Intestinal renal metabolism of L-citrulline and L-arginine following enteral or parenteral infusion of L-alanyl-L-[2,15N]glutamine or L-[2,15N]glutamine in mice

Petra G. Boelens,1 Paul A. M. van Leeuwen,1 Cornelis H. C. Dejong,2 and Nicolaas E. P. Deutz2

1Department of Surgery of the Vrije Universiteit University Medical Center, Amsterdam; and 2University of Maastricht and Nutrition and Toxicology Research Institute, Maastricht, The Netherlands

Submitted 24 January 2005; accepted in final form 25 May 2005

Boelens, Petra G., Paul A. M. van Leeuwen, Cornelis H. C. Dejong, and Nicolaas E. P. Deutz. Intestinal renal metabolism of L-citrulline and L-arginine following enteral or parenteral infusion of L-alanyl-L-[2,15N]glutamine or L-[2,15N]glutamine in mice. Am J Physiol Gastrointest Liver Physiol 289: G679 –G685, 2005. First published July 28, 2005; doi:10.1152/ajpgi.00026.2005.—Previously, we observed increased plasma arginine (ARG) concentrations after glutamine (GLN)-enriched diets, in combination with clinical benefits. GLN delivers nitrogen for ARG synthesis, and the present study was designed to quantify the interorgan relationship of exogenous L-GLN or GLN dipeptide, by enteral or parenteral route, contributing to intestinal citrulline (CIT) and renal de novo ARG synthesis in mice. To study this, we used a multicatheterized mouse model with Swiss mice (n = 43) in the postabsorptive state. Stable isotopes were infused into the jugular vein or into the duodenum (per group either free L-[2,15N]GLN or dipeptide L-ALA-L-[2,15N]GLN, all with L-[ureido-13C-2H3]CIT and L-[guanidino-15N2-2H2]ARG) to establish renal and intestinal ARG and CIT metabolism. Blood flow was measured using 14C-para-aminohippuric acid. Net intestinal CIT release, renal uptake of CIT, and net renal ARG efflux was found, as assessed by arteriovenous flux measurements. Quantitatively, more de novo L-[2,15N]-CIT was produced when free L-[2,15N]GLN was given than when L-ALA-L-[2,15N]GLN was given, whereas renal de novo L-[2,15N]-ARG was similar in all groups. In conclusion, the intestinal-renal axis is hereby proven in mice that L-[2,15N]GLN or dipeptide were both converted into de novo renal L-[2,15N]ARG; however, not all was derived from intestinal L-[2,15N]CIT production. In this model, the feeding route and form of GLN did not influence de novo renal ARG production derived from GLN.

A need for L-arginine (L-ARG) may develop under conditions of metabolic stress such as surgical trauma and sepsis (19). Renal L-ARG synthesis is completely dependent on the availability of circulating L-citrulline (CIT), and the small intestine is the major source of circulating L-CIT (12, 26). L-Glutamine (GLN) and/or L-glutamate (GLU) are the substrates for L-CIT production in the gut (25). This pathway, also described as the intestinal-renal axis, was also suggested from the findings of increased plasma L-ARG levels in trauma patients receiving enteral L-GLN (15). Because the L-ARG contents of the feedings in that study were similar in the study group and in the control group, we suggested that renal L-ARG synthesis accounted for the increase in L-ARG plasma values in patients receiving L-GLN-enriched nutrition (15). This pathway of exogenous delivered L-GLN serving as a substrate for intestinal L-CIT production and renal L-ARG synthesis has not yet been quantified by stable isotope technology across the intestinal-renal axis in mice in vivo.

It has been recognized that an adequate availability of L-ARG is important during metabolic stress because L-ARG is the physiological precursor for the synthesis of nitric oxide (NO), which is identified as the endothelium-dependent relaxing factor, a mediator of immune responses, a neurotransmitter, and a signaling molecule (9). L-ARG depletion compromises these important functions. Several studies provide evidence that supports the clinical relevance of supplementing L-ARG (19). Sufficient supply of L-ARG is thus of nutritional and physiological importance, and, for that reason, our hypothesis is that by increasing the availability of L-GLN, via increased intestinal L-CIT production, renal L-ARG synthesis might increase.

