APPENDIX

Calculations

1 First pass extraction

The first pass extraction (FPE) of Arg was calculated based on the simultaneous intragastric and intravenous infusion of the U-$^{13}C_6$ and 1,2 $^{13}C_2$ Arg tracers (respectively) as,

$$FPE_{Arg} = \left[i_{Arg6} \bullet \left(\frac{E_{i6}}{E_{Arg6}}\right) - i_{Arg2} \bullet \left(\frac{E_{i2}}{E_{Arg2}} - 1\right) \right] \div \left[i_{Arg6} \bullet \left(\frac{E_{i6}}{E_{Arg6}}\right) \right] * 100 \qquad \text{Eq. [1]}$$

where FPE_{Arg} is the first pass extraction of arginine in %, i_{Arg6} is the intragastric infusion rate of U-$^{13}C_6$ arginine (µmol•kg^{-1}•h^{-1}), i_{Arg2} is the intravenous infusion rate of 1,2 $^{13}C_2$ arginine (µmol•kg^{-1}•h^{-1}), E_{i6} and E_{i2} the isotopic enrichment of the infused U-$^{13}C_6$ and 1,2 $^{13}C_2$ arginine, and E_{Arg6} and E_{Arg2} the corresponding plasma arginine enrichments (mpe). Note that for the intravenous tracer, the infusion rate of the tracer is taken into account (the -1 in the equation); because U-$^{13}C_6$ arginine replaced unlabeled dietary arginine, however, no correction applies to this tracer.

2 Rate of appearance

The rate of appearance (Ra) of arginine, ornithine, citrulline and phenylalanine were calculated from the isotopic dilution of the intravenously infused tracers at plateau enrichment, as

$$Ra_M = i_m \bullet \left(\frac{E_{im}}{E_M} - 1\right) \qquad \text{Eq. [2]}$$

where Ra_M is the rate of appearance (entry rate or flux) of the metabolite *M* (µmol•kg^{-1}•h^{-1}), i_m is the intravenous infusion rate of the tracer *m* (µmol•kg^{-1}•h^{-1}), E_{im} is the enrichment of the infused tracer *m* in the infusate and E_M is the plasma enrichment of metabolite *M* at plateau (mpe).

This is the classical isotopic dilution equation (1), which measures the entry rate of the unlabeled molecule. Note that in these experiments the dietary precursors (proline and glutamine) were ~30% enriched and that the tracers replaced isomolar quantities of the amino acid present in the amino acid solution infused intragastrically. For these reasons, the rate of appearance of citrulline has to be adjusted to include the contribution of these dietary tracers as described in Eq. 6.

3 Rates of conversion

The total rates of conversion (Rc) of plasma ornithine to plasma citrulline ($Rc_{Orn\rightarrow Arg}$), plasma arginine to plasma ornithine ($Rc_{Arg\rightarrow Orn}$), plasma arginine to plasma citrulline ($Rc_{Arg\rightarrow Cit}$) were determined as follows

$$Rc_{x\rightarrow y} = Ra_y\left(\frac{E_y/(100-E_y)}{E_x/(100-E_x)}\right) \qquad \text{Eq. [3]}$$

where $Rc_{X\rightarrow Y}$ is the conversion of precursor *X* into product *Y* (μmol•kg^{-1}•h^{-1}), Ra_Y is the plasma rate of appearance of product *Y*, determined from the steady state enrichments of the intravenously infused tracers ([5,5 D_2] ornithine, U-$^{13}C_6$ arginine, ^{15}N citrulline) ; E_X and E_Y, the respective plasma enrichments of the precursors and products ([5,5 D_2] ornithine and [5,5 D_2] citrulline; U-$^{13}C_6$ arginine + 1,2,3,4,5 $^{13}C_5$ arginine and U-$^{13}C_5$ ornithine; U-$^{13}C_6$ arginine + 1,2,3,4,5 $^{13}C_5$ arginine and U-$^{13}C_5$ citrulline). Note that both arginine M+6 and M+5 are the precursors for ornithine M+5 and citrulline M+5 and that, in theory, the M+5 label can recycle indefinitely. Eq. 3 takes into account this infinity recycling. The conversion of a precursor into a product can be also express as the percentage of the Ra precursor (% fate of the precursor) or the Ra product (% Ra product originated from the precursor, Eq. 3' and 3" respectively).

