Published in final edited form as:

Clin Nutr. 2008 August ; 27(4): 513–522. doi:10.1016/j.clnu.2008.04.014.

Adaptation to a long term (4 weeks) arginine- and precursor (glutamate, proline and aspartate)-free diet[☆]

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Summary

Background & aims—It is not known whether arginine homeostasis is negatively affected by a “long term” dietary restriction of arginine and its major precursors in healthy adults. To assess the effects of a 4-week arginine- and precursor-free dietary intake on the regulatory mechanisms of arginine homeostasis in healthy subjects.

Methods—Ten healthy adults received a complete amino acid diet for 1 week (control diet) and following a break period, six subjects received a 4-week arginine, proline, glutamate and aspartate-free diet (APF diet). The other four subjects continued for 4 weeks with the complete diet. On days 4 and 7 of the first week and days 25 and 28 of the 4-week period, the subjects received 24-h infusions of arginine, citrulline, leucine and urea tracers.

Results—During the 4-week APF, plasma arginine fluxes for the fed state, were significantly reduced. There were no significant differences for citrulline, leucine or urea fluxes. Arginine *de novo* synthesis was not affected by the APF intake. However, arginine oxidation was significantly decreased.

Conclusions—In healthy adults, homeostasis of arginine under a long term arginine- and precursor-free intake is achieved by decreasing catabolic rates, while *de novo* arginine synthesis is maintained.

Keywords

Arginine; Metabolism; Stable isotopes; Synthesis; Oxidation

Introduction

Arginine serves multiple functions in addition to its role as a substrate in protein synthesis; it is required for detoxification of ammonia, for the synthesis of nitric oxide,¹ agmatine,

[☆]In memoriam Dr Vernon R. Young.

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Conflict of interest statement None of the authors have any conflict of interest with the funding agencies.

polyamines and creatine.² Arginine also plays a role in the nutritional rescue of reduced gene expression³ and in the translation control of inducible nitric oxide (iNOS) mRNA.⁴ An arginine deficiency impairs B cell maturation,⁵ and supplementation or restriction of arginine intake have been advocated as therapeutic modalities for different pathophysiologic conditions.^{6,7} Despite the functional and physiological significance of arginine, the whole body response and the homeostatic mechanisms elicited during a “long term” dietary restriction of arginine and its immediate metabolic precursors (proline, glutamate and aspartate), have not been previously investigated in healthy adults. Therefore, we have examined whole body, *in vivo* homeostasis of arginine in healthy adults under conditions of a long term (4 weeks) restriction of dietary arginine and its major precursors (ornithine, proline, glutamate and aspartate). This information is important to enhance our understanding of the significance of arginine in human nutrition. We also investigated leucine and urea kinetics as indicators of the dynamic status of whole body protein metabolism during this period of dietary arginine restriction.

Materials and methods

Subjects

Ten healthy adult subjects (9 men and 1 woman; 26 ± 2 years; body weight 75.5 ± 16 kg) participated in these studies. They were studied at the Clinical Research Center (CRC) of the Massachusetts Institute of Technology (MIT). All were in good health, as determined by history and physical examination, analysis of blood cell count, biochemical profile and normal urine analysis. The purpose of the study and potential risks involved were fully explained to each subject. Written consent was obtained. The protocol was approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Executive and Policy Committee of the MIT CRC. The subjects received financial compensation for their participation in the experiments. All remained healthy throughout the study period. Six subjects participated in the arginine- and precursor-free study and 4 different subjects participated in the control studies. Only 4 subjects were studied in the control group because this group received adequate arginine intake throughout the course of the study and no changes in arginine metabolism were expected under conditions of adequate arginine intake.

