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Plasma arginine and citrulline kinetics in adults given adequate and arginine-free diets

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ABSTRACT **The fluxes of arginine and citrulline through plasma and the rate of conversion of labeled citrulline to arginine were estimated in two pilot studies (with a total of six adult subjects) and in a dietary study with five healthy young men. These latter subjects received an L-amino acid-based diet that was arginine-rich or arginine-free each for 6 days prior to conduct, on day 7, of an 8-hr (first 3 hr, fast; final 5 hr, fed) primed continuous intravenous infusion protocol using L-[*guanidino*-^{13}C]arginine, L-[5,5-$^{2}H_2$]citrulline, and L-[5,5,5-$^{2}H_3$]leucine, as tracers. A pilot study indicated that citrulline flux was about 20% higher ($P < 0.05$) when determined with [*ureido*-^{13}C]citrulline compared with [$^{2}H_2$]citrulline, indicating recycling of the latter tracer. Mean citrulline fluxes were about 8–11 μmol·kg^{-1}·hr^{-1} for the various metabolic/diet groups and did not differ significantly between fast and fed states or arginine-rich and arginine-free periods. Arginine fluxes (mean ± SD) were 60.2 ± 5.4 and 73.3 ± 13.9 μmol·kg^{-1}·hr^{-1} for fast and fed states during the arginine-rich period, respectively, and were significantly lowered ($P < 0.05$), by 20–40%, during the arginine-free period, especially for the fed state, where this was due largely to reduced entry of dietary arginine into plasma. The conversion of plasma citrulline to arginine approximated 5.5 μmol·kg^{-1}·hr^{-1} for the various groups and also was unaffected by arginine intake. Thus, endogenous arginine synthesis is not markedly responsive to acute alterations in arginine intake in healthy adults. We propose that arginine homeostasis is achieved largely via modulating arginine intake and/or the net rate of arginine degradation.**

The physiological needs by tissues and organs for arginine are met via the endogenous synthesis of arginine and/or arginine supplied by the diet. For the U.S. population the latter amounts to about 5.4 g daily per capita (1). The rates of endogenous arginine synthesis in the immature rat (2, 3), guinea pig (4), cat (5, 6), dog (7–9), chicken (10), rabbit (11), and pig (12) are not sufficient to support normal growth or maintain function, and so these species require a source of dietary arginine. The quantitative importance of dietary arginine for maintenance of physiologic function in humans is less clear (1, 13). Under some pathological conditions, including liver disease and genetic errors of urea-cycle enzyme activity (13–15), there is, or may be, a dietary need for arginine, implying inadequate rates of endogenous arginine synthesis. Few estimates of these rates in human subjects and of their quantitative responses to nutritional, hormonal, and pathological factors are available. However, this information is needed to enhance our understanding of the relative importance of endogenous and exogenous sources of arginine for maintaining arginine homeostasis and meeting its physiologic functions. The latter include, in addition to its role as a substrate for protein synthesis, its function as a precursor of nitric oxide (16) and of creatine and its participation as arginyl-tRNA in the process of ubiquitin-dependent protein degradation (17). Therefore, we have begun to use stable-isotope tracer techniques to explore, noninvasively, kinetic and regulatory aspects of arginine metabolism in adult human subjects (18, 19). Here we report results of a study in young men who were given for 7 days an arginine-rich diet and then, for another 7 days, an arginine-free diet. Our kinetic model involves L-[*guanidino*-^{13}C]arginine and L-[5,5-$^{2}H_2$]citrulline as tracers, to estimate plasma arginine and citrulline fluxes as well as the rate of transfer of plasma citrulline into the arginine pool. From the present findings, and our recent studies (19), we propose an integrative scheme of body arginine homeostasis and balance, which defines the metabolic basis for the conditional indispensability of dietary arginine under various pathophysiological conditions (1, 13, 14).