4 Contribution of plasma arginine to the synthesis of citrulline follows two possible two routes

The contribution of plasma arginine to citrulline follows two possible routes: 1) uptake of arginine and conversion to ornithine in tissues, ornithine appearance in plasma and usage by enterocyte (PlArg→PlOrn→Cit; Supplemental Fig. 1-1) and 2) uptake of arginine by the gut, conversion to ornithine and usage directly in enterocyte (PlArg→EntOrn→Cit; Supplemental Fig. 1-2). The precursor-intermediate-product equations below take into account these two sources

$$Rc_{PlArg\rightarrow PlOrn\rightarrow Cit} = Rc_{Arg\rightarrow Orn} \bullet Rc_{Orn\rightarrow Cit} \div Ra_{Orn} \qquad \text{Eq. [4]}$$

where $RC_{PlArg\rightarrow PlOrn\rightarrow Cit}$ is the rate of conversion of plasma arginine to citrulline through plasma ornithine obtained by multiplying the rate of conversion of plasma arginine into plasma ornithine (Eq. 3) by the proportion plasma ornithine that is converted into citrulline (Eq. 3'),

and

$$Rc_{PlArg\rightarrow EntOrn\rightarrow Cit} = Rc_{Arg\rightarrow Cit} - Rc_{PlArg\rightarrow PlOrn\rightarrow Cit} \qquad \text{Eq. [5]}$$

where $RC_{Arg \rightarrow EntOrn \rightarrow Cit}$ is the rate of conversion of plasma arginine into citrulline through ornithine produced in the enterocyte, obtained by difference between the total rate of arginine conversion to citrulline (Eq. 3) and the rate of arginine conversion to citrulline through plasma ornithine (Eq. 4).

5 Contribution of dietary precursors to the rate of appearance of citrulline and ornithine

The contribution of the different labeled precursors to the synthesis of citrulline (or ornithine) was calculated from the recovery of the tracers in plasma citrulline (or ornithine) as

$$Cit_{rec\_p} = \left(Ra_{cit} \bullet E_{cit\_p} / (100 - E_{cit\_p})\right) \qquad \text{Eq. [6]}$$

where Cit_{rec_p} is the recovery of the dietary labeled precursor *p* as citrulline (or ornithine) in peripheral plasma (μmol•kg^{-1}•h^{-1}), E_{cit_p} is the isotopic plasma enrichment of citrulline (or ornithine) due to the infusion of the labeled *p* precursor (mpe), Ra_{cit} is the entry rate of unlabeled citrulline (or ornithine) as calculated above (μmol•kg^{-1}•h^{-1}; Eq. [2]).

The contribution of the labeled precursor can also be calculated as the percentage of the tracer recovered as citrulline (or ornithine) as

$$Cit_{rec\_p}\% = \frac{Cit_{rec\_p}}{i_p} \bullet 100 \qquad \text{Eq. [7]}$$

where $Cit_{rec\_p}\%$ is the percentage recovery of the infused tracer as citrulline (or ornithine), i_p is the infusion rate of the *p* precursor (μmol tracer•kg^{-1}•h^{-1}) and the other variables as defined above. This equation allows for the comparison of the incorporation of different labeled molecules when the unlabeled molecules are not present in the diet (i.e., ammonia or ornithine) or the infusion rates are different.

