The Journal of Nutrition
Methodology and Mathematical Modeling

Arginine and Ornithine Are the Main Precursors for Citrulline Synthesis in Mice[1–3]

Juan C. Marini*

USDA/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX

Abstract

Recent isotopic tracer studies in mice, piglets, and humans have produced conflicting results as to the main carbon skeleton precursor for citrulline and arginine synthesis. This may be due in part to the different tracers infused and models used to interpret the stable isotope data. Furthermore, previous studies usually investigated a single precursor, which prevented the direct comparison among multiple precursors. To further elucidate the contribution of different precursors to citrulline synthesis, all possible enteral and plasma precursors of citrulline were studied in a mouse model during the postabsorptive and postprandial period using multitracer protocols. In addition, three different models were used to interpret the stable isotope data. The utilization of the classic precursor-product equation, developed for i.v. infused tracers but also used to include i.g. tracers, grossly overestimated the contribution of enteral precursors. Regardless of the model employed, dietary and plasma arginine were the main precursors for citrulline synthesis during feeding and plasma arginine during feed deprivation. The contribution of arginine was directly at the site of citrulline synthesis and through plasma ornithine. The predominant role of arginine and ornithine seen in this study supports the observations in mice, piglets, and humans suggesting that ornithine amino transferase is a pivotal enzyme in this pathway. J. Nutr. 142: 572–580, 2012.

Introduction

Due to their roles in multiple metabolic processes in health and disease (1–3), citrulline and arginine are two amino acids that have elicited considerable interest (2,4,5). Circulating citrulline, the immediate precursor for the endogenous synthesis of arginine, is synthesized mainly in the enterocyte (6) from many possible precursors (**Fig. 1**). Minor contributions to circulating citrulline can originate elsewhere from the action of NO synthase on arginine (7) and dimethylarginine dimethylaminohydrolase on dimethylarginine (8). Enteral citrulline enters the porta vein, bypasses the liver (9), and can be used by many different cell types for arginine synthesis.

The seminal work by Windmueller and Spaeth (10) on glutamine utilization by the gut and their finding that citrulline is one of the main products of intestinal metabolism has been understood as proof that glutamine is utilized for the synthesis of citrulline (11,12). Evidence that glutamine supplementation increases citrulline production and citrulline plasma concentrations (12,13) and that citrulline incorporates ^{15}N from 2-^{15}N glutamine (14–17) seemed to further support this view. Tracer studies concluded that 80% of the citrulline was derived from glutamine and thus that glutamine was the main precursor for citrulline synthesis (18). However, Windmueller and Spaeth (6) clearly stated in their review article, "Source and fate of circulating citrulline," that "intestinally derived citrulline, [is] an end product of glutamine nitrogen metabolism," implying a different fate for glutamine carbon. Furthermore, glutamine is an important respiratory fuel for the small intestine (10) and thus may increase citrulline synthesis by means other than just providing more precursors for citrulline production. Finally, 2-^{15}N glutamine may not be the best tracer to determine precursor-product relationships (19).

The case for glutamine as the main precursor for citrulline synthesis has been contested by us in mouse models (19–21) and by others in piglets (22) and humans (23–25). This has generated some controversy (19,26,27), but multiple physiological factors may contribute to the differences found among different research groups. Feeding vs. feed deprivation, luminal vs. arterial precursors, and neonate vs. adult, together with species differences, are just a few of the physiological factors that may affect the true utilization of different precursors for citrulline synthesis. Other factors that add to this controversy are the choice of tracer and the model used to interpret the data. We have shown that different results are obtained when ^{15}N glutamine or $^{13}C_5$ carbon skeleton-labeled glutamine were utilized to trace the glutamine precursor pool (19). Based on these data, we concluded that dietary glutamine acts as nitrogen donor but is a poor carbon precursor for the synthesis of citrulline. Recently, Tomlinson et al. (23,25) reported that proline is the main precursor for citrulline synthesis. However,

[1] Supported by USDA 2533771314 and NIH KO1 RR24173.
[2] Author disclosures: J. C. Marini, no conflicts of interest.
[3] Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents http://jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: marini@bcm.edu.

Manuscript received October 26, 2011. Initial review completed November 18, 2011. Revision accepted December 14, 2011.
First published online February 8, 2012; doi:10.3945/jn.111.153825.

the use of oral tracers together with a model originally developed for i.v. tracers make their data subject to different interpretation (26).

Studies designed to determine the role of the different precursors have for the most part focused on a single precursor and on a single (arterial or enteral) route of precursor utilization. For these reasons, precursor utilization had to be compared among different studies conducted in different species, different protocols, different physiological stages, etc. The objective of the present research was to systematically investigate all possible enteral and arterial precursors for citrulline synthesis in the postabsorptive and postprandial periods in a mouse model. A secondary objective was to compare the model used by Tomlinson et al. (23–25) with the one we used previously (21) for the interpretation of the tracer data.

Materials and Methods

Animals and treatments

General. Young adult male Institute of Cancer Research mice (6 wk old) purchased from Harlan Laboratories were used for all the experiments. Mice were housed in a specific pathogen-free facility and had access to an irradiated 18% crude protein pelleted feed (Harlan Teklad, Rodent Diet 2920x). Dietary proximate analysis was as follows: protein (185 g/kg), gross energy (14.1 MJ/kg), fat (60 g/kg), fiber (28 g/kg), and ash (46 g/kg). Autoclaved reverse osmosis water was available at all times. Mice were under a 12-h-light cycle (0600–1800 h) in a temperature-controlled (22 ± 2°C) and humidity-controlled (55 ± 5%) environment. All animal procedures were authorized by the Baylor College of Medicine Institutional Animal Care and Use Committee.

Gastric and tail vein catheterization procedures were previously described in detail (19). Mice recovered their presurgery body weight within 5 d and infusions were conducted at least 7 d after surgery.

Infusions and sampling. On the day of the infusion, feed was removed at 0700 h and mice weighed at 0930 h. After a 3-h feed deprivation, mice were restrained and the tail vein catheterized for the infusion of different tracers (Cambridge Isotopes Laboratories). The tail vein and gastric catheters were then connected to syringe infusion pumps (PHD2000, Harvard Apparatus) and mice were infused for 4 h. We previously showed that plateau isotopic enrichment not only for the tracers infused, but also for their products, is reached with this infusion protocol (19).

Tracer infusions. Different tracer infusions were conducted to determine the contribution of all possible dietary and plasma precursors to the synthesis of citrulline in the postprandial and postabsorptive periods. To mimic the effect of feeding, mice were i.g. infused with a complete mixture of amino acids and glucose (**Supplemental Table 1**). In this group, unlabeled amino acids were replaced by an equimolar amount of the corresponding i.g. tracer. To avoid a supplementation effect of the i.v. tracers, both labeled and unlabeled precursors were i.v. infused at a rate of 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (for arginine, proline, and ornithine) and 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (for glutamine and glutamate).

Postprandial (fed) arm. Eight groups of mice ($n = 5$) were continuously i.g. infused with an amino acid and dextrose mixture to maintain a steady-state fed condition. Additionally, arginine, glutamine, glutamate, and proline were i.v. infused (Supplemental Table 1).

