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Plasma Arginine and Ornithine Are the Main Citrulline Precursors in Mice Infused with Arginine-Free Diets[1–3]

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Abstract

Dietary arginine is the main dietary precursor for citrulline synthesis, but it is not known if other precursors can compensate when arginine is absent in the diet. To address this question, the contributions of plasma and dietary precursors were determined by using multitracer protocols in conscious mice infused i.g. either an arginine-sufficient diet [Arg(+)] or an arginine-free diet [Arg(−)]. The plasma entry rate of citrulline and arginine did not differ between the 2 diet groups (156 ± 6 and 564 ± 30 μmol·kg^{-1}·h^{-1}, respectively); however, the entry rate of ornithine was greater in the mice fed the Arg(+) than the Arg(−) diet (332 ± 33 vs. 180 ± 16 μmol·kg^{-1}·h^{-1}). There was a greater utilization of plasma ornithine for the synthesis of citrulline (49 ± 4 vs. 36 ± 3 μmol·kg^{-1}·h^{-1}, 30 ± 3% vs. 24 ± 2% of citrulline entry rate) in the mice fed the Arg(−) diet than the Arg(+) diet. The utilization of plasma arginine did not differ between the 2 diet groups for citrulline synthesis, either through plasma ornithine (~29 ± 3 μmol·kg^{-1}·h^{-1}) or at the site of citrulline synthesis (~12 ± 3 μmol·kg^{-1}·h^{-1}). The contribution of dietary proline to the synthesis of citrulline was mainly at the site of citrulline production (17 ± 1 μmol·kg^{-1}·h^{-1}), rather than through plasma ornithine (5 ± 0.4 μmol·kg^{-1}·h^{-1}). Dietary glutamine was utilized only at the site of citrulline synthesis (4 ± 0.2 μmol·kg^{-1}·h^{-1}). Dietary glutamine and proline made a greater contribution to the synthesis of citrulline in mice fed the Arg(−) diet but remained minor sources for citrulline production. Plasma arginine and ornithine are able to support citrulline synthesis during arginine-free feeding. J. Nutr. 140: 1432–1437, 2010.

Introduction

The metabolism of citrulline, a nonprotein amino acid, has recently received renewed attention due to its involvement in arginine synthesis and nitric oxide metabolism (1–3). Furthermore, it has been suggested that citrulline per se may have additional roles such as increasing protein synthesis and nitrogen retention, possibly by activating the mammalian target of rapamycin pathway (4,5).

Because little or no citrulline is present in the diet [with the notable exception of watermelon (6)], citrulline is almost entirely of endogenous origin. Citrulline is synthesized by condensation of ornithine and carbamylphosphate by action of ornithine transcarbamylase (7). This enzyme is present only in hepatocytes and enterocytes in the mouse and other species, including humans (8–10). Under physiological conditions, there is no net synthesis of citrulline by the liver due to the channeling of urea cycle intermediates within the urea cycle (11), and thus circulating citrulline originates in the small intestine. The plasma entry rate of citrulline seems to be rather constant under normal conditions and plasma citrulline concentration has been proposed as an indicator of gut mass and functionality (12,13). In addition, it has been shown that a lack of arginine in the diet did not increase citrulline production in rats (14,15), pigs (16), or humans (17), and thus the endogenous synthesis of arginine could not be upregulated when arginine was absent in the diet. More recently it has been shown that the total removal of all possible citrulline precursors (glutamate, glutamine, arginine, and proline) from the diet for an extended period of time produced no changes in citrulline production (18). These findings indicate that citrulline production can be sustained by utilizing exclusively endogenous precursors and that citrulline production may not be amenable to dietary manipulation.

Although the ornithine utilized for the synthesis of citrulline can be synthesized de novo from proline and glutamine by action of ornithine aminotransferase (19), we have shown that at least in adult mice, preformed ornithine resulting from the hydrolysis of dietary arginine is the preferred precursor for citrulline synthesis (20). Furthermore, the production of ornithine utilized for the synthesis of citrulline can be local at the site of citrulline synthesis (i.e. in the enterocytes) or it can occur somewhere else in the body and the ornithine thus generated transported in plasma for its utilization by the small intestine.