Although physiological concentrations of L-GLN are the highest of all circulating amino acids, metabolic stress such as surgery is responsible for a critical reduction of L-GLN’s bioavailability (7). Oudemans-van Straaten et al. (21) concluded in patients admitted to the intensive care unit that low-plasma L-GLN relates to higher age, shock as primary diagnosis, and higher hospital mortality. Moreover, they showed that low-plasma L-GLN represents a risk of poor outcome. Thus it is of crucial importance to prevent or improve low-plasma L-GLN, and many studies show that adding L-GLN to the nutrition of diverse patient populations can improve outcome (7).

Unfortunately, free L-GLN is relatively unstable, and the pharmacological complexity implies that it is therefore less suitable to be a compound of a shelf-stored, water-based nutritional formula (14). The GLN dipeptide L-alanyl-L-GLN, which is stable in watery solution, is rapidly hydrolyzed in mammals to free L-GLN and L-alanine (L-ALA) and seems to be a good alternative (2–4). However, it is not known whether the metabolic fate of enteral dipeptide-derived L-GLN is similar to the fate of enteral free L-GLN in relationship to L-ARG production.

Although we found that free L-GLN-enriched enteral nutrition increased plasma L-ARG concentrations (15), most of the studies investigating GLN-enriched parenteral nutrition did not show changes in plasma L-ARG. Because the gut has a key role in GLN’s conversion to L-CIT, we hypothesized that L-GLN would enhance L-CIT production when luminally offered to the intestines compared with parenterally delivered L-GLN, and,

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Therefore, enteral GLN could lead to more substrate for renal L-ARG production.

To better understand 1) the metabolic basis of the nutritional requirement of L-GLN, 2) the extent to which GLN serves as a substrate for L-CIT or L-ARG, and 3) whether feeding route (enteral or parenteral) alters this pathway, it is necessary to explore the quantitative aspects of CIT and ARG metabolism during GLN supply. This has, to our knowledge, not been studied before. Therefore, we used a combination of stable isotope tracers with arteriovenous substrate-metabolite balance measurements across the intestines and kidneys to gain more insight into the interorgan metabolism of these specific amino acids.

The purpose of this study was to elucidate whether different routes of delivery of GLN and biological availability coming from the free amino acid or the dipeptide influences the intestinal L-CIT production and, thereby, renal L-ARG production.

**MATERIALS AND METHODS**

Experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, and they were approved by the Ethical Committee of Animal Research of Maastricht University.

**Animals**

Male Swiss mice \(n = 43, 20 \pm 0.5 \text{ g body wt (mean } \pm \text{ SE)}\) were obtained from IFFA Credo Broekman (Somerden, The Netherlands). The mice were fed standard lab chow (SMRA 2131, Hope Pharmas, Woerden, The Netherlands) before the day of the experiments and were subject to standard 12:12-h light-dark cycle periods (7:30 AM to 7:30 PM). Room temperature was maintained at 25°C. Radio music was on to acquaint the animals with noise in the environment to reduce their stress toward noise induced by the caretakers/researchers. All experiments were performed in the postabsorptive state.

**Experimental Design**

Mice received one of four infusions. Mice in the first series were assigned to group 1 or 2, and mice in the second series were assigned to group 3 or 4; group 1 received parenteral L-ALA-[2-15N]GLN infusion, \(n = 11\); group 2 received enteral L-ALA-[2-15N]GLN infusion, \(n = 12\); group 3 received parenteral L-[2-15N]GLN infusion, \(n = 10\); and group 4 received enteral L-[2-15N]GLN infusion, \(n = 10\).

A primed continuous infusion of L-[2-15N]GLN or L-ALA-[2-15N]GLN, depending on the group, was administered for 1 h simultaneously with intravenous infusion of L-ureido-[13C]-H2CIT and L-guanidino-[15N2]-H2ARG (steady-state model). The plasma flow through the intestines and kidneys was measured by using an indicator dilution technique with a primed continuous infusion of 14C para-aminohippuric acid (14C-PAH) (13).