Enteral tracers. The following tracers replaced isomolar quantities of the unlabeled amino acid analogs present in the i.g. infused mixture: infusion 1: $U^{13}C_6$ arginine (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 2: $U^{13}C_5$ glutamine (prime, 200 $\mu mol \cdot kg^{-1}$; continuous, 200 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 3: $U^{13}C_5$ glutamate (prime, 200 $\mu mol \cdot kg^{-1}$; continuous, 200 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); and infusion 4: $U^{13}C_5$ proline (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) to determine the contributions of dietary arginine, glutamine, glutamate, and proline to the synthesis of citrulline, respectively.

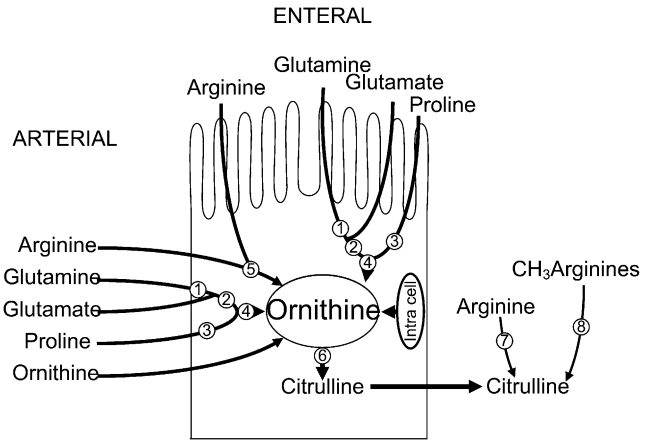

FIGURE 1 Precursors for the synthesis of citrulline. Multiple precursors can be utilized in the synthesis of citrulline. Enteral amino acids (arginine, glutamine, glutamate, and proline) and plasma amino acids (arginine, glutamine, glutamate, proline, and ornithine) together with intracellular sources are the main contributors to the ornithine used for citrulline synthesis. The enzymes involved in the synthesis of ornithine are: *1*) glutaminase; *2*) pyrroline 5 carboxylate synthase; *3*) proline oxidase; *4*) OAT; and *5*) arginase. Ornithine is converted into citrulline by action of ornithine transcarbamylase (6). A minor contribution to circulating citrulline is made by NO synthase (7) and dimethylarginine dimethylaminohydrolase (8). OAT, ornithine aminotransferase.

Parenteral tracers. The following tracers replaced the unlabeled amino acid analogs infused i.v.: infusion 5: $U^{13}C_6$ arginine (prime, 25 $\mu mol \cdot kg^{-1}$; continuous, 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 6: $U^{13}C_5$ glutamine (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 7: $U^{13}C_5$ glutamate (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); and infusion 8: $U^{13}C_5$ proline (prime, 25 $\mu mol \cdot kg^{-1}$; continuous, 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) to determine the contributions of plasma arginine, glutamine, glutamate, and proline to the synthesis of citrulline, respectively.

Postabsorptive (feed-deprived) arm. Four groups of mice ($n = 5$) were continuously i.v. infused: arginine, glutamine, glutamate, and proline (**Table 1**).

Parenteral tracers. The following tracers replaced the unlabeled amino acid analogs infused i.v.: infusion 9: $U^{13}C_6$ arginine (prime, 25 $\mu mol \cdot kg^{-1}$; continuous, 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 10: $U^{13}C_5$ glutamine (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); infusion 11: $U^{13}C_5$ glutamate (prime, 100 $\mu mol \cdot kg^{-1}$; continuous, 100 $\mu mol \cdot kg^{-1} \cdot h^{-1}$); and infusion 12: $U^{13}C_5$ proline (prime, 25 $\mu mol \cdot kg^{-1}$; continuous, 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$).

In addition, ^{15}N(ureido) citrulline (prime, 7 $\mu mol \cdot kg^{-1}$; continuous, 7 $\mu mol \cdot kg^{-1} \cdot h^{-1}$), 5,5 $^2H_2$ ornithine (prime, 25 $\mu mol \cdot kg^{-1}$; continuous, 25 $\mu mol \cdot kg^{-1} \cdot h^{-1}$), and $^2H_2$ (ring) phenylalanine (prime, 10 $\mu mol \cdot kg^{-1}$; continuous, 10 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) were i.v. infused to determine the *Ra* of citrulline, ornithine, and phenylalanine and the *Rc* of plasma ornithine into circulating citrulline.

After a 4-h infusion, blood was drawn from the submandibular bundle, centrifuged at 1500 × *g* for 10 min at 4°C, and plasma kept frozen at −80°C until analysis.

Sample analysis

Plasma amino acid isotopic enrichments were determined as their dansyl derivatives by liquid chromatography (28) using a TSQ Quantum Ultra system (Thermo Finnigan). The following parent-daughter ion transitions were monitored: arginine (*m/z* 408→170, 410→170, 413→170, and 414→170), glutamine (*m/z* 380→170, 382→170, and 385→170), glutamate (*m/z* 381→170, 383→170, and 386→170), proline (*m/z* 349→170, 351→170, and 354→170), ornithine (*m/z* 599→170, 601→170, and 604→170), citrulline (*m/z* 409→170, 411→170, 414→170, 415→170, 409→392 and 410→392), and phenylalanine (*m/z* 399→170 and 404→170).

TABLE 1 Plasma isotopic enrichment of citrulline and its precursors after the infusion of labeled amino acids in fed mice[1]

	Plasma enrichment						
	$^{13}C_5$Arg	$^{13}C_6$Arg	$^{13}C_5$Gln	$^{13}C_5$Glu	$^{13}C_5$Pro	$^{13}C_5$Orn	$^{13}C_5$Cit
				mpe			
I.g. tracers							
Inf. 1 arginine	3.56 ± 0.19	4.24 ± 0.74	0.26 ± 0.01	0.18 ± 0.02	0.46 ± 0.02	14.94 ± 0.67	10.18 ± 0.36
Inf. 2 glutamine	0.06 ± 0.02	0.00 ± 0.00[a]	1.39 ± 0.06	0.77 ± 0.04	0.04 ± 0.03[a]	0.38 ± 0.03	0.29 ± 0.03
Inf. 3 glutamate	0.14 ± 0.01	0.01 ± 0.01[a]	0.13 ± 0.01	0.80 ± 0.06	0.06 ± 0.07[a]	0.67 ± 0.09	0.56 ± 0.05
Inf. 4 proline	0.26 ± 0.01	0.00 ± 0.01[a]	0.24 ± 0.03	0.16 ± 0.03	7.13 ± 0.72	0.97 ± 0.11	1.07 ± 0.12
I.v. tracers							
Inf. 5 arginine	0.44 ± 0.06	4.62 ± 0.25	0.04 ± 0.01	0.01 ± 0.02[a]	0.04 ± 0.01	2.28 ± 0.16	0.98 ± 0.03
Inf. 6 glutamine	0.04 ± 0.01	0.01 ± 0.00[a]	3.05 ± 0.09	0.40 ± 0.03	0.01 ± 0.00	0.36 ± 0.03	0.15 ± 0.01
Inf. 7 glutamate	0.01 ± 0.00	0.01 ± 0.01[a]	0.66 ± 0.03	11.80 ± 2.20	0.01 ± 0.00	0.34 ± 0.02	0.06 ± 0.01
Inf. 8 proline	0.05 ± 0.01	0.00 ± 0.00[a]	0.05 ± 0.01	0.06 ± 0.01	2.34 ± 0.17	0.37 ± 0.03	0.17 ± 0.02
	D_2Arg		D_2Gln	D_2Glu	D_2Pro	D_2Orn	D_2Cit
				mpe			
Inf. 1–8 ornithine	1.06 ± 0.03		−0.03 ± 0.03[a]	0.06 ± 0.02	0.07 ± 0.03	7.59 ± 0.19	2.79 ± 0.09

[1] Values are means ± SEM, n = 5 or 40 (ornithine tracer). [a]Not different from 0, $P > 0.05$. Inf., infusion number; mpe, mole percent enrichment.