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Our objective in this work is to determine the contribution of the endogenous and dietary precursors involved in the synthesis of citrulline when arginine, the main dietary precursor, is absent from the diet.

Materials and Methods

Animals and housing. Young adult male Institute of Cancer Research mice (6 wk old) were used in 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 2920×). 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- (22 ± 2°C) and humidity- (55 ± 5%) controlled environment. All animal procedures were authorized by the Baylor College of Medicine Institutional Animal Care and Use Committee.

Gastric catheterization. The procedure and postoperative care has been described in detail elsewhere (20). Mice recovered their presurgery body weight within 5 d and infusions were conducted at least 7 d after surgery.

*Treatments, infusions, and sampling.*On the day of the infusion, feed was removed at 0700 h and mice weighed at 0930 h. After 3 h of feed deprivation, mice were restrained and a tail vein catheter was inserted as previously described (21). The tail vein catheter and the gastric catheter were then connected to syringe infusion pumps (PHD2000, Harvard Apparatus). In addition to the different tracers (Cambridge Isotope Laboratories) described below, a liquid amino acid and glucose solution similar to the one used in our previous report [arginine-sufficient [Arg (+)][7] diet (20)] was infused i.g. at a rate of 20 mL·kg^{-1}·h^{-1} to maintain a fed steady state. For the arginine-free [Arg(–)] diet, an isonitrogenous amount of alanine replaced arginine (**Table 1**). Tracers infused i.g. replaced equimolar amounts of the unlabeled amino acids.

The infusion protocols lasted 4 h, which we have shown previously was sufficient to reach plateau enrichment of the primed-continuously infused tracers and their products (20). The first 4 infusion protocols were designed to determine the contribution of plasma arginine (and ornithine) to the synthesis of citrulline under different dietary arginine availabilities, and the contribution of dietary proline and glutamine to citrulline synthesis when arginine is absent in the diet (**Table 2**). These 4 infusion regimens were: infusion 1: Arg(+) diet and i.v. infusion of [U-$^{13}C_6$] arginine (50 mL·kg^{-1}·h^{-1}; prime 50 mL·kg^{-1}·h^{-1}; n = 10 mice); infusion 2: Arg(–) diet and i.v. infusion of [U-$^{13}C_6$] arginine (50 mL·kg^{-1}·h^{-1}; prime 50 mL·kg^{-1}·h^{-1}; n = 10 mice); infusion 3: Arg(–) diet and i.g. infusion of [U-$^{13}C_5$] proline (240 mL·kg^{-1}·h^{-1}; prime 240 μmol·kg^{-1}; n = 10 mice); infusion 4: Arg(–) diet and i.g. infusion [U-$^{13}C_5$] glutamine (200 μmol·kg^{-1}·h^{-1}; prime 200 μmol·kg^{-1}; n = 10 mice).

In addition, these infusion protocols included the i.v. infusion of [5,5 D_2] ornithine (22 μμmol·kg^{-1}·h^{-1}; prime 22 μmol·kg^{-1}), [^{15}N (ureido)] citrulline (7 μmol·kg^{-1}·h^{-1}; prime 7 μmol·kg^{-1}), and [ring-D_5] phenylalanine (10 μmol·kg^{-1}·h^{-1}; prime 10 μmol·kg^{-1}).

An additional infusion (infusion 5) was conducted to determine the first pass utilization of arginine: Arg(+) diet and simultaneous continuous infusion of [U-$^{13}C_6$] arginine (enteral; μmol·kg^{-1}·h^{-1}; prime 95 μmol·kg^{-1}) and [1,2 $^{13}C_2$] arginine (i.v.; 30 μmol·kg^{-1}·h^{-1}; prime 30 μmol·kg^{-1}; n = 6 mice).

After a 4-h infusion, blood was drawn from the submaxibular bundle, centrifuged at 1500 × g for 15 min at 4°C and plasma was kept frozen at −80°C until analysis.