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MATERIALS AND METHODS

Subjects and Experimental Design. Eleven healthy, adult males [age, 22 ± 3 years; body weight, 77 ± 3 kg (mean ± SD)] participated in the study. They were investigated at the Clinical Research Center of the Massachusetts Institute of Technology. All were in good health, as established by medical history, physical examination, analysis of blood cell count, routine biochemical profile, and urinalysis. Their daily intake was calculated to maintain body weight, based on a dietary history and an estimate of the subject's usual level of physical activity. The subjects were encouraged to maintain their customary levels of physical activity but did not participate in competitive sports.

The purpose of the study and the potential risks involved were fully explained to each subject. Written consent was obtained and the protocol was approved by the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects and the Executive and Policy Committee of the Massachusetts Institute of Technology Clinical Research Center. All subjects received financial compensation for their participation in the experiments and they remained healthy throughout.

Pilot studies. Two subjects participated in an initial pilot experiment designed to help establish appropriate tracer doses of citrulline for our subsequent studies. They consumed for 2 days an adequate diet, and this was followed by a low-dose (0.29 μmol·kg^{-1}·hr^{-1}) intravenous citrulline tracer infusion study on day 3 (see below). This experiment was repeated about 4 weeks later when we studied a higher (2.05 μmol·kg^{-1}·hr^{-1}) citrulline tracer dose, otherwise using similar experimental conditions. Four additional subjects took part in a second pilot study, which was conducted to assist interpretation of the isotopic data generated in our main, diet study (see below). These subjects consumed the adequate experimental diet (also see below) for 2 days and on day 3 they were given primed, simultaneous constant intravenous

tracer infusions of L-[5,5-$^2H_2$]citrulline and L-[*ureido*-^{13}C]citrulline, each at known infusion rates of ≈0.98 μmol·kg^{-1}·hr^{-1}.

Diet study. Five subjects participated in this investigation, which was concerned with the effects of arginine-rich (adequate) and arginine-free intakes on plasma arginine and citrulline kinetics. They consumed the arginine-rich diet for 6 days and on the morning of day 7 they received primed, constant intravenous tracer infusions of L-[*guanidino*-^{13}C]arginine, L-[5,5-$^2H_2$]citrulline, and L-[5,5,5-$^2H_3$]leucine. This tracer study was followed immediately by a second experimental diet period during which an arginine-free intake was given for 6 days. Again, on the morning of day 7, a second intravenous infusion protocol, also with the above tracers, was carried out.

Diets. For the pilot studies and during the first 7-day phase of the diet study, each subject received a complete L amino acid-based diet (formulated largely from the amino acid pattern of hen's egg white and whole egg powder), which provided the equivalent of about 1 g of "protein" (N × 6.25) per kg of body weight per day. The major energy source was provided in the form of protein-free wheat starch cookies. The composition of the diet has been described (19).

For the diet experiment, each subject received for 7 days the arginine-rich L amino acid diet and during the next 7-day period, subjects received the same diet except that it was based on an isonitrogenous, arginine-free, L amino acid mixture formula, as described (19). The daily intake, prior to the tracer infusion studies, was consumed as three separate meals at 0800, 1200, and 1700. Meals were prepared in the Clinical Research Center by the dietary staff.

Tracer Infusion Studies. The isotope infusion protocols for the pilot and dietary studies lasted 8 hr. For the first 3 hr, subjects remained in the postabsorptive state (fast state) following an overnight fast, which began at about 1900. This 3-hr phase was followed immediately by a 5-hr fed state during which subjects received small, equal meals at 30- or 60-min intervals (see below). Details of the procedures followed immediately before and during the infusion protocol have been described (19, 20).

In the first pilot study, a relatively low priming dose (0.29 μmol·kg^{-1}) was followed by a constant infusion (0.29 μmol·kg^{-1}·hr^{-1}) of L-[5,5-$^2H_2$]citrulline. Simultaneously, L-[*guanidino*-^{13}C]arginine was given as a primer dose (5.3 μmol·kg^{-1}) and then as a constant infusion at 5.3 μmol·kg^{-1}·hr^{-1}; a [$^2H_3$]leucine priming dose (4.2 μmol·kg) and continuous infusion (4.2 μmol·kg^{-1}·hr^{-1}) were given along with the citrulline and arginine tracers. Four weeks later this experiment was repeated in the same two subjects; on this occasion a larger tracer dose of citrulline (priming, 2.0 μmol·kg; continuous infusion, 2.0 μmol·kg^{-1}·hr^{-1}) was administered, together with [^{13}C]arginine and [$^2H_3$]leucine tracers at the rates used for the first infusion during this pilot study.