Calculations

Ra *and first pass splanchnic extraction.* The Ra[4] (also known as entry rate or flux) is determined by isotopic dilution (29). This implies that the tracer is infused directly into the pool that is sampled (i.e., i.v. infusion and plasma sample) where the tracer is diluted by the tracee, which originates from dietary or endogenous sources, entering the pool.

Thus, the Ra of the different amino acids was calculated as

$$Ra_M = iIV_M \cdot \left(\frac{E_{iIV}}{E_{IVM}} - 1\right), \qquad \text{Eq. 1}$$

where Ra_M is the Ra of the unlabeled metabolite M (μmol · kg^{-1} · h^{-1}), iIV_M is the i.v. infusion rate of the tracer (μmol · kg^{-1} · h^{-1}), E_{iIV} is the enrichment of the infused i.v. tracer, and E_{IVM} is the plasma enrichment of metabolite M at isotopic plateau enrichment (mpe). The Ra of citrulline and ornithine was adjusted to include the contribution of the labeled enteral precursors as previously described (21).

The FPE of the i.g. infused tracer was calculated as

$$FPE_M = \left[iIG_M \cdot \left(\frac{E_{iIG}}{E_{IGM}}\right) - iIV_M \cdot \left(\frac{E_{iIV}}{E_{IVM}} - 1\right)\right] \div \left[iIG_M \cdot \left(\frac{E_{iIG}}{E_{IGM}}\right)\right] \cdot 100, \qquad \text{Eq. 2}$$

where FPE_M is the FPE of the M amino acid (%), iIV_M and iIG_M are the i.v. and i.g. infusion rates of the M amino acid, respectively, and E_{IVM} and E_{IGM} are the plasma isotopic enrichments of the M amino acid due to the i.v. and i.g. infusions, respectively.

Rc *of a precursor into a product.* Three different approaches were followed to determine the contribution of the different precursor to the synthesis of citrulline.

Classic precursor-product equation for a tracer infused i.v., as used by Tomlinson et al. (23) in humans and Urschel et al. (22) in piglets for oral/enteral tracers.

$$Rc_{prec \rightarrow prod} = Ra_{prod} \cdot \left(\frac{E_{prod}/(100 - E_{prod})}{E_{prec}/(100 - E_{prec})}\right), \qquad \text{Eq. 3}$$

where $Rc_{prec \rightarrow prod}$ is the Rc of a precursor into its product (μmol · kg^{-1} · h^{-1}), Ra_{prod} is the plasma Ra product determined from the steady-state enrichments of the i.v. infused tracer, and E_{prec} and E_{prod} are the respective plasma enrichments of the precursor and product due to the infusion of the labeled precursor. Note that the implicit assumption for citrulline synthesis is that the plasma enrichment of the precursor represents the enrichment of the precursor at the site of citrulline synthesis. Also, this model as used by Tomlinson et al. (23) assumes that the Ra of precursors and product can be determined with the use of oral tracers, and therefore the underlying assumption is that there is no first pass splanchnic tracer disappearance.

This equation was also utilized to determine the conversion of plasma arginine into plasma citrulline (a proxy for NO synthesis), plasma citrulline into plasma arginine (de novo arginine synthesis) (30), and plasma ornithine into plasma citrulline.

Precursor-intermediate product model as used by Marini et al. (21). This model does not make any assumptions regarding the enrichment of the precursor/s at the site of citrulline synthesis. It is based on mass balance; in other words, it relies on the recovery of label in plasma citrulline from the infused precursor. Thus, the contribution of the infused labeled precursor to the synthesis of citrulline can be calculated as

$$Cit_{rec\_prec} = Ra_{cit} \cdot \left(E_{Cit\_prec}/\left(100 - E_{Cit\_prec}\right)\right), \qquad \text{Eq. 4}$$

where Cit_{rec_prec} is the recovery of the labeled precursor as citrulline in peripheral plasma (μmol label · kg^{-1} · h^{-1}), E_{cit_prec} is the isotopic plasma enrichment of citrulline due to the infusion of the labeled precursor (mpe), and Ra_{cit} is the Ra of citrulline as calculated above.

The contribution of the labeled precursor can also be calculated as the percentage of the tracer recovered as citrulline as

$$Cit_{rec\_prec}\% = Cit_{rec\_prec} \cdot 100/i_{prec}, \qquad \text{Eq. 5}$$

where Cit_{rec_p}% is the percentage of the infused tracer recovered as citrulline (%) and i_{prec} is the infusion rate of the precursor (μmol tracer · kg^{-1} · h^{-1}) and the other variables as defined above. Assuming that the tracer and tracee are metabolically undistinguishable, then the relative contribution of the dietary (for i.g. tracers) or plasma (for i.v. tracers) unlabeled precursor is identical to the labeled tracer. This can then be expressed as an absolute rate by multiplying Cit_{rec_prec}% by the rate of intake of the dietary precursor (for i.g. tracers) or by Ra precursor (for i.v. tracers).

$$Prec_{to}Cit = Cit_{rec\_prec}\% \cdot DP/100 \qquad \text{Eq. 6}$$

or

$$Prec_{to}Cit = Cit_{rec\_prec}/(E_{prec}/(100 - E_{prec})), \qquad \text{Eq. 6'}$$

where $Prec_{to}Cit$ is the rate of precursor utilization (μmol · kg^{-1} · h^{-1}) for the synthesis of citrulline from dietary sources (Eq. 6) or from plasma

[4] Abbreviations used: AIC, Akaike's Information Criterion; FPE, first pass extraction; mpe, mole percent enrichment; OAT, ornithine aminotransferase; *Ra*, rate of appearance; *Rc*, rate of conversion.

(Eq. 6′); *DP* is the rate of intake of the precursor and the other variables as defined above.

The contribution of the precursor can also be expressed as a percentage of *Ra*citrulline as

$$Prec_{to}Cit\% = Prec_{to}Cit \cdot 100/Ra_{Cit}, \quad \text{Eq. 7}$$

where $Prec_{to}Cit\%$ is the contribution of the precursor to the synthesis of citrulline in percentage. Similar calculations can be conducted to obtain the recovery of tracer in plasma ornithine and the contribution of the precursors to circulating ornithine.