The protocol of Hallemeech et al. (13) was used. Briefly, after the mice were weighed, anesthesia was induced by an intraperitoneal injection of a mixture of ketamine (62.5 mg/kg body wt; Nimatek, AUV, Cuyk, The Netherlands) and medetomidine (400 \(\mu\)g/kg body wt; Domitor, Farmos, Espoo, Finland). A subcutaneous injection of 1.5 ml saline was given in the neck. Anesthesia was maintained with a continuous subcutaneous infusion of a mixture of ketamine (17.5 mg·kg body wt·1·h-1) and medetomidine (112 \(\mu\)g·kg body wt·1·h-1). During the surgical procedures, the mice were kept at 37°C using a temperature controller (Technical Service, Maastricht University) and heat pads. The jugular vein, carotid artery, portal vein, right renal vein, mesenteric vein, and duodenum were catheterized using a 30-gauge needle fitted to a Silastic tube (Silastic Medical Grade tubing 0.40-mm inner diameter, 0.84-mm outer diameter, Dow Corning Medical Products, Midland, MO) and were fixed with cyanoacrylate (Cyanolit 201, Het Rubberhuis, Maastricht, The Netherlands) (13).

\[1^4\text{C]}\text{PAH Infusion Protocol for Blood Flow Measurement}\]

The nontoxic indicator [14C]PAH (New England Nuclear Life Science Products, Boston, MA) was used for plasma flow measurements as described by Hallemeech et al. (13).

**Tracer Infusion Protocol**

A primed-constant infusion of stable isotopes (Cambridge Isotope Laboratories, Andover, MA; the dipeptide tracer was a kind gift of Prof. D. E. Matthews, University of Vermont, Burlington, VT) was given in the jugular vein (per mouse an equimolar amount of either intravenous L-[2-15N]GLN [2.65 mg t-GLN per experiment (1 h)] or intravenous L-ALA-[2-15N]GLN [4.24 mg t-alanyl-GLN per experiment, equivalent to 2.86 mg GLN (1 h)]; either enteral L-[2-15N]GLN or enteral L-Ala-[2-15N]GLN; all groups simultaneous with intravenous L-[ureido-13C]-H2CIT and L-[guanidino-15N2]-H2ARG (Table 1). The GLN or dipeptide tracer was administered in the duodenum in the enteral groups. The mice receiving infusions of the GLN or dipeptide stable isotopes in the jugular vein were called the intravenous groups. In a pilot experiment, the time to reach tracer steady state in mice was confirmed to be 20–30 min, as previously shown (8, 24). In the experiments of this study, we therefore sampled blood 40 min after the start of the primed continuous infusion from the carotid artery, portal vein, or renal vein. Arterial amino acid concentrations and whole body results of the same series of mice were described separately (P. G. Boelens, unpublished observations).

**Blood Sampling and Processing**

For hematocrit determinations, a microhematocrit tube (Vitrex, Herlev, Denmark) was filled with blood and centrifuged for 3 min at 6.8 g at room temperature in an EBA 12 centrifuge (Hettich, Dépxé, de Bilt, Netherlands). Hematocrit was determined by dividing the length (in mm) of the red blood cell section by the totally filled length of the tube. Blood was collected in heparinized cups (Sarstedt, Nümbrecht, Germany) on ice and centrifuged (4 min, 4°C, 8,500 g) to obtain plasma.

For 14C-PAH determinations, 20 \(\mu\)l of whole blood were added to 280 \(\mu\)l of 8.6% trichloroacetic acid and thoroughly mixed for deproteinization and centrifuging (4 min, 4°C, 8,500 g, Eppendorf 5413 centrifuge).

For determination of amino acid concentrations and tracer-tracer ratios (TTR), 65 \(\mu\)l of plasma were added to 4 mg solid 5′-sulfosalicylic acid, vortexed, frozen in liquid nitrogen, and stored at −80°C.

**Analysis of Samples**

For 14C-PAH determinations, 250 \(\mu\)l of supernatant were mixed with 6 ml scintillation fluid (Ultima Gold, Packard, Groningen, The Netherlands), and radioactivity was counted on a Wimspectral 1414 counter (Wallac, EG&G, Breda, The Netherlands).

