. Total renal arginine production from citrulline was 3.0 ± 0.7 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), representing 72% of net renal citrulline uptake and 74% of net renal arginine release. The kidneys were responsible for 75% of de novo arginine synthesis at the whole-body level.

DISCUSSION

This study shows the qualitative and quantitative effects of an intravenous supplement of glutamine on whole-body and renal metabolism of glutamine, citrulline, and arginine in humans in the postabsorptive state. The use of the \([2-^{15}N]\)glutamine tracer showed that almost all circulating citrulline derived from glutamine. Approximately half of the circulating plasma citrulline was used for arginine production. The kidneys were responsible for 75% of whole-body de novo arginine synthesis, with a production rate of approximately 3.0 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\).

This study’s effect of the intravenous glutamine supplement on the metabolism of citrulline formation and arginine production should be interpreted in the background of results obtained in previous stable isotope studies with a similar design. The study by Ligthart-Melis et al (12) is comparable to the current study, because similar intravenous stable isotopes were used in human subjects in the postabsorptive state during abdominal surgery to determine renal metabolism. In those patients, the whole-body glutamine flux was 240 ± 14 \( \mu \)mol \( \cdot \) kg\(^{-1}\) \cdot h\(^{-1}\), whereas in our patients receiving a supplement of glutamine, the whole-body total glutamine flux was almost doubled and the endogenous glutamine flux was ∼40% higher. In both studies, half of circulating citrulline was used for de novo arginine synthesis. However, in our study, the glutamine supplement caused an increase in citrulline concentrations, and renal arginine production

![FIGURE 1](https://academic.oup.com/ajcn/article-abstract/100/5/1385/4576623 by guest on 30 November 2018)
from citrulline was 3.0 ± 0.7 μmol·kg⁻¹·h⁻¹ compared with only 1.5 ± 0.7 μmol·kg⁻¹·h⁻¹ in the study without glutamine administration (12). Thus, renal arginine production seems to be doubled in humans receiving 0.5 g alanyl-glutamine · kg⁻¹ · d⁻¹ intravenously compared with previous results in humans without glutamine supplementation. This was also reflected by the significant increase of arginine plasma concentration during glutamine infusion compared with baseline in the current study.

Arginine is an important regulator of the immune system, cell homeostasis, and protein synthesis, and it plays important roles in multiple metabolic pathways (24). In addition, arginine is the sole precursor for nitric oxide generation, a signaling agent with a crucial role in immunity, inflammation, and organ perfusion (25). As demonstrated in previous studies and the present study, citrulline can be converted into arginine, mainly in the kidneys (12, 26). This de novo arginine synthesis has been shown to be the main regulator of plasma arginine concentrations (11). Plasma arginine concentrations can be affected in pathologic conditions in which the disposal capacity of arginine is enhanced (5, 27–31). Thus, in various pathologic conditions, de novo arginine synthesis appears to be insufficient. Systemic arginine deficiency contributes to immunosuppression, inflammation disorders, and vascular dysfunction in sick patients, which may lead to concomitant morbidity and mortality (4). Metabolic studies suggested that an arginine deficiency is related to a decreased glutamine availability, affecting intestinal citrulline formation and subsequently arginine production (11). Conversely, after glutamine supplementation, an increase in citrulline and arginine plasma concentrations is found (7, 10, 32). Other intermediates of the intestinal-renal axis have been suggested to enhance arginine production as well. For example, the supplementation of ornithine α-ketoglutarate resulted in increased citrulline and arginine concentrations, whereas ornithine supplementation did not (33). Furthermore, citrulline regulates de novo arginine synthesis, and citrulline supplementation could augment a similar effect on arginine production, as shown in the current study. However, glutamine is still considered the premier precursor of arginine via the intestinal-renal axis. In fact, in this study, we confirmed the existence of this precursor role and demonstrated that glutamine supplementation enhances renal de novo arginine synthesis from citrulline in comparison with previously published results (12).

Glutamine administration showed to be beneficial in several pathologic conditions (7, 10). Recently, the administration of high doses of glutamine in critically ill patients with multiorgan failure became controversial after possible adverse effects were found in these patients (22). However, this clinical trial included patients with kidney failure and liver failure, which are both contraindications for glutamine supplementation. After combination of available studies on glutamine supplementation, however, it was found that glutamine still may prevent and limit infections, improve recovery from injury, and positively affect mortality (34–36). Because glutamine is the substrate for citrulline and arginine, (part of) the effects of glutamine supplementation could be mediated by its derivatives. As described before (11), de novo arginine production from citrulline is the regulating factor in optimizing plasma arginine concentrations in the body. Excessive arginine supplementation could also have adverse effects, probably from the excessive formation of nitric oxide and oxidative metabolites, subsequently leading to oxidative stress (6). Glutamine supplementation is suggested to be a more physiologic way of correcting arginine concentrations and subsequently achieving both glutamine and arginine benefits. In light of previous work, our results seem to support this by showing the qualitative and quantitative effects of the glutamine supplement on renal de novo arginine synthesis from citrulline.