Note that at this stage, the model does not distinguish between the many routes in which the precursor can label the product. The i.v. infusion of an additional ornithine tracer allows for the determination of the contribution of plasma ornithine to the synthesis of citrulline and for the calculation of indirect contribution of a precursor through this intermediate. Thus,

$$RC_{Prec \rightarrow PlOrn \rightarrow Cit} = Prec_{to}Cit \cdot Rc_{Orn \rightarrow Cit}/Ra_{Orn}, \quad \text{Eq. 8}$$

where $RC_{Prec \rightarrow PlOrn \rightarrow Cit}$ is the *Rc* (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) of the precursor to citrulline through plasma ornithine obtained by multiplying the *Rc* of the precursor into plasma ornithine (Eq. 3) by the proportion plasma ornithine that is converted into citrulline, and

$$RC_{Prec \rightarrow EntOrn \rightarrow Cit} = Prec_{to}Cit - RC_{Prec \rightarrow PlOrn \rightarrow Cit}, \quad \text{Eq. 9}$$

where $RC_{Prec \rightarrow EntOrn \rightarrow Cit}$ is the *Rc* of the precursor into citrulline through ornithine produced in the enterocyte, obtained by the difference between the total rate of product conversion to citrulline (Eq. 6) and the rate of precursor conversion to citrulline through plasma ornithine (Eq. 8).

Multi-factorial model. This model does not make any assumptions regarding the enrichment of the precursor/s at the site of citrulline synthesis. Furthermore, it simultaneously estimates the direct contribution of the different precursor to the synthesis of citrulline. The only assumption of this model is that the enrichment of citrulline is a linear function of the enrichment of each of the different precursors multiplied by its fractional contribution to the synthesis of citrulline. In fact, this model is an expansion (and rearrangement) of Eq. 3, in which the contribution of multiple precursors is simultaneously solved. Thus,

$$\frac{E_{Cit}}{(100 - E_{Cit})} = \frac{E_{p1}}{(100 - E_{p1})} \cdot FContrib_{p1} + ... + \frac{E_{pn}}{(100 - E_{pn})} \cdot FContrib_{pn}, \quad \text{Eq. 10}$$

where E_{cit} is the measured $^{13}C_5$ citrulline plasma enrichment, E_{pi} the measured $^{13}C_5$ plasma ($^{13}C_5$+$^{13}C_5$ for arginine) or dietary enrichment of the *i* precursor (from 1 to 8 precursors for the postprandial and 1 to 4 for the postabsorptive arms of the study), and $FContrib_{pi}$ is the fractional contribution of each one of these precursors to the synthesis of citrulline.

For the 5,5 $^2H_2$ ornithine tracer, citrulline and the other precursors M+2 enrichments were included in the calculations. The absolute contribution of each precursor (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) can be obtained by multiplying the fractional contribution by *Ra*citrulline.

$$ContribPrec_i = FContribPrec_i \cdot Ra_{Cit}. \quad \text{Eq. 11}$$

Similar calculations can be conducted to obtain the contribution of the precursors to circulating ornithine and then the contribution of the different precursors to the synthesis of citrulline through plasma ornithine can be estimated.

Data analysis

Data were statistically analyzed as complete randomized designs utilizing the proc mixed procedure of SAS (v. 9.2, SAS Institute). The effect of feeding or feed deprivation was tested for significance at the 5% level. Values in the text are means ± SEM. A *t* test was used to determined if the contribution of the different precursors to the synthesis of citrulline and ornithine were different from zero.

The proc reg procedure of SAS was used for the determination of the fractional contributions of the different precursors to citrulline and ornithine synthesis utilizing the multi-factorial model. AIC, a measure of the relative goodness of fit of the statistical model, was used for model selection.

Results

The i.g. and i.v. infusion of labeled precursors resulted in a wide range of labeling of plasma ornithine, citrulline, and arginine, even taking into account for the different infusion rates (**Tables 1** and **2**). The appearance of $^{13}C_5$ arginine when $^{13}C_6$ arginine was infused implies that the infused arginine tracer was converted to ornithine, which subsequently was used for citrulline synthesis and arginine production (Tables 1 and 2). Note that a small amount of $^{13}C_5$ arginine was present in the infusate (Supplemental Fig. 1); the values in Tables 1 and 2, however, were adjusted to reflect just the recycling of arginine. Higher citrulline enrichments ($P < 0.001$) were achieved with i.g. tracers than with the i.v. infusion of identical tracers, even after correcting for the different infusion rates.

Some of the interconversions among precursors resulted in very small enrichments that, although statistically different from zero, were below the analytical limit of quantification. However, the detection of these enrichments was possible due to the virtual absence of background enrichment for the M+5 isotopologues.

TABLE 2 Plasma isotopic enrichment of citrulline and its precursors after the infusion of labeled amino acids in feed-deprived mice[1]

	Plasma enrichment						
	$^{13}C_5$Arg	$^{13}C_6$Arg	$^{13}C_5$Gln	$^{13}C_5$Glu	$^{13}C_5$Pro	$^{13}C_5$Orn	$^{13}C_5$Cit
				mpe			
I.v. tracers							
Inf. 9 arginine	0.42 ± 0.03	5.78 ± 0.29	0.05 ± 0.01	0.05 ± 0.02[a]	0.14 ± 0.01	5.60 ± 0.41	1.93 ± 0.15
Inf. 10 glutamine	0.05 ± 0.01	0.02 ± 0.02[a]	4.82 ± 0.35	0.59 ± 0.02	0.09 ± 0.01	0.58 ± 0.08	0.46 ± 0.05
Inf. 11 glutamate	−0.01 ± 0.01[a]	0.00 ± 0.01[a]	0.89 ± 0.04	13.61 ± 2.53	0.07 ± 0.01	0.52 ± 0.06	0.17 ± 0.02
Inf. 12 proline	0.05 ± 0.01	0.00 ± 0.01[a]	0.06 ± 0.01	0.06 ± 0.02[a]	7.46 ± 0.34	0.56 ± 0.07	0.42 ± 0.04
	D_2Arg		D_2Gln	D_2Glu	D_2Pro	D_2Orn	D_2Cit
				mpe			
Inf. 9–12 ornithine	1.50 ± 0.06		0.04 ± 0.03[a]	0.05 ± 0.03[a]	0.10 ± 0.01	11.98 ± 0.61	5.70 ± 0.22

[1] Values are means ± SEM, $n = 5$, except for the ornithine tracer, $n = 20$. [a]Not different from 0, $P > 0.05$. Inf., infusion number; mpe, mole percent enrichment.

Ra and FPE of amino acids involved in the synthesis of citrulline. The *Ra* of all the amino acids measured, with the exception of glutamate, was greater during feeding than during the postabsorptive period (**Table 3**) ($P < 0.001$). *Ra*citrulline was also ~70% greater during feeding ($P < 0.001$). Glutamate was almost completely utilized during its first pass through the splanchnic tissues. Glutamine and arginine were removed to a similar extent (~74–78%), whereas only 22% of the dietary proline was removed during its first pass (Table 3).

The *Rc* of arginine to citrulline, an estimate for NO production, was greater during feeding than during the postabsorptive period (Table 3) and accounted for ~3% of *Ra*citrulline ($P = 0.019$). The *Rc* of citrulline to arginine was greater during feeding than during feed deprivation and was the fate of roughly two-thirds of *Ra*citrulline ($P < 0.001$). It also represented a contribution of 16 and 26% to *Ra*arginine in the postprandrial and postabsorptive periods ($P < 0.001$). The *Rc* of plasma ornithine into citrulline was greater ($P < 0.001$) during feeding than during feed deprivation; however, ornithine made a larger contribution ($P < 0.001$) during the postabsorptive state (39 vs. 30%). The absolute recycling rate of plasma arginine to plasma arginine during feeding was greater than during the postabsorptive period ($P = 0.020$). However, when this recycling was expressed as the percentage of *Ra*arginine, the physiological states did not differ ($P = 0.06$). There was no difference ($P = 0.42$) in the absolute *Rc* of arginine into ornithine, but a greater proportion ($P < 0.001$) of plasma ornithine originate from plasma arginine during feed deprivation (89%) than during feeding (44%).