Diet

The experimental diets used are shown in Table 1. Each subject received an L-amino acid-based diet supplying the equivalent of about 1 g protein ($N \times 6.25$) (kg body wt^{-1}). For the arginine- and precursor-free study (APF), the amino acid mixture was devoid of arginine, proline, aspartic and glutamic acid. Citrulline and ornithine, two non-dietary amino acids, were not supplied. Both diets were maintained isonitrogenous and recommended intakes of indispensable amino acids were equal in both groups. The major energy source was provided in the form of protein-free wheat starch cookies, as previously described.⁸ The energy intake was maintained constant within each individual and ranged between 35 and 55 $\text{kcal kg}^{-1} \text{ day}^{-1}$, according to diet history and an approximation of the subject's physical activity level. Energy was provided as 35–40% fat and 55–60% as beet sucrose and wheat starch carbohydrates. Flavoring agents (Vivonex flavor packets; Norwich Eaton Pharmaceuticals, Norwich, NY) were added to improve the taste of the amino acid mixture. Beet sucrose was used to avoid changes in the background ^{13}C -isotope enrichments of the expired carbon dioxide between the fasted and fed states, particularly during the tracer studies. Vitamins were supplied as a daily supplement to meet or exceed recommended dietary allowances.⁹ Multi-mineral and choline supplements were also provided.⁸ The total daily intake before the tracer infusion studies was consumed as three separate meals at 08:00, 12:00 and 17:00 h. At least two of these three meals were eaten in the CRC under the supervision of the dietary staff.

Study design

There were two study groups, control and APF. Subjects were assigned to each group. Within each group, subjects were randomized to receive either the 1-week or 4-week adequate intake for the control group, or the 1-week adequate or 4-week APF for the APF group, as depicted in Fig. 1. There was a break period of 1 week between the 1- and 4-week dietary periods. The subjects received two 24-h tracer infusions during each diet period (days 4 and 7 of the first week; and days 25 and 28 of the 4-week period). Plasma ammonia concentrations were obtained every week throughout the study.

Tracer protocols

The subjects were admitted as inpatients to the CRC infusion room at 06:30 h on the morning of the tracer study. Weight was obtained after voiding and then they remained in bed during the infusion study. The procedures followed have been described previously.^{8,10} Each infusion study period lasted a total of 24 h. During the first 12 h (0–720 min) the subjects received their daily protein and energy intake, provided as small, isonitrogenous, isocaloric meals every hour. For the following 12 h (720–1440 min) the subjects remained fasting.

Blood and expired air samples

Blood and breath samples were collected at baseline and every hour throughout the 24-h period. Blood was collected in chilled, heparin-containing tubes and was centrifuged immediately at $3000 \times g$ for 15 min at 4 °C; the plasma was stored at –20 °C until analyzed. Breath samples were collected as described previously,⁸ and stored at room temperature until analyzed by isotope ratio mass spectrometry (MAT Delta E; Finnigan, Bremen, Germany). Total carbon dioxide production was measured by indirect calorimetry (Deltatrack; Sormedics, Anaheim, CA) on four occasions throughout the 24-h period.

Infusion studies

Following an overnight fast, the subjects received on dietary day 4 of the 1-week control period and on day 25 of the subsequent 4-week diet period, a 24-h primed, constant, intravenous infusion of L-[guanidino-¹⁵N₂,5,5,²H₂]arginine ($5 \mu\text{mol kg}^{-1}$; $5 \mu\text{mol kg}^{-1} \text{h}^{-1}$), L-[1-¹³C]leucine ($4 \mu\text{mol kg}^{-1}$; $4 \mu\text{mol kg}^{-1} \text{h}^{-1}$) and ¹³C urea ($77 \mu\text{mol kg}^{-1}$ and $7 \mu\text{mol kg}^{-1} \text{h}^{-1}$), respectively, for prime and constant infusion rates (Fig. 1). On days 7 and 28 of these respective periods (control and 4-week period), they received for 24 h L-[¹³C-ureido,5,5,²H₂]citrulline and L-[5-¹³C]arginine, at rates of $1 \mu\text{mol kg}^{-1}$; $1 \mu\text{mol kg}^{-1} \text{h}^{-1}$ and $5 \mu\text{mol kg}^{-1}$; $5 \mu\text{mol kg}^{-1} \text{h}^{-1}$, respectively, for the prime and constant infusions. The bicarbonate pool was primed with $0.8 \mu\text{mol kg}^{-1}$ of ¹³C sodium bicarbonate (99% atom, Cambridge Isotopes Laboratories), in each tracer infusion study.