For the second pilot study, two citrulline tracers (L-[5,5-$^2H_2$]citrulline and L-[*ureido*-^{13}C]citrulline) were given simultaneously, using priming doses of 0.98 μmol·kg^{-1} and continuous infusion rates of 0.98 μmol·kg^{-1}·hr^{-1}. In both pilot studies, beginning after 180 min of tracer infusion, subjects were given six equal meals at hourly intervals, with intake being equivalent to 1/12th of the total daily intake as provided by the experimental diet during the preceding diet period.

In the diet study, priming doses of L-[*guanidino*-^{13}C]arginine (2.7 μmol·kg^{-1}), L-[5,5-$^2H_2$]citrulline (1.0 μmol·kg^{-1}), and [$^2H_3$]leucine (4.0 μmol·kg^{-1}) were administered over 3 min and then followed immediately by constant, known intravenous tracer infusions at rates of about 2.7, 1.0, and 4.0 μmol·kg^{-1}·hr^{-1}, respectively. After 180 min subjects were given small meals every 30 min for the next 5 hr, providing two-thirds of the previous day's amino acid and energy intake. Baseline blood samples were drawn before the tracers were given, and at 20-min intervals during the last hour of the initial 3-hr tracer period, and again frequently during the last 2 hr of the 5-hr fed phase. These were kept on ice until centrifuged. Plasma was removed and stored at −20°C prior to analysis. Infusates of tracers were prepared from sterile, pyrogen-free powders of high chemical purity and high isotopic enrichment. Tracers (≥98% isotopic purity) were obtained from Tracer Technologies (Somerville, MA).

Analysis of Enrichment of Plasma Arginine, Citrulline, and Leucine. To determine the plasma isotopic enrichment of the ^{13}C and ^{2}H isotopologs of arginine and of citrulline, 200 μl of plasma was extracted essentially by the procedure of Adams (21). A methyl ester was first formed by using methanol and acetyl chloride, and N-trifluoroacetylation followed according to Nissim *et al.* (22). Analysis of the methyl ester trifluoroacetyl derivatives was carried out by on-column injection onto an HP5890 series II gas chromatograph coupled to an HP5988A mass spectrometer (Hewlett–Packard). Selective ion monitoring of arginine and citrulline was conducted on the $[M-20]^-$ ions, using negative chemical ionization with methane as the reagent gas. These ions correspond to a loss of HF from the molecular ion. Selective ion monitoring was carried out at m/z 361, 362, and 363 for natural, ^{13}C-labeled, and ^{2}H-labeled citrulline, respectively, and at m/z 456, 457, and 458 for unlabeled, ^{13}C-labeled, and ^{2}H-labeled arginine, respectively.

Measurement of the plasma [$^2H_3$]leucine enrichments was determined in samples extracted as for arginine. A *tert*-butyldimethylsilyl derivative was prepared (23) and analyzed with a similar GC/MS system as for arginine and citrulline, using splitless injection and electron impact ionization. Selective ion monitoring was carried out at m/z 302 ($[M-57]^+$) for unlabeled leucine and m/z 305 for the ^{2}H-labeled species.

All plasma samples were measured in duplicate and determined against calibration standards. Multiple linear regression was used to calculate the individual plasma isotope enrichments, which were expressed as mole fractions above the value determined from blood drawn prior to tracer administration.

Model of Citrulline and Arginine Kinetics. Plasma amino acid fluxes were calculated from the mean of the plasma isotopic enrichment values for the last 1 hr of the fast and last 2 hr of the fed phases of the tracer period by using steady-state isotope dilution equations, as for previous studies with leucine (18, 24).