Precursor contribution to the synthesis of citrulline using the conventional precursor-product model. The utilization of the classic precursor-product equation, developed for i.v. infused tracers but used here to include also i.g. tracers, accounted for 306 and 81% of the citrulline produced in the postabsorptive and postprandial periods, respectively (**Table 4**). Dietary and plasma arginine and ornithine were the main precursors for the synthesis of citrulline.

Precursor contribution to the synthesis of citrulline using the precursor-intermediate–product model. The model accounted for 131 and 112% of *Ra*ornithine. Dietary arginine and plasma arginine were the main precursors for the synthesis of plasma ornithine during feeding and plasma arginine during feed deprivation (**Supplemental Table 2**). The precursor-intermediate–product model accounted for ~74% of *Ra*citrulline during the postprandial period but only for 45% of the plasma citrulline during postabsorption (**Table 5**).

A greater recovery of the arginine tracer in citrulline and ornithine was observed during the postprandial and postabsorptive periods compared to the other precursors infused ($P < 0.001$) (Supplemental Table 2; Table 4). For the i.g. infused arginine, 39 and 18% of the tracer were recovered in citrulline and ornithine, respectively. For the i.v. infused arginine, 19 and 8% of the tracer were recovered as plasma ornithine and citrulline during feeding, whereas 27 and 8% were recovered in these two amino acids during feed deprivation. Approximately 4% of the i.g. infused proline was recovered in citrulline and ornithine, but ~5% was recovered of the i.v. infused proline in both the postprandial and postabsorptive periods. The recovery of glutamine and glutamate tracers as ornithine + citrulline was <1% regardless of infusion route or physiological state.

A fraction of the precursors was converted into plasma ornithine before being used for citrulline synthesis by the gut. During feeding, approximately one-half of the contribution of the precursors to the synthesis of citrulline was direct and the remaining 50% was through plasma ornithine. During feed deprivation, a larger part of the precursors (75%) was converted into ornithine before utilization in the synthesis of citrulline.

Multifactorial model. This model accounted for 102 and 109% of the plasma ornithine (**Supplemental Table 3**) produced during the postprandrial and postabsorptive periods, respectively. The model selected for the feeding period using the AIC included dietary arginine and proline and plasma arginine, glutamine, and proline (adjusted $R^2 = 0.99$), whereas it included all four plasma precursors for the feed deprivation period (adjusted $R^2 = 0.99$). Dietary arginine and plasma arginine were the main precursors for plasma ornithine in the fed phase and plasma arginine during feed deprivation. Plasma glutamine

TABLE 3 *Ra*, interconversions, and first pass splanchnic extraction of citrulline precursors in fed and feed-deprived mice[1]

	Fed	Feed-deprived	$P <$	FPE
Ra	$\mu mol \cdot kg^{-1} \cdot h^{-1}$			%
Arginine	525 ± 29	412 ± 19	0.011	74 ± 3
Glutamine	3174 ± 92	2027 ± 166	0.001	78 ± 1
Glutamate	861 ± 152	749 ± 164	0.63	94 ± 1
Proline	1065 ± 72	313 ± 14	0.001	22 ± 3
Phenylalanine	239 ± 7	163 ± 6	0.001	
Ornithine	214 ± 8	141 ± 10	0.001	
Citrulline	181 ± 4	108 ± 4	0.001	
Rc				
Arginine to citrulline	4.6 ± 0.41	3.3 ± 0.21	0.019	
Citrulline to arginine	137.0 ± 3.26	68.0 ± 7.75	0.001	
Ornithine to citrulline	49.2 ± 1.49	38.2 ± 2.39	0.001	
Arginine to arginine	50.5 ± 7.59	29.9 ± 1.33	0.020	
Arginine to ornithine	90.9 ± 11.31	103.3 ± 7.40	0.42	

[1] Values are means ± SEM, n = 5, 20 (ornithine, citrulline, and phenylalanine *Ra* and citrulline to arginine, and ornithine to citrulline conversions), or 40 (fed). FPE, first pass splanchnic extraction; *Ra*, rate of appearance; *Rc*, rate of conversion.

TABLE 4 Contribution of the different precursors to the synthesis of citrulline in fed and feed-deprived mice calculated using the conventional precursor-product model[1]

	Fed	Feed-deprived
	%	
Dietary precursors		
Arginine	140.3 ± 14.7	
Glutamine	21.0 ± 2.4	
Glutamate	71.5 ± 9.7	
Proline	14.1 ± 0.6	
Plasma precursors		
Arginine	18.8 ± 0.9	29.7 ± 1.2
Glutamine	4.8 ± 0.3	9.0 ± 0.8
Glutamate	0.6 ± 0.1	1.3 ± 0.2
Proline	7.4 ± 1.2	5.3 ± 0.6
Ornithine	27.3 ± 0.8	35.2 ± 1.6
Total	305.8 ± 17.9	80.5 ± 2.2

[1] Values are means ± SEM, n = 5, 20 (ornithine, feed-deprived), or 40 (fed).

TABLE 5 Tracer recover and precursor contribution to plasma citrulline in fed and feed-deprived mice calculated using the precursor-intermediate-product model[1]