Some specific patient populations may benefit most from a supplement of glutamine because they show disease-related low glutamine and arginine concentrations. In combination with data from the literature, our results indicate that in these specific patient populations, intravenous glutamine supplementation could restore renal arginine production. However, the aim of this study was to investigate the effect of glutamine administration on arginine production at whole-body level as well as in the kidneys under the most physiologic condition possible. The included patients did not have kidney failure and liver failure. Therefore, we concluded that glutamine supplementation, when compared with baseline, doubled the arginine plasma concentration during glutamine infusion compared with previous results in humans without glutamine administration (12).
not have metabolic disorders, organ failures, or aberrant diets; glutamine, citrulline, and arginine concentrations were in the normal ranges; and the tracer protocol was conducted in the exploratory phase of surgery. Although our results suggest that an intravenous supplement of glutamine could correct depleted arginine concentrations in patients with a pathologic induced arginine deficiency, quantitative and qualitative studies using a stable isotope technique in these specific patient populations should be performed in the future. Furthermore, the patients in this study had an average high BMI and were relatively older, representative of patients with abdominal aortic aneurysm, which may have influenced our metabolic measurements.

The use of stable isotope tracers to quantify glutamine to arginine metabolism has been a subject of discussion in the past years, ever since a study by Marini et al (15) showed that the use of a [2,15N]glutamine tracer may overestimate the quantitative contribution of glutamine to arginine in mice because of nitrogen recycling. However, studies in humans showed that citrulline plasma concentrations increase after glutamine supplementation, which is confirmed by our study. This is possible only when a substantial part of the carbon skeleton of glutamine is used for citrulline formation. An excellent study by Tomlinson et al (16) evaluated this complexity of the glutamine to arginine pathway in humans in the fed state by using both a C-labeled and an N-labeled glutamine tracer. Similar to our results, they found that the N-labeled tracer showed a contribution of glutamine [M+1] to arginine [M+1] synthesis that exceeded the citrulline to arginine conversion. Furthermore, they demonstrated that the labeled N-atom was found in various locations of the ornithine, citrulline, and arginine molecules. Thus, although they demonstrated that the N-atom from glutamine is used for ornithine formation and the equivalent isotopomers of citrulline and arginine, the way it contributes to the formation of the isotopomers remains indeterminate. Our results showed citrulline [M+1] and arginine [M+1] enrichments, but ornithine enrichments were not significant, indicating a complex contribution of the N-atom in this pathway. Consequently, we agree with Tomlinson et al (16) that the N-labeled glutamine tracer can provide qualitative information on the glutamine-to-citrulline and arginine pathway, yet quantitative results should be interpreted with caution because outcomes may overestimate the contribution of glutamine to citrulline and arginine formation. More important, quantitatively they confirmed with a [1,13C]glutamine tracer that the carbon skeleton of glutamine is used for ~50% of de novo arginine synthesis in humans, supporting the existence of the glutamine-citrulline-arginine pathway (16). With this in mind, future stable isotope studies to elucidate glutamine to arginine metabolism in humans should be performed by using a carbon-labeled glutamine tracer.

In conclusion, this study was conducted to investigate the way in which a therapeutic dose of parenteral glutamine affects the synthesis of arginine at the whole-body level and in the kidneys specifically. Although the amino acid kinetics was examined in only 7 patients, our data consistently showed that during glutamine supplementation, most of the circulating citrulline derived from glutamine, and 49% was used for de novo arginine synthesis in the kidneys. The intravenous glutamine supplement resulted in significantly higher glutamine, citrulline, and arginine plasma concentrations. To our knowledge, this is the first quantitative study showing that renal arginine production from citrulline is enhanced in patients receiving an intravenous glutamine supplement when comparing these results with previously published data. In conclusion, an intravenous supplement of glutamine dipeptide enhances de novo arginine synthesis in the kidneys of humans in the postabsorptive state.

The authors’ responsibilities were as follows—NB, SJHB, and MARV: contributed to the study design; NB, SJHB, and AB: contributed to the implementation of the study; NB, SJHB, and JL: conducted the research and collected the data; WW: supervised during surgical procedures; JEO and HS: performed mass spectrometry and amino acid concentration analyses; NB and MARV: performed calculations and statistical analysis; HS, JBrG, and APJH: helped with interpretation of the data; NB, SJHB, and JEO: drafted the manuscript; JL, HS, WW, AB, JBrG, APJH, and MARV: critically reviewed the manuscript; and PAMvL: had primary responsibility for all aspects of the study and for final content. PAMvL reports receiving fees from Fresenius Medical Care for clinical consultation. None of the other authors reported a conflict of interest related to the study. Fresenius Kabi provided alanyl-glutamine (Dipeptiven) for this study, and the Vivax Foundation supported this work with a research grant; both entities did not participate in data collection, data analysis, data interpretation, or writing of the manuscript.

REFERENCES