Sample analysis. Plasma citrulline, arginine, ornithine, and phenylalanine were determined as their dansyl derivatives by LC-MS utilizing a TSQ Quantum Ultra System (Thermo Finnigan). Citrulline isotopic enrichment was determined by monitoring the transitions mass:charge ratio (*m/z*) 409 to 392, 410 to 392 (ureido nitrogen), 412 to 394, and 414 to 397 for citrulline; m/z 408 to 170, 413 to 170, and 414 to 170 for arginine; *m/z* 599 to 170, 601 to 170, and 604 to 170 for ornithine; and *m/z* 399 to 170 and 404 to 170 for phenylalanine.

Plasma amino acid concentrations were measured by reverse-phase HPLC of their phenyl isothiocyanate derivatives (PicoTag, Waters).

Calculations. The calculations to estimate the different rates of appearance, rates of conversion, and rates of tracer recovery are reported in the Supplemental Appendix. Briefly, rate of appearance (Ra) of the different amino acids was calculated from the isotopic dilution of the i.v. infused tracer and the first pass extraction of arginine from the calculated disappearance of the i.g. tracer with respect of the peripherally infused tracer. The recovery of the i.g. tracer in circulating citrulline was calculated by multiplying the plasma citrulline enrichment due to the i.g. tracer by the Ra of citrulline. We used the precursor-intermediate-product approach to determine the site of production of the ornithine used for citrulline synthesis. This model does not rely on any assumption regarding the enrichment of the precursor pool, but rather on the rate of infusion of the i.g. tracers and their recoveries as plasma citrulline. Note that the arginine tracer utilized (U$^{13}C_6$ arginine) allows for the distinction between citrulline generated in the synthesis of nitric oxide (U$^{13}C_6$ citrulline) and the citrulline synthesized through ornithine (U$^{13}C_5$ citrulline).

Data analysis. Data were analyzed statistically as complete randomized designs utilizing the proc mixed procedure of SAS (v. 9.2, SAS Institute) with day of infusion as a random effect of the model. The fixed effect of arginine in the diet (infusion 1 vs. infusions 2, 3, and 4) and the utilization of dietary precursors when arginine is absent from the diet (infusion 3 vs. infusion 4) were tested for significance at the 5% level. Values in the text are means ± SEM.

Results

Contribution of plasma arginine and ornithine to the synthesis of citrulline. The first pass extraction of arginine, determined by the simultaneous infusion of 2 different arginine tracers via the i.g. and i.v. routes, was 69 ± 0.9%. The lack of arginine in the diet did not change ($P > 0.20$) the plasma Ra of citrulline, arginine, or phenylalanine, but reduced the entry rate

TABLE 1 Rate of i.g. infusion of amino acid and glucose in mice infused with an Arg(+) or Arg(–) diet for 4 h

	Arg(+)	Arg(–)
Infusion rate, *μmol·kg^{-1}·h^{-1}*		
L-Tryptophan	69	69
L-Tyrosine	77	77
L-Phenylalanine	374	374
L-Leucine	916	916
L-Valine	628	628
L-Isoleucine	515	515
L-Lysine	620	620
L-Threonine	617	617
L-Histidine	264	264
L-Proline	759	759
L-Arginine	301	0
L-Alanine	680	1884
L-Aspartate	701	701
L-Serine	621	621
L-Glutamate	876	876
L-Glutamine	638	638
Glycine	606	606
L-Methionine	203	203
L-Cysteine	154	154
Dextrose	12,890	12,890

[7] Abbreviations used: Arg(+) diet, arginine-sufficient diet; Arg(–) diet, arginine–free diet; *m/z*, mass:charge ratio; Ra, rate of appearance.