Analytical methods

We have previously described, in detail, the treatment of blood and expired air samples for determination of isotopic enrichment and analysis of plasma arginine, leucine, citrulline and urea concentrations and enrichment.^{8,10,11}

Materials

L-[1-¹³C]Leucine (99 atom %), L-[guanidino-¹⁵N₂,5,5,²H₂]arginine (99 atom %), L-[¹³C-ureido-5,5,²H₂]citrulline (99 atom %) and L-[5-¹³C]arginine (99 atom %), were purchased from MassTrace, Woburn, MA. NaH¹³CO₃ (95 atom %) and ¹³C-urea (99 atom %) were purchased from Cambridge Isotope Laboratories, Andover, MA.

Tracer model of amino acid kinetics

Plasma amino acid fluxes ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) were calculated from the mean of the plasma isotopic enrichment values, according to standard, steady state isotope-dilution principles.^{8,12} The leucine flux for the present study was determined from the enrichment of plasma ^{13}C -leucine rather than from α -keto-[1- ^{13}C]isocaproic acid.

The rate of citrulline conversion to arginine (net *de novo* arginine synthesis) ($Q_{\text{cit}} \rightarrow \text{arg}$ $\mu\text{mol kg}^{-1} \text{h}^{-1}$) was determined essentially according to the tracer models of Clarke and Bier¹³ and Thompson et al.,¹⁴ as previously described.^{14,15}

The rate of $^{13}\text{CO}_2$ release from ^{13}C -labeled arginine (arg ox) or rate of leucine oxidation (leu ox), based on measurement of plasma arginine and leucine enrichments, and $^{13}\text{CO}_2$ output in expired air, was calculated as previously described. In calculating leucine oxidation the contribution made to $^{13}\text{CO}_2$ output via the hydrolysis of [^{13}C]urea was assumed to be insignificant.⁸ The ^{13}C retention factor for estimating arginine oxidation was obtained from 6 healthy subjects receiving a constant infusion of L-[5- ^{13}C]glutamic acid.¹⁶ These studies gave a fractional recovery of $^{13}\text{CO}_2$ of 0.40 and 0.52, respectively, for fasting and fed states in healthy subjects. L-[5- ^{13}C]glutamic acid was used to determine fractional retention of ^{13}C , because its ^{13}C carbon is a more direct precursor for estimation of arginine oxidation, than bicarbonate or acetate. For estimation of leucine oxidation we used values of 0.77 and 0.85, respectively, for the post-absorptive and fed periods for bicarbonate recovery, as obtained previously in healthy subjects.⁸

Statistical methods

Plasma amino acid concentrations, fluxes, arginine synthesis rates, as well as arginine and leucine oxidation were tested for normality using the Kolmogorov–Smirnov test and no significant departures were detected for any of the variables.¹⁷ To evaluate differences between fed and fast metabolic states and to determine the effect of the APF diet compared to the adequate diet, a repeated-measures analysis of variance (ANOVA) using a mixed-model approach was performed.^{18,19} Two specific questions were addressed: (i) to verify that the two groups (APF and control) were not significantly different at the start of the study, a repeated-measures ANOVA on week 1 only (baseline data) was conducted on each variable with group as the between-subjects effect and fed/fast state as the within-subjects effect; (ii) to test the effect of the APF diet compared to the adequate diet, repeated-measures ANOVA on the change from week 1 to week 4 was performed, with group as the between-subjects effect and fed/fast state as the within-subjects effect. For variables with significant diet by state interactions, simple effects testing was used in the mixed-model analysis to evaluate differences between fed and fast states within the APF and control groups separately. Two-tailed values of $P < 0.05$ were considered statistically significant. Analysis of the data was performed using the GLM procedure in the SPSS statistical package (version 12.0, SPSS Inc., Chicago, IL). Data are presented in terms of the mean and standard deviation (SD) for all continuous variables.