**Table 1. Tracer prime and infusion rates**

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Prime, nmol (in bolus of: 0.04 ml/mouse)</th>
<th>Infusion, nmol/min (in: 0.2 ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-[2-15N]GLN</td>
<td>3,000</td>
<td>250</td>
</tr>
<tr>
<td>L-ALA-[2-15N]GLN</td>
<td>3,000</td>
<td>250</td>
</tr>
<tr>
<td>L-[ureido-13C]-H2CIT</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>L-[guanidino-15N2]-H2ARG</td>
<td>60</td>
<td>5</td>
</tr>
</tbody>
</table>

GLN, l-glutamine; ALA, t-alanine; CIT, L-citrulline; ARG, t-arginine.
Plasma amino acid concentration TTR were measured using a fully automated LC-MS system, using precolumn derivatization with o-phthalaldehyde. Detailed information on the accuracy and precision of this method were given before (23, 24).

Calculations

Net balances across organs. Plasma flows across the portally drained viscera (Fig. 1A) and the kidneys (Fig. 1B) were calculated using the indicator dilution technique with [14C]PAH as described recently (13). As shown in Fig. 1A, portally drained viscera (PDV) amino acid fluxes were calculated by multiplying the portal venous-arterial concentration differences with the mean PDV plasma flow. Amino acid fluxes are expressed in nanomoles per 10 g body wt per minute. As shown in Fig. 1B, renal substrate fluxes were calculated by multiplying the renal venous-arterial concentration difference with the mean renal plasma flow and are expressed in nanomoles per 10 g body wt per minute.

\[ NB = \text{plasma flow} \times ([\text{venous}] - [\text{arterial}]) \]

where NB is net balance. A positive flux indicates net release (efflux) and a negative flux reflects net uptake (influx) by the organ.

Organ Balance Tracer Methodology

TTR is an equivalent of specific activity. Therefore, formulas were derived from metabolic studies using radioactive tracers; when necessary, a correction for the contribution of lower isotopomers was performed as described by Wolfe (27). The amino acid stable isotope tracers were used to obtain information regarding the net fluxes. With the use of these tracers, organ disposal and production rates can be calculated.

Intestinal-renal amino acid turnover was calculated in a two-compartment model (27). Substrate NB or fluxes were calculated by multiplying the plasma venous-arterial concentration difference by the plasma flow. The tracer NB across an organ (TNB), the disposal, and production rate (nmol·10 g body wt⁻¹·min⁻¹) across the portally drained viscera and kidneys were calculated as

\[ \text{TNB} = \text{plasma flow} \times ([\text{arterial}] \times \text{TTR}_a) - ([\text{venous}] \times \text{TTR}_v) \]

where TTR_a and TTR_v are the TTRs of the measured amino acid in the arterial plasma and venous plasma, respectively.

Disposal was calculated as

\[ \text{disposal} = \frac{\text{TNB}}{\text{TTR}_a} \]

TTR_a was used as a surrogate precursor pool enrichment, because TTR_v, in contrast to TTR_a, more accurately represents the precursor pool TTR (27).

Production of an amino acid across the organ was calculated as

\[ \text{Production} = \text{disposal} + \text{NB} \]

where NB is the plasma venous-arterial concentration difference multiplied by plasma flow. PDV are defined as the total of all portal-drained viscera, which, in addition to the spleen and pancreas, largely represent the intestines. These are therefore referred to as the “gut” or “intestine” in the RESULTS and DISCUSSION.

Organ Amino Acid Kinetics

Calculations on the organ de novo synthesis rate of ARG are based on the same principle of isotopic conversion used for whole body calculations. The organ TNB of the isotopic metabolic product must be corrected for its loss by extraction across the organ. A good approximation of this fractional extraction can be obtained by the following partial dilution of the isotopomers.

Fractional extraction (FE) is the fraction of the total product that bypasses metabolism in the organ and so appears in the output and is used for correction of NB.

\[ \text{FE}_{\text{ARG}} = \left(\frac{\text{TTR}_a - 2\text{DARG} \times [A] - \text{TTR}_v - 2\text{DARG} \times [R]}{\text{TTR}_a - 2\text{DARG} \times [A]}\right) \times \left(\frac{\text{R}}{\text{TTR}_v \times 2\text{DARG} \times [A]}\right) \]

where R is the ARG concentration from the renal vein or the renal TTR (TTR_R) and A is the arterial ARG concentration or arterial TTR (TTR_A) of ARG or CIT. The term \( 1 - \text{FE}_{\text{ARG}} \) represents the fraction of total product that bypasses metabolism and so appears in the output and is used for correction of NB_{15NARG}.