The rate of conversion of plasma citrulline to arginine ($Q_{c\to a}$) was determined essentially according to the tracer models of Clarke and Bier (25) and Thompson *et al.* (26), used in these earlier cases for measurement of the conversion of labeled phenylalanine to tyrosine. Thus

$$Q_{c\to a} = Q_a\left(\frac{E_a}{E_c}\right)\left(\frac{Q_c}{i_c + Q_c}\right),$$

where Q_a and Q_c are the plasma arginine and citrulline fluxes (μmol·kg^{-1}·hr^{-1}) estimated independently from the primed, constant infusions of [^{13}C]arginine and [$^2H_2$]citrulline, respectively; E_a and E_c, are the respective plasma enrichments of [$^2H_2$]arginine and [$^2H_2$]citrulline; and i_c is the rate of infusion of labeled citrulline. The expression $Q_c/(i_c + Q_c)$ corrects for the contribution of the tracer citrulline infusion to $Q_{c\to a}$.

Evaluation of Data. Data were analyzed by a repeated measures procedure: subject by diet (arginine-free or arginine-rich) by condition (fast or fed) factorial. When a significant diet–condition interaction was observed, the analysis was repeated separately for each diet and conditions were compared. Plasma amino acid values were compared by a paired t test. $P \le 0.05$ was considered statistically significant.

Table 1. Citrulline and arginine fluxes and conversion of citrulline to arginine ($Q_{c\to a}$) at two tracer doses of citrulline (pilot study 1)

Citrulline dose	Subject	Citrulline		Arginine		$Q_{c\to a}$	
		Fast	Fed	Fast	Fed	Fast	Fed
High	1	9.8	8.0	49.2	55.4	4.7	5.8
	2	8.5	9.0	51.7	62.6	4.6	5.8
Low	1	8.0	6.7	49.3	56.2	5.3	5.2
	2	6.9	6.1	46.1	50.4	4.9	4.2

Values are $\mu mol \cdot kg^{-1} \cdot hr^{-1}$.

RESULTS AND DISCUSSION

To our knowledge, there have been no published estimates of plasma citrulline fluxes in healthy adults under defined dietary conditions. Hence, from the first pilot study we determined, as summarized in Table 1, plasma fluxes of [5,5-$^2H_2$]citrulline to be 6–10 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ during the fast and fed states in these two subjects. Based on these initial data we chose an intermediate citrulline tracer dose for use in the diet study; our purpose was to achieve an enrichment of plasma arginine in the range of about 0.01 mole fraction.

The plasma fluxes of citrulline and arginine while subjects consumed the arginine-rich (adequate) or arginine-free diets are recorded in Table 2. Citrulline fluxes were similar during the fed and fasted states and did not change during the 7-day period of consuming an arginine-free diet. Also, the rate of conversion of plasma citrulline to plasma arginine ($Q_{c\to a}$) was about 5–6 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. Again, this conversion rate was similar during the fast and fed states and was unaffected by ingestion of the arginine-free diet.

Arginine flux was lowered by giving the arginine-free diet, especially during the fed phase of the tracer study. Mean arginine intake with the arginine-rich intake during the fed state was 36 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. We (27) have estimated an ≈38% first-pass disappearance of dietary arginine within the splanchnic region; 62% of the dietary intake appears in the peripheral circulation. Assuming that this value applies to the present study, the difference between the fed-state arginine fluxes for the arginine-rich and arginine-free diets is largely, if not entirely, attributable to the ≈22 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ (36 × 0.62) decline in the entry of dietary arginine into the circulation when the arginine-free diet was ingested. Therefore, the lower arginine flux during the fed state probably reflects quantitatively a diminished supply of dietary arginine, rather than a change in the rate of entry, into the plasma compartment, of endogenous arginine coming from tissue protein breakdown and/or *de novo* synthesis. The similar plasma leucine fluxes (Table 2) under the two arginine-intake conditions imply comparable rates of leucine, and therefore of arginine, release into plasma from the turnover of body proteins and thus supports our reasoning that the lower, fed-state arginine flux, with the arginine-free intake, is due to the altered rate of entry of dietary arginine into the peripheral circulation. However, in this same context, the ≈20% decrease in plasma arginine flux during the fasting state with the arginine-free diet is not as readily understood, because the leucine fluxes were also the same during this state with the two diets. It is possible that the ratio of the plasma leucine and arginine fluxes under different conditions is the consequence of more complex metabolic relationships rather than simply one of turnover of these amino acids from the same protein-bound amino acid pools.