Results

Table 2 shows the plasma amino acid values obtained. There were marked differences ($P > 0.05$) in plasma amino acid concentrations for the fed and fasted state as previously observed.^{8,11} In contrast to the plasma concentrations of arginine, ornithine, proline, glutamate, aspartate and leucine which decreased during the fasted state, when adequate intake was given, plasma citrulline was higher ($P > 0.05$) during the fasted state at 1 and 4 weeks for the control and APF groups, and this is also consistent with previous data.¹⁵

There was a significant effect of the 4-week APF diet on plasma amino acid concentrations. Plasma arginine, ornithine, proline and aspartate were significantly decreased ($P > 0.001$) during the fed and fasted states when the subjects received the 4-week APF dietary period. The lowest arginine concentrations were observed when the APF diet had been consumed for 4 weeks ($P < 0.001$), but interestingly the lowest concentration was observed during the fed period. Plasma citrulline values were similar to those previously reported in healthy subjects,^{11,15} and did not change with the 4-week APF intake. Plasma leucine concentrations were variable. However, they remained unchanged between the 1-week adequate intake and the 4-week APF intake. Hence, there was no effect of the APF diet on plasma leucine concentrations. All other amino acids not mentioned remained unaffected by the APF diet.

Plasma ammonia concentrations remained unchanged throughout the study period in both the control and APF dietary groups (Table 2). The values tended to be at the upper end of the normal range. This is possibly related to the use of an L-amino acid mixture as the sole source of nitrogen and amino acids.

The plasma isotopic abundances for the infused L-[1-¹³C]leucine and L-[guanidino-¹⁵N₂ 5,5,2H₂]arginine (M + 4) on day 4 of the control period and day 25 of the 4-week period for the control and APF dietary groups are summarized in Table 3 and in Fig. 2A and B, respectively. Those for L-[5-¹³C]arginine and L-[¹³C-ureido,5,5,2H₂]citrulline (M + 3) (citrulline) infused on days 7 and 28 are also summarized in Table 3 and Fig. 3A and B, respectively. The plasma enrichment values of L-[¹³C-guanidino-5,5,2H₂]arginine (M + 3) formed from the L-[¹³C-ureido, 5,5,2H₂]citrulline tracer, reflecting net *de novo* arginine synthesis, are presented also in Table 3 and in Fig. 3A and B for the control and APF groups, respectively. We could not detect plasma enrichment of [¹⁵N,5,5,2H₂]citrulline derived from L-[guanidino-¹⁵N₂ 5,5,2H₂]arginine, and therefore, we could not estimate rates of NO synthesis. In all cases, including that for [¹³C]urea (Table 3), a steady state isotopic enrichment plateau was achieved. Plasma fluxes of arginine, leucine, citrulline and urea were calculated from these isotopic enrichments.

Table 4 summarizes the plasma fluxes of arginine, citrulline, leucine, urea and the net rate *de novo* of arginine synthesis during the fed and fast states for the control diets and the 4-week APF dietary intake. There was a difference ($P = 0.001$) in plasma arginine fluxes, regardless of the tracer used, for the fast and fed state, as previously observed.^{11,15} However, there was no difference in plasma arginine fluxes between weeks 1 and 4 of the control group. In the APF group receiving adequate intake during week 1, there was a difference ($P < 0.001$) in plasma arginine fluxes between fed and fasted state. There was no difference between the arginine fluxes for the fed and fasted state following 4 weeks of consuming the APF diet. Furthermore, the plasma fluxes of arginine for the fed state of week 4 of the APF period, as determined with either the L-[guanidino-¹⁵N₂5,5,2H₂]arginine (M + 4) or the L-[5-¹³C]arginine (M + 1) tracer, were significantly lower ($P < 0.05$) than those obtained during the control group receiving adequate diets at 1 and 4 weeks, and from week 1 of the APF group when an adequate arginine intake was given.