TABLE 2 Infusion protocols followed in conscious mice to determine the contribution of different precursors to the synthesis of citrulline[1,2]

	Arginine in diet	i.g. Tracer	i.v. Tracers
Infusion 1	+		U-$^{13}C_6$ Arg, D_2 Orn, ^{15}N Cit, D_5 Phe
Infusion 2	—		U-$^{13}C_6$ Arg, D_2 Orn, ^{15}N Cit, D_5 Phe
Infusion 3	—	U-$^{13}C_5$ Pro	D_2 Orn, ^{15}N Cit, D_5 Phe
Infusion 4	—	U-$^{13}C_5$ Gln	D_2 Orn, ^{15}N Cit, D_5 Phe
Infusion 5	+	U-$^{13}C_6$ Arg	1,2$^{13}C_2$ Arg

[1] D_2 Orn, [5,5 D_2] ornithine; ^{15}N Cit, [^{15}N(ureido)] citrulline; D_5 Phe, [D_5(ring)] phenylalanine.
[2] Infusion rates are reported in the text.

of ornithine ($P < 0.001$; **Table 3**). However, plasma arginine and ornithine concentrations were both reduced ($P < 0.001$; **Table 4**). The rate of conversion of plasma ornithine into citrulline was greater ($P < 0.003$) in mice fed the Arg(+) diet than in mice fed the Arg(−) diet (Table 3) but represented a smaller proportion ($P < 0.001$) of the Ra of ornithine. Plasma ornithine was the precursor for 30.4 and 23.5% of the circulating citrulline ($P < 0.006$) in mice fed Arg(+) and Arg(–) diets, respectively. The contribution of plasma arginine to the Ra of ornithine tended to be greater ($P = 0.058$) in mice fed the Arg(+) diet than in mice fed the Arg(−) diet, both in absolute terms as well as the proportion of entry rate of arginine ($P = 0.055$). However, a greater portion of plasma ornithine originated from plasma arginine in mice fed the Arg(–) diet than in mice fed the Arg(+) diet ($P < 0.015$).

We observed no differences ($P > 0.10$) between the 2 diet groups for the citrulline derived from plasma arginine either in absolute amounts or as proportion of the entry rate of arginine. Furthermore, plasma arginine was the precursor for ~24% of the citrulline synthesized, regardless of the presence of arginine in the diet (Table 3). This contribution of arginine to citrulline synthesis was ~70% through plasma ornithine and only ~30% at the site of citrulline synthesis (i.e. in the enterocytes).

TABLE 3 Amino acid Ra and product-precursor conversions in mice infused with an Arg(+) or Arg(–) diet for 4 h[1]

	Arg(+)	Arg(–)
Ra, $\mu mol \cdot kg^{-1} \cdot h^{-1}$		
Citrulline†	164 ± 5	154 ± 6
Ornithine†	332 ± 33	180 ± 16*
Arginine‡	591 ± 27	555 ± 31
Phenylalanine†	349 ± 10	356 ± 11
Rate of conversion precursor-product		
Conversion of Orn to Cit †		
$\mu mol \cdot kg^{-1} \cdot h^{-1}$	49.2 ± 3.9	36.3 ± 2.9*
% of RaOrn[2]	15.2 ± 0.9	20.4 ± 1.1*
% of RaCit	30.4 ± 2.8	23.5 ± 1.6*
Conversion of Arg to Orn‡		
$\mu mol \cdot kg^{-1} \cdot h^{-1}$	190.0 ± 18.1	146.7 ± 21.1+
% of RaArg	31.6 ± 2.0	26.4 ± 2.7+
% of RaOrn	57.5 ± 2.2	67.3 ± 2.3*
Conversion of Arg to Cit‡		
$\mu mol \cdot kg^{-1} \cdot h^{-1}$	39.0 ± 1.3	41.5 ± 2.4
% of RaArg	6.7 ± 0.5	7.5 ± 0.3
% of RaCit	23.9 ± 0.7	24.4 ± 1.1
Rate of conversion precursor-intermediate-product		
Conversion of Arg to plasma Orn to Cit‡		
$\mu mol \cdot kg^{-1} \cdot h^{-1}$	28.1 ± 2.2	29.2 ± 3.5
% total plasma Arg contribution	71.7 ± 6.1	71.6 ± 8.1
Conversion of Arg to Orn in enterocytes and used for Cit‡ synthesis		
$\mu mol \cdot kg^{-1} \cdot h^{-1}$	10.9 ± 2.5	12.2 ± 3.7
% total plasma Arg contribution	28.3 ± 6.1	28.4 ± 8.1

[1] Values are means ± SEM, †Arg(+) $n = 10$ and Arg(–) $n = 30$; ‡Arg(+) $n = 10$ and Arg(–) $n = 10$. *Different from Arg(+), $P < 0.01$.
[2] RaOrn, RaCit, and RaArg: ornithine, citrulline, and arginine Ra.