From these findings, the lower, fed-state plasma arginine concentration (Table 3) indicates a reduced entry rate of dietary arginine into the circulation, and the decline in the fasting- and fed-state levels of plasma ornithine (Table 3) is probably a consequence of a reduced rate of arginine degradation, with diminished conversion to ornithine and glutamate (28). Citrulline concentrations were not affected in the fasting condition, although they rose slightly, but significantly ($P < 0.05$), in the fed condition when the arginine-free diet was consumed.

Under steady-state conditions, the plasma citrulline flux (Q_c) of 8–11 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ (Table 2) provides an index of the rate of formation, as well as degradation, of citrulline. Since the metabolic disappearance (degradation) of citrulline from plasma occurs via formation of argininosuccinic acid, with subsequent synthesis of arginine, it is necessary to consider the difference found here between the plasma citrulline flux (Q_c) and the estimated rate of conversion of isotopic citrulline to plasma arginine ($Q_{c\to a}$). The latter rate was about 5–6 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ (Table 2), which is essentially identical to the rate found for arginine synthesis by the kidney of hypertensive patients with normal renal function and determined from arterio–venous difference measurements across this organ (29). This synthesis occurs in the proximal convoluted tubule of the kidney (30–33).

We used L-[5,5-$^2H_2$]citrulline as the tracer, which, in theory, could be recycled through arginine and ornithine pools with return to the plasma compartment. This possibility was confirmed in our second pilot study. As shown in Table 4, citrulline fluxes obtained with the [$^2H_2$]citrulline probe were 82% ($P < 0.05$) of those derived with [*ureido*-^{13}C]citrulline; this latter label would not be recycled, because it is lost as urea during one turn of the urea cycle. Thus, the [$^2H_2$]citrulline underestimates the actual citrulline flux, but these comparative citrulline tracer data may help to explain the metabolic basis for the difference between Q_c and $Q_{c\to a}$, as follows.

First, the cycling between arginine and citrulline via the L-arginine/nitric oxide pathway (34, 35) can be ruled out as an explanation for the difference between the [^{13}C]- and [^{2}H]citrulline fluxes, since both of these labels would be recycled to the same extent. Second, we assume that the 5,5-$^2H_2$ label of citrulline is not recycled via direct flow through the plasma arginine pool, because only a low level of plasma arginine labeling was achieved by giving the citrulline tracer. Thus, part of the labeled citrulline appears to be taken up and converted to arginine at a metabolic site(s) which is separated from the plasma arginine pool and does not equilibrate with it. Subsequently, this plasma-citrulline-derived arginine is metabolized to ornithine and then to citrulline, which reappears as the 5,5-$^2H_2$ compound in plasma. Third, it is assumed that there are no other significant routes of loss of plasma citrulline other than via the flow from citrulline to

Table 2. Plasma citrulline, arginine, and leucine fluxes and conversion of citrulline to arginine ($Q_{c\to a}$) with arginine-rich and arginine-free intakes in young men

Diet	Citrulline		Arginine		$Q_{c\to a}$		Leucine	
	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed
Arg-rich	11.3 ± 3.3	8.3 ± 2.5	60.2 ± 5.4	73.3 ± 13.9*	5.7 ± 1.6	5.2 ± 2.2	106 ± 18	158 ± 26
Arg-free	10.6 ± 3.2	9.7 ± 2.7	49.0 ± 7.5†	47.5 ± 8.8†	5.2 ± 1.3	5.7 ± 1.3	107 ± 15	143 ± 10*

Values are $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ (mean ± SD, $n = 5$).
*Significantly ($P < 0.05$) higher than for the fasting state.
†Significantly ($P < 0.05$) lower than for the arginine-rich diet.