The plasma citrulline fluxes varied between group means of 14.1–24.5 $\mu\text{mol kg}^{-1}\text{h}^{-1}$ among the control and APF diets. There were significant differences ($P < 0.01$) for citrulline fluxes between the fed and fast states when the adequate diet was consumed during weeks 1 and 4 in the control group, or during week 1 of the APF group, but there was no difference when the 4-week APF diet was consumed, and the citrulline flux increased during the APF intake ($P < 0.01$). The plasma fluxes of leucine remained unchanged between the control diets and APF diet. There was an increase ($P < 0.01$) in plasma leucine fluxes during the fed state across dietary groups, as previously observed for adequate leucine intakes.⁸ Urea fluxes tended to be slightly, but not significantly lower after 4 weeks with both the adequate and APF diets although

the decline was small (~8%). The urea fluxes were about as expected for the total nitrogen intake given in this study.²⁰

The net synthesis rates of arginine varied between group means of 6.9–11 $\mu\text{mol kg}^{-1} \text{h}^{-1}$ among the different diet-time groups. There was no difference in arginine synthesis between the control or APF groups.

Fig. 4 shows the rates of $^{13}\text{CO}_2$ production ($V^{13}\text{CO}_2$) with the arginine and leucine tracers for the control group at 1 and 4 weeks (A) and for the APF dietary group at 1 and 4 weeks (B). Table 5 presents results for ^{13}C enrichment of expired air and the appearance rate of $V^{13}\text{CO}_2$ in both groups. Arginine oxidation rates, estimated from the recovery of [5- ^{13}C] glutamate, were significantly lower ($P < 0.01$) for the fast state when compared to the fed state, in the control group. In the APF group, the same effect of the fast/fed state was observed in week 1 of the adequate dietary intake of the APF group, but not when the APF diet was consumed for 4 weeks, where distinctively, the arginine oxidation rates were the lowest ($P < 0.001$) during the fed state. There was a significant ($P < 0.001$) change in the rates of arginine oxidation from week 1 to week 4 of the APF but not in the control group. Hence, restriction of dietary arginine and its precursors resulted in a marked reduction in arginine oxidation rate during the fed and fast states. The rates of leucine oxidation were lower for the fasted state when compared to the fed state ($P < 0.001$), as previously reported,⁸ and their values did not differ among the control or APF groups. Likewise, there was no change between weeks 1 and 4 of the APF group Fig. 5.

Discussion

We have investigated *in vivo* whole body arginine, citrulline, leucine and urea metabolism in healthy young adults receiving an adequate or an arginine- and arginine-precursor-free intake for a relatively prolonged period of 4 weeks. Given the multiple functions of arginine^{1,2,21} and the fact that a number of therapeutic approaches have been based on alterations in arginine availability,^{6,7} it is of importance to investigate the longer-term homeostatic mechanisms elicited in response to a prolonged period of dietary deprivation of arginine and of its major immediate precursors. As shown in Fig. 6 the carbon skeletons from alpha-ketoglutarate and oxaloacetate are readily available for synthesis of L-glutamic and L-aspartic acid respectively, and their amino groups are provided by transamination. Therefore, these arginine precursors appear to be accessible. However, whether a long term deprivation of L-arginine and its precursors would be limiting on arginine homeostasis had not been previously explored. Our data show that L-glutamic acid, L-proline and L-aspartic acid are not limiting for endogenous arginine synthesis in healthy adults fed a diet devoid of these precursors over a 4-week period. Previously, we have studied the changes in whole body arginine metabolism over a shorter, 6-day arginine-free diet and observed that (i) arginine fluxes were reduced, especially during the fed period, (ii) net arginine synthesis was not influenced by an acute change in arginine intake, (iii) the rate of conversion of arginine to ornithine was markedly reduced, as was the rate of arginine oxidation, especially in the fed state.^{11,12,15}