\[ \text{NB}_{15NARG} = \text{PF} \times \frac{[\text{R}] \times \text{TTR}_R - [\text{A}] \times \text{TTR}_A}{(1 - \text{FE}_{\text{ARG}})} \]

With the use of this corrected NB_{15NARG}, the organ de novo synthesis of the metabolic product can be calculated similar to disposal in which the arterial substrate enrichment TTR_A is used as the precursor pool because the upstream compartment is thought to best reflect the intracellular delivered enrichment.

Renal ARG synthesis from¹⁵N-CIT = NB_{15NARG}/TTR_A_{15NCT}

According to the same methodology as renal ARG de novo synthesis, intestinal CIT synthesis was calculated.

Statistical Analysis

Results are presented as means ± SE as indicated in the text. For each series, normality was tested by means of the Shapiro-Wilk’s test, and, when data were normal distributed, a Student’s t-test was used to compare differences between the groups for feeding route (enterally vs. parenterally). If data were not normally distributed, a Mann-Whitney U-test for comparisons between the feeding routes was used (SPSS for Windows, release 11.0.1 2001; SPSS, Chicago, Il). Significance was defined as P < 0.05.

RESULTS

Plasma Flow

Intestinal and renal plasma flow was not affected by route of administering L-GLN (Table 2). Net Amino Acid Organ Fluxes

L-GLN fluxes. Intravenous infusion of L-GLN resulted in intestinal GLN influx, whereas enteral administration of L-GLN led to L-GLN efflux (P < 0.05; Fig. 1). Similarly, the dipeptide infusion showed this difference (P < 0.05).

Table 2. Plasma flow and net balance over PDV and kidney

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-ALA-L-GLN IV</td>
<td>L-ALA-L-GLN EN</td>
</tr>
<tr>
<td>Intestinal PF</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Renal PF</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>GLU PDV</td>
<td>47±19</td>
</tr>
<tr>
<td>ALA PDV</td>
<td>186±89</td>
</tr>
<tr>
<td>EAA PDV</td>
<td>49±79</td>
</tr>
</tbody>
</table>

Values are means ± SE in mL·10 g body wt⁻¹·min⁻¹ for plasma flow (PF) and in nmol·10 g body wt⁻¹·min⁻¹ for net balance. Positive amino acid fluxes indicate release in portal vein or renal vein, and negative amino acid fluxes indicate uptake by the splanchic viscera or kidney. IV, intravenous infusion; EV, arterial infusion; PDV, portally drained viscera or intestine. List of all amino acid fluxes available on request. Student’s t-test was used for parametric data. *P < 0.05 is significant for the feeding route. Series 1 had significant differences among the groups. Mann-Whitney U-test was used when data were nonparametric.
All groups released L-CIT in the portal vein, and, in all groups, uptake of L-CIT by the kidney was observed, but there were no differences among the groups (Figs. 2 and 3).

L-ARG fluxes. L-GLN or the dipeptide, by either the enteral or parenteral route, resulted in intestinal ARG uptake and renal ARG release without differences between the groups (Figs. 2 and 3).

**Effect of L-GLN on Renal L-CIT and L-ARG Metabolism**

**Renal L-CIT.** The kidneys showed more disposal of L-CIT than production, representing the net uptake of L-CIT. Renal L-CIT production was higher in mice receiving the dipeptide intravenously compared with the enteral group ($P < 0.05$; Fig. 3).

**Renal L-ARG.** Renal production of L-ARG was higher than renal L-ARG disposal, reflecting the net release of L-ARG in all groups. Renal L-ARG disposal and production were higher in the intravenous group after dipeptide infusion but were lower in the intravenous group when free L-GLN was given ($P < 0.05$; Fig. 3).

**De Novo Intestinal L-[2,15N]CIT From L-[2,15N]GLN**

L-[2,15N]GLN and L-ALA-L-[2,15N]GLN, given either enterally or parenterally, were both converted into L-[2,15N]CIT by the PRV. De novo 14 $\pm$ 2 nmol·10 g body wt $^{-1}$·min $^{-1}$ L-[2,15N]CIT was produced when L-ALA-L-[2,15N]GLN was given intravenously and enterally (16 $\pm$ 3 nmol·10 g body wt $^{-1}$·min $^{-1}$).