TABLE 4 Plasma amino acid concentration in mice infused with an Arg(+) or Arg(–) diet for 4 h[1]

	Arg(+)	Arg(–)
	μmol/L	
Arginine	119 ± 10	72 ± 5*
Citrulline	97 ± 5	86 ± 4
Ornithine	164 ± 14	72 ± 5*
Proline	216 ± 20	208 ± 9
Glutamine	645 ± 27	636 ± 24
Glutamate	55 ± 3	53 ± 3
Phenylalanine	91 ± 6	90 ± 3
Tyrosine	75 ± 4	81 ± 3
Tryptophan	79 ± 6	87 ± 5
Threonine	342 ± 37	341 ± 19
Histidine	18 ± 5	17 ± 3
Lysine	689 ± 38	652 ± 29
Leucine	261 ± 18	260 ± 10
Isoleucine	164 ± 12	163 ± 6
Valine	408 ± 22	408 ± 16
Methionine	126 ± 8	133 ± 5
Cystine	55 ± 16	48 ± 9
Cysteine	298 ± 70	307 ± 47
Taurine	329 ± 18	293 ± 13
Aspartate	10 ± 1	10 ± 1
Asparagine	38 ± 2	40 ± 1
Glycine	249 ± 21	241 ± 11
Serine	188 ± 17	194 ± 9
Alanine	662 ± 32	730 ± 29

[1] Values are means ± SEM, Arg(+) $n = 10$ and Arg(–) $n = 30$. *Different from Arg(+), $P < 0.01$.

Contribution of dietary glutamine and proline to the synthesis of citrulline in mice infused with the Arg(−) diet. The recovery of tracer into citrulline from i.g. infused proline was higher ($P < 0.001$) than for glutamine in absolute terms (**Table 5**) as well as a percentage of infused tracer. The i.g. infusion of the glutamine tracer did not label plasma ornithine and thus no label was recovered in circulating ornithine (Table 5).

The contribution of dietary proline to citrulline synthesis was higher ($P < 0.001$) than the contribution of glutamine. This implies that 14% of the citrulline produced originated from dietary proline, but only 3% from dietary glutamine (Table 5). Whereas dietary proline contributed 24 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ to circulating ornithine (or 16.5% of the ornithine entry rate), dietary glutamine did not produce any plasma ornithine. Approximately 77% of the contribution of proline to citrulline synthesis was at the site of citrulline synthesis (i.e. in the enterocytes) and the rest was through plasma ornithine; glutamine contribution, however, was only at the site of citrulline synthesis.

Discussion

Contribution of plasma arginine and ornithine to the synthesis of citrulline. Feeding an arginine-free diet did not

TABLE 5 Utilization of dietary proline and glutamine for the synthesis of citrulline in mice infused with Arg(–) diet for 4 h[1]