In the present study we extended the period of dietary restriction to 4 weeks, including limiting the intakes of the major, immediate dietary precursors of arginine (proline, glutamate, and aspartate). Nevertheless, in qualitative terms, the response to a 4-week APF restriction appears to be similar to that following a 1-week restriction of dietary arginine alone.^{11,15} Thus, a reduced rate of arginine oxidation with maintenance of endogenous arginine synthesis, which occurs at a rate of about one-third of the dietary intake level, supports the notion¹² that arginine homeostasis is maintained initially via modulations in the rate of arginine oxidation, via conversion to ornithine and subsequently to glutamate. Whether this response requires a change in the levels/activities of arginase isoenzymes^{22,23} and/or of ornithine aminotransferase²² and/

or whether it is a consequence of the lower concentrations of arginine in the plasma, reflecting smaller intracellular pools, cannot be determined from the present study. The maintenance of *de novo* arginine synthesis in spite of the long term arginine- and precursor-free intake is probably due to preservation of glutamine availability for intestinal synthesis of citrulline and export to the kidney, where *de novo* synthesis of arginine takes place.

At the zero arginine intake we estimate the rate of arginine oxidation to be about $240 \mu\text{mol kg}^{-1} \text{ day}^{-1}$, with an estimated *de novo* synthesis rate of approximately $260 \mu\text{mol kg}^{-1} \text{ day}^{-1}$. On this basis it would appear that the body has adjusted to the low intakes of arginine and its precursors by effectively lowering the catabolism of arginine and so maintaining body arginine equilibrium. For the adequate diet, the subjects appeared to be in a positive arginine balance (balance = intake + *de novo* synthesis – oxidation); and whole body arginine balance was estimated to be $6 \mu\text{mol kg}^{-1} \text{ day}^{-1}$. This compares with an estimated leucine balance (correcting the oxidation by a factor of 1.3²⁴ to provide the equivalent of a ketoisocaproic acid-based measurement of intracellular leucine) of $3 \mu\text{mol kg}^{-1} \text{ day}^{-1}$. At the end of the fourth week with the APF diet the mean arginine balance was $0.9 \mu\text{mol kg}^{-1} \text{ day}^{-1}$ whereas the leucine balance was $-0.8 \mu\text{mol kg}^{-1} \text{ day}^{-1}$. It appears that given some uncertainty associated with the precise estimation of arginine oxidation, our subjects' body amino acid balances were in approximate equilibrium for both leucine and arginine.

The purpose of including [¹³C]leucine in this study was in reference to its use as an indicator of the overall status of whole body leucine balance^{24,25} and as an approximation to estimate amino acid requirements via application of the so-called indicator amino acid oxidation approach.^{26,27} It was thought that if an arginine-precursor-free diet caused deterioration in whole body amino acid homeostasis, as would be expected if the intake of one of the specific indispensable amino acids was inadequate, this would be an indication that arginine availability was limiting. According to the leucine balance estimates above, the APF diet did not bring about an increased negative leucine balance. Leucine balance was also stable throughout the 4-week dietary period when subjects consumed the control (adequate) diet.

On the basis of these findings we conclude that in healthy adults, and exclusively from the nutritional endpoint, arginine is a dispensable amino acid, even when the intakes of the immediate metabolic precursors (glutamate, proline, aspartate) are restricted, while maintaining nitrogen intake at a sufficient level. The “functional requirements” of arginine remain to be established and may be different from nutritional requirements. In the present case nitrogen intake was $160 \text{ mg N kg}^{-1} \text{ day}^{-1}$, which exceeds that necessary to maintain mean nitrogen balance in healthy adults who receive an adequate supply of indispensable amino acids.⁸ It has been suggested,²⁸ based on the theory presented by Katagiri and Nakamura,²⁹ that a preformed source of α -amino nitrogen was necessary for maintenance of the nitrogen economy of the host and that the primary amino acid in this case would be glutamate. However, the present APF diet contained alanine and serine that presumably serve as adequate sources of α -amino N.