**Effect of L-GLN on Intestinal L-CIT and L-ARG Metabolism**

**Intestinal L-CIT.** Intestinal production of L-CIT was higher than its disposal in all groups, indicating the intestinal efflux of L-CIT (Fig. 2). Mice in series 1 that were given the dipeptide appeared to have lower CIT productions than mice in series 2 receiving free L-GLN.

**Intestinal L-ARG.** In all groups, disposal was higher than production, pointing at L-ARG utilization by the gut. Intestinal disposal of L-ARG was higher in the mice receiving free L-GLN enterally compared with the parenteral group ($P < 0.05$; Fig. 2).

**Fig. 1.** L-Glutamine (GLN) net balance over the portally drained viscera (PDV). Top: the L-GLN flux over the PDV without correction of the enteral (EN) contribution of L-GLN supply. Bottom: the net flux of L-GLN by the PDV, i.e., the enteral groups were corrected for the enteral supply of GLN. Positive amino acid fluxes indicate release in portal vein, and negative amino acid fluxes indicate uptake by the PDV. Values are means ± SE in nmol·10 g body wt (BW) $^{-1}$·min $^{-1}$. *$P < 0.05$ was considered significant between the enteral versus parenteral route, per series.

**Fig. 2.** Intestinal L-citrulline (CIT; top) and L-arginine (ARG; bottom) metabolism, showing the production, disposal, and substrate net balance over the PDV. Positive amino acid fluxes indicate release in portal vein, and negative amino acid fluxes indicate uptake by the splanchnic viscera. Values are means ± SE in nmol·10 g body wt $^{-1}$·min $^{-1}$. **$P < 0.05$ for mice receiving free L-GLN (series 2).**
Almost twice as much de novo \([L-2,15N]CIT\) was measured in the free \([L-2,15N]GLN\) groups [intravenous group: 34 ± 5 nmol·10 g body wt⁻¹·min⁻¹; enteral group: 35 ± 6 nmol·10 g body wt⁻¹·min⁻¹ (Fig. 4)].

**De Novo Renal \(L-[2,15N]ARG\) From \(L-[2,15N]CIT\)**

Approximately 25% of \([L-2,15N]GLN\) and \([L-ALA]-[L-2,15N]GLN\), given either enterally or parenterally, were both converted via \(L-[2,15N]CIT\) into \([L-2,15N]ARG\) by the kidney. The rate of ARG production by the kidney was not significantly different among the groups (Fig. 4).

**DISCUSSION**

**Major Observations**

Our study explored for the first time the interorgan pathway from exogenous \(L-GLN\) to endogenous \(L-ARG\) in mice using stable isotope technology. We quantified the effect of the feeding route, enterally or parenterally, on de novo intestinal \(L-CIT\) production and de novo renal \(L-ARG\) production following free \(L-GLN\) or dipeptide \(L\text{-}[L-GLN]\) administration.

**Validity of the Model**

The use of a multicatheterized mice model under steady-state conditions enabled in vivo flux measurements across the organs, crucial for our hypothesis (8, 13). Moreover, the unique combination of different types of stable isotope tracers in mice allowed the simultaneous quantification of the important metabolic routes. The model was used to study the disposal, production, and net balances of \(L-ARG\) and \(L-CIT\) as well as de novo synthesis by the kidneys and gut.