Table 3. Plasma concentrations of free citrulline, ornithine, arginine, and leucine in young men with arginine-rich and arginine-free intakes

Amino acid	Arginine-rich		Arginine-free	
	Fast	Fed	Fast	Fed
Citrulline	38 ± 7	27 ± 5*	37 ± 4	35 ± 5†
Arginine	181 ± 24	210 ± 19	141 ± 22†	136 ± 20†
Ornithine	51 ± 3	70 ± 12*	20 ± 4†	50 ± 6*†
Leucine	162 ± 18	202 ± 41	163 ± 29	204 ± 30*

Values (μmol/liter, mean ± SD, $n = 5$) are for samples taken at the end of the first 3 hr (fast) and at the end of the 5-hr fed phase of the 8-hr tracer infusion.
*Different ($P < 0.05$) from fast.
†Different ($P < 0.05$) from arginine-rich.

ornithine ($Q_{c\rightarrow o}$, involving an inaccessible arginine pool and an "ornithine site") and via the conversion of plasma citrulline to arginine ($Q_{c\rightarrow a}$) in the kidney.

Based on these considerations, tracer/tracee mass balance equations were developed and further details of the model will be provided upon request. Applying these equations to our dietary conditions, we arrive at the following interpretation of our data. If we assume a 20% recycling of the [$^2H_2$]citrulline label, the nonrecycling flux, which equals the rate of citrulline production (appearance) via this cycle ($Q_{o\rightarrow c}$), is ≈12 μmol·kg^{-1}·hr^{-1}. Of this, 5.5 μmol·kg^{-1}·hr^{-1} is converted to arginine that appears in plasma. This latter conversion rate ($Q_{c\rightarrow a}$) is estimated from the $^2H_2$ enrichments of citrulline and arginine and a simultaneous infusion of guanidino-labeled arginine, analogous to determination of the plasma phenylalanine-to-tyrosine conversion rate proposed by Clarke and Bier (25). This plasma citrulline-to-arginine conversion ($Q_{c\rightarrow a}$) equals the net citrulline production ($Q_{o\rightarrow c} - Q_{c\rightarrow o}$). A large fraction of the flow from plasma citrulline back to ornithine then leaves the system, probably via conversion to glutamate, and about 2.4 μmol·kg^{-1}·hr^{-1} is recycled. Correcting the total citrulline production rate ($Q_{o\rightarrow c}$) for the recycling component gives the measured flux of the recycled [5,5-$^2H_2$]citrulline. This equals 10.6 μmol·kg^{-1}·hr^{-1}, and it reflects the recycling-corrected flow of citrulline from its site of production through the plasma site. On leaving the plasma site, part of the flow of citrulline feeds the conversion of plasma citrulline to arginine and another part flows back to ornithine (i.e., $Q_{c\rightarrow o}$), where it then leaves the cycle, at the ornithine site. Thus, the flux of the recycling tracer is always larger than the plasma citrulline-to-arginine conversion rate alone.

Despite the complexity of these *in vivo* isotopic data, they are coherent; both Q_c and $Q_{c\rightarrow a}$ did not change between the arginine-rich and arginine-free diets. On this basis, we conclude that the net synthesis rate of arginine was not affected by a 7-day arginine-free intake. Using a less direct tracer model approach, involving comparison of plasma arginine and leucine fluxes, we (19) concluded previously that an arginine-free intake did not affect the rate of endogenous arginine synthesis. Our observations are consistent with those of Dhanakoti *et al.* (31), who found that renal arginine synthesis in the adult rat was unaffected by the intake of dietary arginine, and of Hartman and Prior (36), who reported no change in intestinal citrulline synthesis in rats given an arginine-free, glutamate-supplemented diet.

Table 4. Comparison in healthy young men of two citrulline tracers for measurement of plasma citrulline flux

Citrulline tracer	Flux, μmol·kg^{-1}·hr^{-1}	
	Fast	Fed
ureido-^{13}C	12.6 ± 3.2 (100%)	10.7 ± 2.2 (100%)
5,5-$^2H_2$	10.3 ± 2.9 (81 ± 6%)	8.7 ± 1.6 (82 ± 5%)

Values (mean ± SD, $n = 4$) for [5,5-$^2H_2$]citrulline are significantly ($P < 0.05$) lower than those for [*ureido*-^{13}C]citrulline.