The contribution of the intragastrically infused dietary precursors can then be calculated by dividing the recovery of the labeled precursor by the corresponding enrichment of the tracer in the infusate as

$$Dietary\_P\_contribution = \frac{Cit_{rec\_p}}{E_{ip}} \bullet 100 \qquad \text{Eq. [8]}$$

where *Dietary_P_Contribution* is the contribution of the dietary (labeled and unlabeled) precursor *P* to the synthesis of citrulline (or ornithine; μmol•kg^{-1}•h^{-1}), Cit_{rec_p} is the recovery of the labeled precursor *p* as citrulline (or ornithine) in peripheral plasma (μmol•kg^{-1}•h^{-1}; Eq. [6]), and E_{ip}, the enrichment of the intragastrically infused *p* precursor (mpe).

Finally, the relative importance of the different dietary precursors to rate of appearance of citrulline can be calculated as

$$Citorigen\% = \frac{Dietary\_P\_Contribution}{RaCit} \bullet 100 \quad \text{Eq. [9]}$$

where *Citorigen%* is the contribution, as percentage, of the dietary precursor *P* to the production of citrulline (note that this equation is valid even in the event of hepatic extraction of citrulline) and RaCit, is the adjusted entry rate of citrulline as described above.

6 Contribution of dietary precursors to the synthesis of citrulline also follows two possible two routes

The contribution of the dietary precursors to citrulline follows two possible routes, 1) absorption of the precursor, conversion to ornithine and usage directly in enterocyte (DPrec→EntOrn→Cit; Supplemental Fig. 1-3) or 2) absorption of the precursor, conversion to ornithine in tissues, ornithine appearance in plasma and usage by enterocyte (DPrec→PlOrn→Cit; Supplemental Fig. 1-4). This last route can be estimated by calculating the amount of tracer that follows this route as

$$PlOrn^* \rightarrow Cit^* = Orn_{rec\_p} \bullet Rc_{Orn \rightarrow Cit} \div Ra_{Orn} \quad \text{Eq. [10]}$$

where *PlOrn*→Cit** is the rate at which the plasma labeled ornithine (μmol tracer•kg^{-1}•h^{-1}), originated from the intragastrically infused tracers, is converted into citrulline. This is calculated multiplying the rate of recovery of the dietary tracer as plasma ornithine determined in Eq. 6, by the proportion of plasma ornithine that is converted into citrulline (Eq. 3'). The incorporation of tracer into citrulline by this route results in an enrichment of plasma citrulline that can be calculated as

$$Ecit(orn) = PlOrn^* \rightarrow Cit^* \bullet 100 / RaCit \quad \text{Eq. [11]}$$

where *Ecit(orn)* is the enrichment of citrulline (mpe) due to conversion of labeled plasma ornithine into citrulline, and the other variables as defined above. The contribution of tracer (an also tracee) through this route can be calculated as a percentage of the total measured citrulline enrichment (*ECit*) as

$$Rc_{DPrec \rightarrow PlOrn \rightarrow Cit}\% = Ecit(orn) \bullet 100 / ECit \quad \text{Eq. [12]}$$