	Tracer recovered		Precursor contribution					
			Total		Through PI ornithine		Directly	
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	% infused	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	%RaCit	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	%RaCit	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	%RaCit
Fed								
Dietary precursors								
Arginine	17.8 ± 1.0	17.7 ± 1.0	53.4 ± 3.2	30.5 ± 1.1	22.3 ± 2.7	12.7 ± 1.2	31.0 ± 0.8	17.9 ± 0.7
Glutamine	0.6 ± 0.1	0.3 ± 0.0	1.8 ± 0.3	0.9 ± 0.1	0.8 ± 0.0	0.4 ± 0.0	1.1 ± 0.3	0.5 ± 0.1
Glutamate	0.9 ± 0.1	0.5 ± 0.1	4.0 ± 0.5	2.4 ± 0.2	1.4 ± 0.1	0.9 ± 0.0	2.6 ± 0.4	1.6 ± 0.2
Proline	1.8 ± 0.2	1.7 ± 0.1	13.6 ± 1.2	8.1 ± 0.9	3.4 ± 0.8	2.1 ± 0.5	10.1 ± 0.4	6.0 ± 0.4
Plasma precursors								
Arginine	1.9 ± 0.1	7.5 ± 0.4	35.7 ± 2.6	18.8 ± 1.0	21.2 ± 1.9	11.1 ± 0.8	14.5 ± 1.2	7.6 ± 0.5
Glutamine	0.3 ± 0.0	0.3 ± 0.0	9.2 ± 0.6	4.8 ± 0.3	5.4 ± 0.4	2.8 ± 0.3	3.8 ± 0.3	2.0 ± 0.1
Glutamate	0.1 ± 0.0	0.1 ± 0.2	1.0 ± 0.3	0.6 ± 0.1	1.3 ± 0.3	0.7 ± 0.1	−0.3 ± 0.1[a]	−0.2 ± 0.1[a]
Proline	0.3 ± 0.1	1.3 ± 0.2	14.8 ± 2.6	7.4 ± 1.2	8.5 ± 1.4	4.3 ± 0.6	6.3 ± 1.3	3.2 ± 0.6
Total Fed			133.5 ± 5.1	73.5 ± 2.2	64.3 ± 3.7	35.0 ± 1.7	69.3 ± 2.1	38.6 ± 1.2
Feed-deprived								
Plasma precursors								
Arginine	2.0 ± 0.1	8.0 ± 0.5	30.5 ± 2.2	29.7 ± 1.2	26.5 ± 3.1	26.2 ± 3.0	4.0 ± 3.2[a]	3.6 ± 3.0[a]
Glutamine	0.5 ± 0.0	0.5 ± 0.0	9.7 ± 1.0	9.0 ± 0.8	4.5 ± 0.3	4.2 ± 0.4	5.2 ± 0.8	4.8 ± 0.5
Glutamate	0.2 ± 0.0	0.2 ± 0.0	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.3	1.3 ± 0.4	0.0 ± 0.3[a]	−0.1 ± 0.2[a]
Proline	0.5 ± 0.1	1.9 ± 0.2	6.0 ± 0.9	5.3 ± 0.6	3.0 ± 0.3	2.7 ± 0.2	3.0 ± 0.6	2.6 ± 0.5
Total feed-deprived			47.6 ± 2.6	45.3 ± 1.6	35.4 ± 9.9	34.4 ± 9.4	12.2 ± 3.4	10.9 ± 3.1

[1] Values are means ± SEM, n = 5. Values were obtained using Eq. 4–9. [a]Not different from 0, $P > 0.05$. RaCit, Racitrulline.

contributed ~10% of the precursor for plasma ornithine synthesis during both physiological states.

The multifactorial model accounted for 60 and 44% of the citrulline (**Table 6**) produced during the postprandial and postabsorptive periods, respectively. The selected model included (adjusted R^2 = 0.99) dietary arginine and glutamine and plasma arginine, proline, and ornithine for the feeding phase. Only plasma ornithine was included in the model during feed deprivation (R^2 = 0.97).

Discussion

The recent controversy on the precursors used for citrulline and arginine synthesis is due at least in part to the different tracers and models used to interpret the data. We previously addressed the use of different tracers and the implications of utilizing ^{15}N compared to ^{13}C labeled molecules (19). The utilization of U-$^{13}C_n$ tracers ensures that the carbon skeleton of the precursor amino acid is traced into its product, citrulline, and thus a precursor-product relationship can be established.

In the present work, all possible dietary and plasma precursors for citrulline synthesis were investigated. This allowed for a direct comparison among the different precursors. Dietary and plasma arginine were by far the main precursors for citrulline synthesis regardless of the model used. The failure to account for 100% of the citrulline produced was due to the utilization of unlabeled precursors released from protein breakdown or synthesized within the enterocyte; as expected, this contribution was greater during feed deprivation than during feeding.

Ra and FPE of amino acids involved in the synthesis of citrulline. The *Ra* of arginine, citrulline, and glutamine were within values previously measured in mice (19,21,31). *Ra*ornithine, however, was slower than previously measured in fed (21) and feed-deprived mice (31). This may have been due to changes in protein metabolism, because *Ra*phenylalanine was similarly affected. No values for glutamate and proline have been reported so far in mice and thus the present results cannot be compared with the literature.

TABLE 6 Precursor contribution to plasma citrulline in fed and feed-deprived mice calculated using the multifactorial model[1]

	Full model		AIC selected model	
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	%RaCit	$\mu mol \cdot kg^{-1} \cdot h^{-1}$	%RaCit
Fed				
Dietary precursors				
Arginine	17.6 ± 1.0	9.7 ± 0.6	17.6 ± 1.0	9.7 ± 0.6
Glutamine	0.5 ± 0.8[a]	0.3 ± 0.4[a]	0.7 ± 0.7	0.4 ± 0.4
Glutamate	2.0 ± 1.1[a]	1.1 ± 0.6[a]		
Proline	3.4 ± 5.8[a]	1.9 ± 3.2[a]		
Plasma precursors				
Arginine	11.2 ± 6.1	6.2 ± 3.4[a]	11.4 ± 6.1	6.3 ± 3.4
Glutamine	4.2 ± 9.4[a]	2.3 ± 5.2[a]		
Glutamate	−0.7 ± 2.0[a]	−0.4 ± 1.1[a]		
Proline	10.5 ± 10.7[a]	5.8 ± 5.9[a]	16.5 ± 4.0	9.1 ± 2.2
Ornithine	61.0 ± 1.6	33.7 ± 0.9	61.0 ± 1.6	33.7 ± 0.9
Total Fed	109.7 ± 16.8	60.2 ± 9.3	107.2 ± 7.6	59.2 ± 4.2
Feed-deprived				
Plasma precursors				
Arginine	−11.0 ± 11.5	−6.1 ± 6.4[a]		
Glutamine	8.3 ± 13.3	4.6 ± 7.4[a]		
Glutamate	−0.9 ± 3.9	−0.5 ± 2.2[a]		
Proline	4.3 ± 8.4	2.4 ± 4.7[a]		
Ornithine	78.4 ± 2.9	43.3 ± 1.6	76.9 ± 2.3	42.5 ± 1.3
Total feed-deprived	79.1 ± 20.1	43.7 ± 11.2	76.9 ± 2.3	42.5 ± 1.3

[1] Values are means ± SEM, n = 5. Values were obtained using Eq. 10 and 11. [a]Not different from 0, $P > 0.05$. AIC, Akaike's Information Criterion; RaCit, Racitrulline.

The extensive FPE of arginine (74%) was similar to the one previously reported by us in ICR and C57/B6J wild-type mice (20,21) but higher than those reported in other species [rats, 33% (32); piglets, 40–50% (33,34); and humans, 33% (35)]. Glutamate and glutamine are amino acids that undergo extensive enteral and hepatic utilization during first pass (36). The current data in conscious mice were almost identical to those reported in an ex-vivo study in rats by Windmueller and Spaeth (37,38). Proline, by contrast, is known to undergo a modest FPE (36,39), which was reflected by the 22% extraction observed in the present study in mice.

Rates of arginine and citrulline interconversions. The *Rc* of arginine to citrulline has been used as a proxy for NO production (30). The rates measured in this study were slightly greater during feeding than during feed deprivation, but as found previously in this laboratory (31) and by others [for a current review, see (40)], it accounted for a small percentage of *Ra*arginine. The *Rc* of citrulline to arginine has been dubbed de novo arginine production and measures the amount of plasma arginine that originates from citrulline. In this study, it represented 16 and 26% of the *Ra*arginine for the feed-deprived and fed conditions, respectively. Roughly two-thirds of the citrulline produced was accounted for as plasma arginine, which indicates the ability of other cell types besides the proximal tubule cells of the kidney to use citrulline to meet local arginine needs (41).

The recycling of plasma arginine was ~8% of *Ra*arginine. This recycling of plasma arginine has also been reported in piglets (34) and humans (42,43) when *Ra*arginine was determined in vivo with two different arginine labels, one tracing the guanidino group and the second one, the carbon skeleton.