There is a clear inter-organ and intracellular compartmentation to arginine metabolism *in vivo* (31, 33, 37, 38). Our previous tracer studies (18, 19, 27) have indicated that the rapid turnover of arginine within the hepatic urea cycle is effectively isolated from the metabolism of arginine within the cytosolic free amino acid pool of the liver and from the arginine pools within the peripheral circulation and tissues. Against this background, and together with the present and previous findings (19), we now propose that whole-body arginine homeostasis in healthy adults is achieved principally via a modulation in the level of dietary arginine intake and/or with regulation in the rate of arginine degradation; changes in the net rate of arginine synthesis in extrahepatic tissues do not appear to be an important contributor to arginine homeostasis (Fig. 1). Consistent with this proposal is a report showing that ornithine oxidation is reduced in mice given an arginine-free diet (39). Further, our hypothesis predicts that those conditions which greatly increase the metabolic demand for arginine, such as major trauma (40, 41), severe sepsis, or marked catch-up growth (14), would precipitate a conditional deficiency of arginine unless there was an adequate supply of arginine or citrulline available from exogenous (enteral or parenteral) sources. Also, this scheme anticipates that, in comparison with citrulline and arginine, exogenous ornithine would serve as a relatively poor substrate for maintaining body arginine balance; evidence from experimental (42, 43) clinical (14, 44–46) studies favors this suggestion.

In conclusion, the net rate of *de novo* arginine synthesis in human subjects appears to be unaffected by acute changes in the dietary intake level of the amino acid; evidently, arginine homeostasis is achieved by changing the rate of arginine

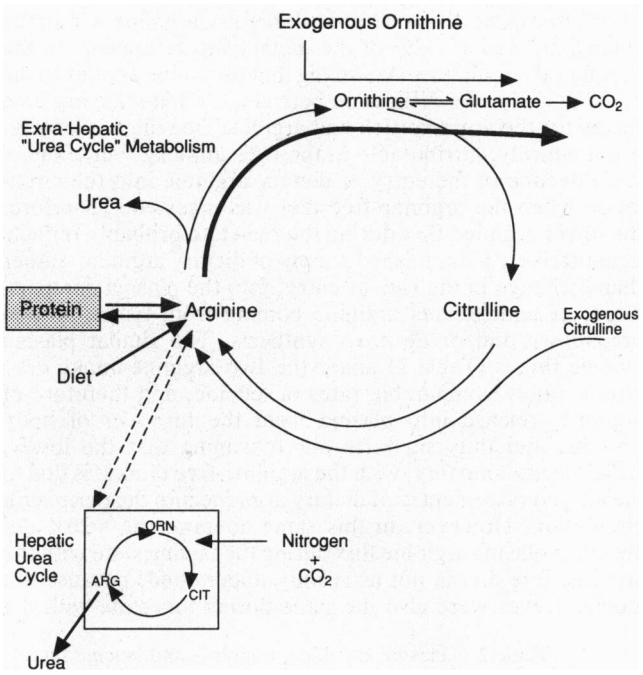

FIG. 1. Metabolic relationships between extrahepatic "urea-cycle" arginine and its precursors, ornithine and citrulline. The heavy arrow is intended to convey the idea that arginine homeostasis is achieved via alterations in the dietary intake level of arginine and its rate of catabolism to ornithine and glutamate. The net synthesis of arginine, via conversion of ornithine to citrulline (in intestine) and of citrulline to arginine (in kidney), is assumed to be less influenced by a regulatory control. In the hepatic urea cycle: ARG, arginine; ORN, ornithine; CIT, citrulline.

degradation relative to the dietary intake level and metabolic state. It follows that conditions which substantially increase arginine utilization and turnover would lead to a dietary requirement for this amino acid and so this explains why, from a metabolic standpoint, it is conditionally indispensable in human nutrition.

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