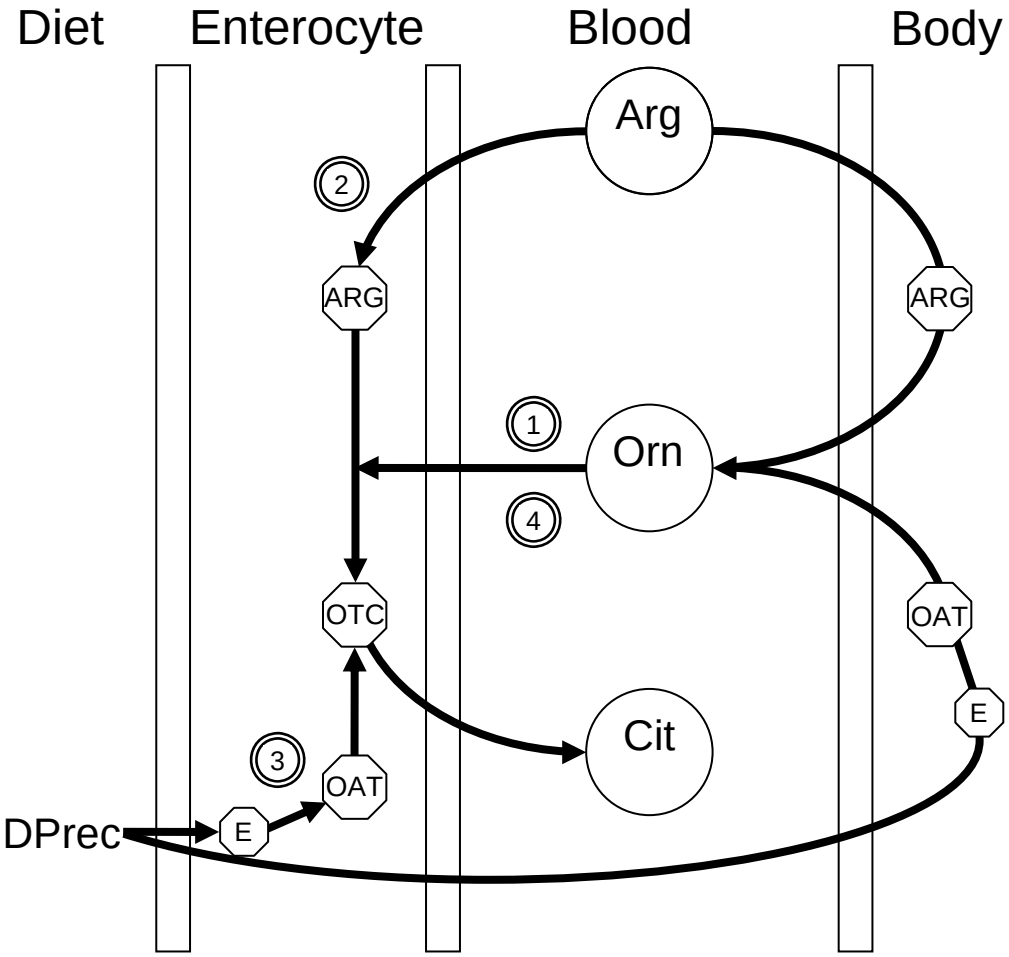
and thus the enrichment in plasma citrulline due to the utilization of the dietary precursor in the enterocyte can be calculated as

$$Rc_{DPrec \rightarrow EntOrn \rightarrow Cit}\% = 100 - Rc_{DPrec \rightarrow PlOrn \rightarrow Cit}\% \quad \text{Eq [13]}$$

where $Rc_{DPrec \rightarrow PlOrn \rightarrow Cit}\%$ is the percentage of precursor converted into citrulline through plasma ornithine and $Rc_{DPrec \rightarrow EntOrn \rightarrow Cit}\%$ is the percentage of precursor converted into citrulline at the site of citrulline synthesis (enterocyte). To obtain the absolute rates ($\mu mol \bullet kg^{-1} \bullet h^{-1}$) this two variables are multiplied by *Dietary_P_Contribution* (Eq. 12' and 13', respectively).

Literature Cited

1. 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(3):737-744.



Supplemental Figure 1. Precursor-Intermediate-Product model. Plasma arginine can be utilized for the synthesis of citrulline after conversion elsewhere in the body to ornithine, which enters the plasma compartment, and then is used by the enterocyte to produce citrulline (1), or directly in the enterocytes, after producing ornithine locally (2). The dietary precursors (DPrec) can be utilized for the synthesis of ornithine and citrulline directly in the enterocyte compartment (3), or they can produce ornithine elsewhere in the body, which can return to the small intestine to produce citrulline (4). ARG, arginase; OTC, ornithine transcarbamylase; OAT, ornithine amino transferase; E, enzymes for the conversion of proline to glutamate semialdehyde (GSA; proline oxidase) or glutamine to GSA (glutaminase and pyrroline-5-carboxylate synthase).