Precursor contribution to the synthesis of citrulline. The limitations of the classic precursor-product model applied to i.g. tracers were evident in this dataset. The unrealistic accounting for 306% of the citrulline produced indicates that this model overestimated the contribution of the precursors used, mainly when i.g. tracers were infused. This was due to the fact that this precursor-product model utilizes the plasma enrichment of the precursor as the enrichment of the precursor at the site of citrulline synthesis. The utilization of dietary amino acids during first pass intestinal metabolism has been well documented (36) and because citrulline is synthesized in the gut, the true enrichment of the precursors in the enterocytes during the administration of i.g. tracers is likely to be greater than in peripheral plasma. Furthermore, a large removal of the tracer during first pass metabolism results in a lower plasma enrichment and thus in a greater contribution of the precursors. This was clear in the case of glutamate, in which a small contribution to the synthesis of citrulline together with an extensive FPE resulted in the apparent contribution of >70% to *Ra*citrulline. A third source of bias when using this model has been that *Ra*product has also been determined with an i.g. tracer.

The precursor-intermediate–product model outlined by us (21) is based on mass balance and does not depend on any assumption regarding the enrichment of the precursors at the site of citrulline synthesis. However, because the assumption that the recycling of the label through precursors other than ornithine is negligible and prone to some double accounting. This model accounted for 74 and 45% of the *Ra*citrulline for the fed and feed-deprived states, respectively.

The strength of the multifactorial approach is that by solving a set of simultaneous equations it can estimate the direct contribution of all possible precursors. The limitation of this model, however, is that additional variability is introduced due to the use of multiple animals to obtain data, and any analytical error is not confined to that measurement but is propagated through the whole system. Additionally, because there is a large range in the contribution of the different precursors to the synthesis of citrulline, the error in the measurement of a main precursor may result in the contribution of minor precursors being undetectable (i.e., not being different from zero). The multifactorial model accounted for ~61 and 44% of *Ra*citrulline and thus the double accounting seen with the precursor-intermediate–product was ~13 and 1% in the fed and feed-deprived periods, respectively.

Biochemical basis for precursor utilization. Arginine and ornithine were quantitatively the main precursors for the synthesis of citrulline. Regardless of the model used, the raw enrichment data showed that the infusion of labeled arginine and ornithine resulted in a greater citrulline enrichment than when other precursors were infused. Thus, it seems then that the ornithine available for citrulline synthesis, rather than originating from the de novo pathway, comes from preformed sources.

The biochemical basis for these observations relies on the activity of one enzyme, OAT. This enzyme can either synthesize ornithine from glutamate semialdehyde (from glutamate, glutamine, or proline) or dispose of ornithine (44) (Fig. 1). In adults, the catabolic route seems to predominate (45,46). Furthermore, this directionality of OAT has been supported by in vivo studies showing that OAT inhibition in adult mice increased the plasma ornithine concentration (47,48), whereas in suckling piglets, OAT inhibition decreased ornithine levels (49). The inhibition of OAT in transgenic mice results in a paradoxical neonatal hypo-ornithemia and hyper-ornithemia after weaning, which mimics similar findings reported in humans with gyrate atrophy (50). Conversely, the overexpression of OAT results in a decrease in the plasma concentration of ornithine in adult mice (51). This change in the function of OAT seems to imply that different precursors are utilized during the neonatal period and adulthood.

In conclusion, the multiple precursors used for the synthesis of citrulline, together with the reuse of the label for the synthesis of other precursors, makes the quantification of the contribution of the different precursors challenging. The precursor-intermediate–product model offers a good approximation without the need to infuse all possible precursors. Regardless of the model used, the predominant role of arginine and ornithine as the main precursors for citrulline synthesis in fed and feed-deprived mice contrasts with the conclusions arrived by others in piglets and humans. It cannot be determined at this stage if this is due to real physiological differences or to the choice of tracer and models used to interpret the data. A systematic study of all possible citrulline precursors in these species is needed to determine what precursors are utilized in the synthesis of citrulline.

Acknowledgments

The author thanks Inka Cajo Didelija for her help during the infusions and sample preparation. The comments of Doug Burrin and Farook Jahoor on this manuscript are greatly appreciated.

Literature Cited

1. Figueroa A, Trivino JA, Sanchez-Gonzalez MA, Vicil F. Oral L-citrulline supplementation attenuates blood pressure response to cold pressor test in young men. Am J Hypertens. 2010;23:12–6.