	Dietary proline	Dietary glutamine
Dietary tracer recovery, $\mu mol\ tracer \cdot kg^{-1} \cdot h^{-1}$		
Citrulline	6.7 ± 0.49	1.2 ± 0.07*
Ornithine	7.4 ± 0.53	ND[2]
Dietary tracer recovery, *% of tracer infused*		
Citrulline	2.9 ± 0.22	0.6 ± 0.04*
Ornithine	3.2 ± 0.22	ND
Dietary contribution, $\mu mol \cdot kg^{-1} \cdot h^{-1}$		
Citrulline	22.2 ± 1.64	4.0 ± 0.23*
Ornithine	24.7 ± 1.76	ND
Dietary contribution, *% of Ra*		
Citrulline	14.4 ± 0.52	2.9 ± 0.12*
Ornithine	16.5 ± 1.00	ND
Labeled plasma Orn (from dietary tracer) to Cit, $\mu mol\ tracer \cdot kg^{-1} \cdot h^{-1}$	1.56 ± 0.14	ND
Plasma citrulline enrichments, *mpe*		
Due to Orn tracer	1.00 ± 0.05	ND
Measured Cit enrichment	4.32 ± 0.17	0.87 ± 0.04*
Dietary precursor utilization for the synthesis of citrulline, *%*		
At the site of Cit synthesis	23.1 ± 0.56	ND
At the site of Cit synthesis	76.9 ± 0.56	100.0*
Dietary precursor utilization for the synthesis of citrulline, $\mu mol \cdot kg^{-1} \cdot h^{-1}$		
Through plasma ornithine	5.2 ± 0.45	ND
At the site of Cit synthesis	17.0 ± 1.20	4.0 ± 0.23*

[1] Values are means ± SEM, $n = 10$. *Different from proline, $P < 0.001$.
[2] ND, not detectable

affect the rate of citrulline produced in mice, similar to what has been reported previously in other species (14–17). Whereas these previous reports highlighted the lack of upregulation of endogenous arginine synthesis in the face of a perceived arginine deficiency, the aim of the present communication was to determine the precursors for citrulline synthesis when arginine is not present in the diet. Because dietary arginine is the main precursor for the synthesis of citrulline (20), it was expected that other precursors would increase their contribution.

Despite the difference in arginine plasma concentration, the entry rate of arginine did not differ between the mice fed the Arg (–) and Arg(+) diets, which suggests that a large proportion of dietary arginine is utilized during first-pass metabolism and does not enter the peripheral circulation. This agrees with the 69% arginine first pass extraction determined in mice in this experiment, which was considerably higher than values reported in rats [33% (22)], piglets [40% (23)], and humans [33% (24)]. This extensive utilization of dietary arginine resulted in a greater entry rate of ornithine that accounted for ~50% of the dietary arginine.

The similar entry rate of phenylalanine in mice fed the treatment diets suggests that there was no difference in the degradation of endogenous proteins as a consequence of the lack of arginine. Likewise, the long-term (4 wk) deprivation in adult humans of dietary arginine and citrulline precursors in the study of Tharakan et al. (18) showed that there was no increase in protein degradation. This not only indicates that arginine, under the conditions of their study, was a dispensable amino acid, but that the participants were able to maintain the rate of citrulline synthesis utilizing endogenous precursors.

As reported previously (25), plasma ornithine was utilized for the synthesis of citrulline in enterocytic mitochondria and in this study represented 15 and 20% of the fate of circulating ornithine for the mice fed the Arg(+) and Arg(–) diets, respectively. Plasma ornithine was the precursor utilized for 30 and 23% of the citrulline produced in mice fed these 2 diets. The contribution of plasma arginine to circulating ornithine was greater in mice fed the Arg(+) diet than in mice fed the Arg(–) diet, which suggests a greater disposal of arginine when this amino acid is abundant (17). Plasma arginine contributed its carbon skeleton to the synthesis of citrulline by 2 different routes. The main route, accounting for approximately two-thirds of the arginine contribution to citrulline synthesis, was the conversion of plasma arginine into ornithine with the subsequent entry of the ornithine into the plasma and further utilization by the small intestine for the synthesis of citrulline. A secondary route, the uptake of arginine directly by the small intestine and the conversion into ornithine at the site of citrulline synthesis (i.e. in the enterocytes), accounted for one-third of the plasma arginine contribution to citrulline production.

The utilization of arginine for the synthesis of ornithine as the precursor for citrulline synthesis, and the subsequent conversion into arginine, constitutes a seemingly futile arginine-arginine cycle. This same phenomenon has been described in piglets (26) and humans (27) in vivo and in ex vivo preparations (22). This arginine-arginine cycle accounted for ~35% of plasma arginine when piglets were fed arginine-free diets (26). The importance of this cycle is unclear as well as how the cycle can be sustained when arginine is absent from the diet during prolonged periods (18). However, this cycle may arise from the need to regulate arginine concentration in different cell types (28) and, if this is the case, recycling of the ornithine thus generated seems to be more efficient than de novo ornithine production.