**Net Substrate Balance**

There was a remarkable difference between the routes of administering \(L-GLN\) or the dipeptide with respect to the intestinal flux of \(L-GLN\). It is well established that the gut is a major GLN-consuming organ, because it has high glutaminase activity (key enzyme able to hydrolyze GLN) and only low glutamine synthetase activity (5, 17, 22). For example, intravenous \(L-GLN\) infusion demonstrated the expected intestinal \(L-GLN\) uptake, but enteral free \(L-GLN\) infusion showed an opposite effect. Enteral free \(L-GLN\) caused an efflux of \(L-GLN\) from the PRV, which was larger than the enteral infusion rate of \(L-GLN\), suggesting “production or release” of \(GLN\) contrary to “GLN consumption.” Therefore, the most likely explanation for the intestinal \(L-GLN\) efflux in the enteral free \(L-GLN\) group seems to be that \(L-GLN\) is just passing through and is not from either \(L-GLN\) release from protein breakdown or \(L-GLN\) de novo production. Regarding protein breakdown, no differences were found among the four groups in intestinal efflux of essential amino acids (a measure of net protein breakdown in the postabsorptive state). Furthermore, the products of \(GLN\) breakdown, e.g., \(L-GLU\), \(L-ALA\), and \(L-CIT\), were not different with respect to the route of administration of \(L-GLN\), pointing out that at the same time a similar \(L-GLN\) uptake or consumption occurs in all groups. This renders it also less likely that intestinal \(L-GLN\) de novo production from its own degradation products, \(L-GLU\) or \(L-ALA\), might add to the GLN efflux. In rats, during acute liver insufficiency-induced hyperammonemia, a comparable net PRV GLN release was found at very low levels.

**Fig. 3.** Renal \(L-CIT\) (top) and \(L-ARG\) (bottom) metabolism, showing the production, disposal, and substrate net balance over the kidneys. Positive amino acid fluxes indicate release in renal vein, and negative amino acid fluxes indicate uptake by the kidneys. Values are means ± SE in nmol·10 g body wt⁻¹·min⁻¹. *P < 0.05 was considered significant between groups of mice receiving \([L-GLN]\)-alanine (ALA)-GLN (series 1) and **P < 0.05 for mice receiving free \(L-GLN\) (series 2).

**Fig. 4.** Intestinal \([L-2,15N]CIT\) production was higher in series 2, in which mice received free \(L-GLN\), irrespective of the route. Renal de novo \([L-2,15N]ARG\) production was not significantly different for the route. Data are means ± SE in nmol·10 g body wt⁻¹·min⁻¹. Student’s t-test or Mann-Whitney U-test was performed when appropriate.
high ammonia levels, possibly related to glutaminase inhibition and glutamine synthetase stimulation. In our experiment, ammonia was not measured, and, although theoretically degradation of L-GLN in L-GLU and ammonia could have played a role in recycling of L-GLN in the enteral free L-GLN group, the importance of this route is yet unclear (11).

Consistent with previous studies (29, 30), the intestines released L-CIT, and the kidneys took up L-CIT and released L-ARG. The results of the fluxes are an extension of the findings of Houdijk et al. (16). They found an increase in arterial CIT and ARG concentrations concomitant with increased renal CIT uptake and ARG release in rats fed a GLN-enriched diet compared with rats fed a control diet.

Organ Balance

Intestinal CIT and ARG metabolism. Consistent with the intestinal substrate fluxes of L-CIT and L-ARG mentioned before, the data coming from the combined tracer methodology showed the same pattern, being a release of L-CIT by the intestines and an uptake of L-ARG. Feeding route of free L-GLN or the dipeptide did not result in significant differences with regard to the intestinal CIT metabolism. However, the two series of experiments might point at a higher L-CIT production when free L-GLN is given compared with the dipeptide. An explanation for higher L-CIT production could be that degradation of GLN coming from the dipeptide alanyl-L-GLN occurs via other pathways than when free L-GLN is directly given. In this light, it could be hypothesized that GLN dipeptides or their metabolites hinder the enzyme systems necessary for the uptake of GLN or the conversion to L-CIT. Dipeptide given intravenously will be cleared from the plasma mostly by the kidney, and most of the dipeptide will be subsequently cleaved rapidly by cytosolic peptidases because the kidney exhibits the highest intracellular hydrolase activity against short-chain peptides (1, 10, 18). Consequently, part of the administered dipeptides will not reach the gut because they are hydrolyzed in the kidney. Besides that, in the human intestine, the predominant mechanism for assimilation of GLN dipeptides is absorption as intact dipeptide rather than hydrolysis (20). For mice, to the best of our knowledge, this has not been studied before, but a certain amount of the dipeptide could be suggested to be transported to the circulation as such and might consecutively be degraded in plasma or the kidney. If this is true, free L-GLN might be metabolized more efficiently by the enterocytes, but this would have to be investigated more extensively.