2. Cynober L, Moinard C, De Bandt JP. The 2009 ESPEN Sir David Cuthbertson. Citrulline: a new major signaling molecule or just another player in the pharmaconutrition game? Clin Nutr. 2010;29:545–51.
3. Luiking YC, Poeze M, Ramsay G, Deutz NEP. Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production. Am J Clin Nutr. 2009;89:142–52.
4. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, Carey Satterfield M, Smith SB, Spencer TE, Yin Y. Arginine metabolism and nutrition in growth, health and disease. Amino Acids. 2009;37: 153–68.
5. Luiking YC, Engelen MPKJ, Deutz NEP. Regulation of nitric oxide production in health and disease. Curr Opin Clin Nutr Metab Care. 2010;13:97–104.
6. Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. Am J Physiol. 1981;241:E473–80.
7. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. Proc Natl Acad Sci USA. 1990;87:8612–6.
8. Ogawa T, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, Ng,Ng-dimethylarginine dimethylaminohydrolase, from rat-kidney. J Biol Chem. 1989;264:10205–9.
9. Drotman RB, Freedland RA. Citrulline metabolism in the perfused rat liver. Am J Physiol. 1972;222:973–5.
10. Windmueller HG, Spaeth AE. Uptake and metabolism of plasma glutamine by the small intestine. J Biol Chem. 1974;249:5070–9.
11. Fujita T, Yanaga K. Association between glutamine extraction and release of citrulline and glycine by the human small intestine. Life Sci. 2007;80:1846–50.
12. Peters JHC, Wierdsma NJ, Teerlink T, Van Leeuwen PAM, Mulder CJJ, Van Bodegraven AA. The citrulline generation test: proposal for a new enterocyte function test. Aliment Pharmacol Ther. 2008;27:1300–10.
13. Rutten EPA, Engelen M, Wouters EFM, Schols A, Deutz NEP. Metabolic effects of glutamine and glutamate ingestion in healthy subjects and in persons with chronic obstructive pulmonary disease. Am J Clin Nutr. 2006;83:115–23.
14. Boelens PG, Melis GC, van Leeuwen PA, ten Have GA, Deutz NE. Route of administration (enteral or parenteral) affects the contribution of L-glutamine to de novo L-arginine synthesis in mice: a stable-isotope study. Am J Physiol Endocrinol Metab. 2006;291:E683–90.
15. Boelens PG, van Leeuwen PA, Dejong CH, Deutz NE. Intestinal renal metabolism of L-citrulline and L-arginine following enteral or parenteral infusion of L-alanyl-L-[2,^{15}N]glutamine or L-[2,^{15}N]glutamine in mice. Am J Physiol Gastrointest Liver Physiol. 2005;289: G679–85.
16. Ligthart-Melis GC, van de Poll MCG, Boelens PG, Dejong CHC, Deutz NEP, van Leeuwen PAM. Glutamine is an important precursor for de novo synthesis of arginine in humans. Am J Clin Nutr. 2008;87: 1282–9.
17. Ligthart-Melis GC, van de Poll MCG, Dejong CHC, Boelens PG, Deutz NEP, van Leeuwen PAM. The route of administration (enteral or parenteral) affects the conversion of isotopically labeled L-[2-^{15}N] glutamine into citrulline and arginine in humans. JPEN J Parenter Enteral Nutr. 2007;31:343–8.
18. Deutz NEP. The 2007 ESPEN Sir David Cuthbertson Lecture: amino acids between and within organs. The glutamate-glutamine-citrulline-arginine pathway. Clin Nutr. 2008;27:321–7.
19. Marini JC, Didelija IC, Castillo L, Lee B. Glutamine: precursor or nitrogen donor for citrulline synthesis? Am J Physiol Endocrinol Metab. 2010;299:E69–79.
20. Marini JC, Keller B, Didelija IC, Castillo L, Lee B. Enteral arginase II provides ornithine for citrulline synthesis. Am J Physiol Endocrinol Metab. 2011;300:E188–94.
21. Marini JC, Didelija IC, Castillo L, Lee B. Plasma arginine and ornithine are the main citrulline precursors in mice infused with arginine-free diets. J Nutr. 2010;140:1432–7.
22. Urschel KL, Rafii M, Pencharz PB, Ball RO. A multitracer stable isotope quantification of the effects of arginine intake on whole body arginine metabolism in neonatal piglets. Am J Physiol Endocrinol Metab. 2007;293:E811–8.
23. Tomlinson C, Rafii M, Ball RO, Pencharz PB. Arginine can be synthesized from enteral proline in healthy adult humans. J Nutr. 2011;141:1432–6.
24. 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. 2011;301:E267–73.
25. Tomlinson C, Rafii M, Sgro M, Ball RO, Pencharz P. Arginine Is synthesized from proline, not glutamate, in enterally fed human preterm neonates. Pediatr Res. 2011;69:46–50.
26. Ligthart-Melis GC, Deutz NEP. Is glutamine still an important precursor of citrulline? Am J Physiol Endocrinol Metab. 2011;301: E264–6.
27. Ligthart-Melis GC, Vermeulen MAR, Van Leeuwen PAM, Deutz NEP. Glutamine: precursor or nitrogen donor for citrulline synthesis? Am J Physiol. 2010;299:683.
28. Marini JC. Quantitative analysis of ^{15}N-labeled positional isomers of glutamine and citrulline via electrospray ionization tandem mass spectrometry of their dansyl derivatives. Rapid Commun Mass Spectrom. 2011;25:1291–6.
29. Rittenberg D, Foster GL. A new procedure for quantitative analysis by isotope dilution, with application to the determination of amino acids and fatty acids. J Biol Chem. 1940;133:737–44.
30. Castillo L, Beaumier L, Ajami AM, Young VR. Whole body nitric oxide synthesis in healthy men determined from [^{15}N]arginine-to-[^{15}N]citrulline labeling. Proc Natl Acad Sci USA. 1996;93:11460–5.
31. Marini JC, Erez A, Castillo L, Lee B. Interaction between murine spf-ash mutation and genetic background yields different metabolic phenotypes. Am J Physiol Endocrinol Metab. 2007;293:E1764–71.
32. Windmueller HG, Spaeth AE. Metabolism of absorbed aspartate, asparagine, and arginine by rat small intestine in vivo. Arch Biochem Biophys. 1976;175:670–6.
33. Bertolo RF, Brunton JA, Pencharz PB, Ball RO. Arginine, ornithine, and proline interconversion is dependent on small intestinal metabolism in neonatal pigs. Am J Physiol Endocrinol Metab. 2003;284: E915–22.
34. Urschel KL, Shoveller AK, Pencharz PB, Ball RO. Arginine synthesis does not occur during first-pass hepatic metabolism in the neonatal piglet. Am J Physiol Endocrinol Metab. 2005;288:E1244–51.
35. Castillo L, Chapman TE, Yu YM, Ajami A, Burke JF, Young VR. Dietary arginine uptake by the splanchnic region in adult humans. Am J Physiol. 1993;265:E532–9.
36. Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J Nutr. 1998;128:606–14.
37. Windmueller HG, Spaeth AE. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. Arch Biochem Biophys. 1975;171:662–72.
38. Windmueller HG. Glutamine utilization by the small intestine. Adv Enzymol Relat Areas Mol Biol. 1982;53:201–37.
39. Bertolo RF, Burrin DG. Comparative aspects of tissue glutamine and proline metabolism. J Nutr. 2008;138:S2032–9.
40. Siervo M, Stephan BCM, Feelisch M, Bluck LJC. Measurement of in vivo nitric oxide synthesis in humans using stable isotopic methods: a systematic review. Free Radic Biol Med. 2011;51:795–804.
41. Featherston WR, Rogers QR, Freedland RA. Relative importance of kidney and liver in synthesis of arginine by the rat. Am J Physiol. 1973;224:127–9.
42. Castillo L, Sanchez M, Chapman TE, Ajami A, Burke JF, Young VR. The plasma flux and oxidation rate of ornithine adaptively decline with restricted arginine intake. Proc Natl Acad Sci USA. 1994;91: 6393–7.
43. Beaumier L, Castillo L, Ajami AM, Young VR. Urea cycle intermediate kinetics and nitrate excretion at normal and "therapeutic" intakes of arginine in humans. Am J Physiol. 1995;269:E884–96.
44. Markova M, Peneff C, Hewlins MJE, Schirmer T, John RA. Determinants of substrate specificity in omega-aminotransferases. J Biol Chem. 2005;280:36409–16.
45. McGivan JD, Bradford NM, Beavis AD. Factors influencing activity of ornithine aminotransferase in isolated rat-liver mitochondria. Biochem J. 1977;162:147–56.
46. Smith AD, Benziman M, Strecker HJ. The formation of ornithine from proline in animal tissues. Biochem J. 1967;104:557–63.
47. Seiler N, Grauffel C, Dauneanglard G, Sarhan S, Knodgen B. Decreased hyperammonemia and orotic aciduria due to inactivation of ornithine aminotransferase in mice with a hereditary abnormal ornithine carbamoyltransferase. J Inherit Metab Dis. 1994;17:691–703.

48. Alonso E, Rubio V. Participation of ornithine aminotransferase in the synthesis and catabolism of ornithine in mice: studies using gabaculine and arginine deprivation. Biochem J. 1989;259:131–8.
49. Flynn NE, Wu GY. An important role for endogenous synthesis of arginine in maintaining arginine homeostasis in neonatal pigs. Am J Physiol. 1996;271:R1149–55.
50. Wang T, Lawler AM, Steel G, Sipila I, Milam AH, Valle D. Mice lacking ornithine-delta-amino-transferase have paradoxical neonatal hypoornithinaemia and retinal degeneration. Nat Genet. 1995;11:185–90.
51. Ventura G, De Bandt JP, Segaud F, Perret C, Robic D, Levillain O, Le Plenier S, Godard C, Cynober L, Moinard C. Overexpression of ornithine aminotransferase: consequences on amino acid homeostasis. Br J Nutr. 2009;101:843–51.