Contribution of dietary glutamine and proline to the synthesis of citrulline. Dietary proline was a better precursor for citrulline synthesis than dietary glutamine, which agrees with our previous data in mice fed an arginine-sufficient diet (20).

Whereas proline was utilized as a precursor for plasma ornithine, glutamine was not, because the labeling of plasma ornithine by the glutamine tracer was undetectable. This was due not only to the high first-pass extraction of glutamine, but also to the fact that proline seems to be a better substrate for ornithine synthesis, as shown by the utilization of these 2 precursors for the production of ornithine at the site of citrulline production.

Almost 80% of the contribution of dietary proline to the synthesis of citrulline occurred in the small intestine, whereas the remainder was due to the conversion of dietary proline to ornithine elsewhere in the body, which was transported in plasma and utilized by the small intestine for citrulline synthesis. Similar findings have been reported in piglets, in which a more efficient utilization of proline for arginine synthesis was observed when the proline was provided enterally rather than parenterally (23,29). Compared with the Arg(+) diet, the Arg(–) diet resulted in a greater contribution of proline (3.3% vs. 14%) and glutamine (0.4% vs. 3%) to the entry rate of citrulline (20).

Integration and model limitations. In this work, for mice fed the Arg(–) diet, we were able to account for the precursors of 70 μmol·kg^{-1}·h^{-1} of the circulating citrulline, or ~47% of the citrulline entry rate. For the mice fed the Arg(+) diet, we combined the results from this report with our previous findings (20), where dietary arginine, proline, and glutamine contributed 40, 3.3, and 0.4% of the circulating citrulline, respectively. In this way, we can account for ~127 μmol·kg^{-1}·h^{-1} or ~76% of the entry rate of citrulline. Although the calculations for the absolute rates of precursor utilization rely on minimal assumptions, there are 2 reasons why we could not account for 100% of the precursors of the ornithine utilized for the synthesis of citrulline. The first reason is the implicit assumption that the enterocyte is the only source for circulating citrulline. In a previous study in similar mice (25), citrulline generated as a coproduct in the production of nitric oxide accounted for ~6% of the entry rate of citrulline. Other sources, such as the turnover of citrullinated proteins or by action of dimethylarginine dimethylaminohydrolase on dimethylarginine (30), seem to be minor compared with the synthesis of citrulline from ornithine. Besides the enterocyte, the other site of citrulline production from ornithine in the body is the hepatocyte, but under normal conditions, there is no export of citrulline (11,31). Note that liver uptake of citrulline will not affect the calculations, because it does not affect the plasma enrichments. Only citrulline production from other sources can compromise the model employed.

The second reason is that other precursor sources, not determined in the present study, are plasma glutamine and proline, and glutamine and proline resulting from endogenous protein degradation. These sources are probably minor contributors because preformed ornithine seems to be the preferred precursor for citrulline synthesis (20). Arginine originating from protein degradation at the site of citrulline synthesis from endogenous sources (i.e. intestinal protein turnover as well as the digestion and absorption of enteral secretions), however, may account for the rest of the citrulline produced. Unfortunately, there is no direct way to measure the contribution of these endogenous precursors originating locally from protein degradation and we have to calculate them by difference.

In conclusion, citrulline production was maintained despite the absence of the main dietary precursor (arginine) for its synthesis. Dietary proline and glutamine made a greater contribution to citrulline synthesis when arginine was absent in the diet but were not able to completely replace dietary arginine as a precursor. Whereas dietary proline contributes to the synthesis of circulating ornithine, dietary glutamine did not, which suggests that proline is a better precursor for the de novo synthesis of ornithine than glutamine. Plasma arginine was the precursor for 24% of the circulating citrulline, regardless of the presence of arginine in the diet. These results support our previous findings (20) that preformed ornithine is the preferred precursor for citrulline synthesis, rather than de novo ornithine generated by action of ornithine amino transferase.

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