Renal L-CIT and L-ARG metabolism. The kidneys showed L-CIT influx and L-ARG efflux. Renal L-ARG disposal and production were higher in the intravenous dipeptide group than the enteral dipeptide group but lower in the intravenous free L-GLN group compared with the enteral free L-GLN group. This would seem to indicate that renal L-ARG production is more abundant in the intravenous dipeptide as a result of the dipeptide degradation pathway by the kidney (13). Renal ARG metabolism benefits more from enteral free L-GLN application than intravenous free L-GLN, possibly due to hepatic pathways involving aspartate. Thus L-CIT production by the intestines does not seem to directly influence the renal L-CIT uptake or renal L-ARG production, leading us to speculate that L-ARG production is part of a tightly regulated biosystem. Because the arterial values of L-GLN, L-CIT, and L-ARG were above normal, it could be that relatively high plasma concentrations of ARG suppressed endogenous ARG productions in our model (unpublished results). Our data indicate that ARG production is independent of CIT supply, and other substrates might play a role in this as well (26).

De Novo CIT Production by the Intestines From GLU

More de novo CIT was generated in the mice receiving free L-GLN than the mice receiving the dipeptide by the intestines. The feeding route of administration did not make a difference. This might indicate that free GLU is more abundantly converted to CIT by the intestinal wall. These data are consistent with whole body data in similar models (6).

De Novo ARG Production by the Kidney From CIT

About 25% of L-[2,15N]GLN and L-ALA-L-[2,15N]GLN, given enterally or parenterally, was converted via L-[2,15N]CIT into L-[2,15N]ARG by the kidney. The intraorgan rate of ARG production by the kidney was not influenced by the feeding route or molecular form of L-GLN in our model. Because less L-CIT was produced out of L-GLN by the intestines (50%) in the dipeptide groups than renal de novo L-ARG production, L-ARG might also be produced by an extrarenal pathway. Wu and colleagues showed that in cultured endothelial cells, a functioning intracellular ARG-CIT cycle exists, which means that NO and L-CIT are constantly generated from L-ARG. In this cycle, L-CIT is actively converted back into L-ARG, and this cycle can be inhibited by extracellular L-GLN concentrations but not by extracellular L-ARG (28). Apparently, part of the total L-CIT is produced by nonintestinal sources, and recycling in endothelial cells might play a role in this as well.

In conclusion, stable isotope measurements showed that exogenous intravenous or enteral L-GLN or L-alamy-L-GLN generate de novo L-CIT production by the intestines and de novo L-ARG production by the kidneys during surgery. The intestinal-renal axis is hereby proven in mice. Although further research is required, it would seem justified on the basis of the present data to state that the route of administration of exogenous L-GLN does influence the metabolism of L-CIT and L-ARG but that not all L-CIT and L-ARG is coming from GLN by the intestinal-renal axis in this model.

ACKNOWLEDGMENTS

We thank Dwight Matthews for the kind gift of the alanyl-L-[2,15N]GLN tracer. P. G. Boelens participated in all aspects of this study, design, preparations, conduct of the experiments, data analysis, data interpretation, and writing the article. P. A. M. van Leeuwen initiated the study and hypothesis, contributed to the preparations, experiments, and data interpretation as well as revising the article. C. Dejong revised the article and N. E. Deutz designed the model, conducted the experiments, and assisted in data interpretation and writing of article. With special thanks, we give recognition to the laboratory staff of the Nutritional Research Center of the Maastricht University (Dept. of Surgery), in particular to Hans van Eijk for technical support, Gabrie A. ten Have for support in the preparations and conduct of the experiments, Gerdien C. Melis in assisting in data management and data interpretation, and Marieke G. van den Heuvel for assisting in data management and the conduct of the second series of experiments. We thank Elly de Lange-de Klerk for statistical expertise.

GRANTS

P. G. Boelens is a recipient of a fellowship (NWO 920-03-185) of The Netherlands Council for Medical Research of The Netherlands Organization for Health Research and Development.
for Health Research and Development. C. H. C. Dejong is supported by Clinical Fellowship Grant 907-00-033 from The Netherlands Organization for Health Research and Development.

REFERENCES