We were unable to reliably measure the enrichment of $\text{L-[ureido-}^{15}\text{N}, 5, 5, \text{}^2\text{H}_2\text{]citrulline (M + 3)}$ intracellularly derived from the $\text{L-[guanidino-}^{15}\text{N}_2, 5, 5, \text{}^2\text{H}_2\text{]arginine (M + 4)}$ tracer in the present experiment. We infer that an isotopic effect related to the deuterated M + 4 arginine label prevent us from measuring derived M + 3 citrulline. Furthermore, the proportion between the rate of appearance of citrulline and the rates of *de novo* arginine synthesis is about 100% when the [¹³C]citrulline tracer is used,¹⁰ this proportion is decreased to about 50% when a deuterated citrulline tracer is used, as previously shown,¹⁵ and confirmed in the present study. We believe that the deuterated tracers exhibit isotopic/metabolic differences that make them less reliable when estimating arginine and NO kinetics. Hence, we do not know whether dietary arginine availability was a limiting factor for NO synthesis in the present case.

Notwithstanding the fact that exogenous arginine availability appears to be a factor in determining the rate of nitric oxide (NO) synthesis,³⁰ consideration of the kinetic characteristics (K_m) of nitric oxide synthase (NOS) would suggest that despite the fall in the plasma (and presumably, cellular) concentrations of arginine on the APF diet, arginine was still at non-limiting levels.

Our tentative conclusion concerning the dietary dispensability of arginine applies only to healthy subjects and with nutritional balance as an endpoint; we have previously concluded that a source of dietary arginine is required in severely stressed patients¹⁶ and so in this case arginine is a conditionally indispensable amino acid.

Finally, it should be recognized that our conclusions are based on considerations of whole body arginine kinetics and amino acid balance. These may be parameters that are not sufficiently precise to determine the dietary, nutritional and functional significance of arginine in the healthy, non-stressed individual.

Acknowledgments

J.F. Tharakan and L. Castillo carried out the human phase of the experiments. Y.-M. Yu supervised the analysis of the samples for isotopic determinations and A.M. Ajami provided guidance and assistance in the mass spectrometric methods. D. Zurakowski assisted us in the statistical evaluation of the data, R.M. Roth contributed to summarizing, calculating the data and revising the manuscript. V.R. Young and L. Castillo wrote the initial draft of the manuscript and managed the overall project. This study was financially supported by grants NIH DK 62363, DK 15856, DK 40561. 3ARP-amino acid program, Ajinomoto and USDA/ARS Cooperative agreement 25337387.

Abbreviations

APF

arginine- and precursor-free

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Figure 1.

Experimental design used to explore a 4-week adaptation period to an arginine- and precursor-free (APF) diet. The isotopically labeled compounds used during the 24 h tracer infusions are indicated in the bottom of the tracer infusion figure.

Figure 2.

(A) Isotopic enrichment of plasma L-[guanidino-¹⁵N₂,5,5,²H₂]arginine, L-[1-¹³C]leucine and [¹³C]urea during the last 6 or 8 h of the fed and fasting periods in subjects receiving the control diet at 1 and 4 weeks. (B) Isotopic enrichment of plasma L-[guanidino-¹⁵N₂,5,5,²H₂]arginine, [¹³C]leucine and [¹³C]urea during the last 6 or 8 h of the fed and fasting periods in subjects receiving the control diet for 1 week and APF diet during the 4-week period.

Figure 3.

(A) Isotopic enrichment of plasma L-[5-¹³C]arginine, L-[¹³C-ureido, 5,5,²H₂]citrulline and its labeled arginine derivative during the fed and fasting periods in subjects receiving the control diet at 1 and 4 weeks. (B) Isotopic enrichment of plasma L-[5-¹³C]arginine, L-[¹³C-ureido, 5,5,²H₂]citrulline and its labeled arginine derivative in subjects receiving the adequate diet for 1 week and APF diet during the 4-week period.

Figure 4.

(A) Rates of $^{13}\text{CO}_2$ production ($V^{13}\text{CO}_2$) derived from L -[1- ^{13}C]arginine tracer on days 7 and 28 and from L -[1- ^{13}C]leucine tracer on days 4 and 25 for the control group at 1 and 4 weeks. (B) Rates of $^{13}\text{CO}_2$ production ($V^{13}\text{CO}_2$) derived from L -[1- ^{13}C]arginine on days 7 and 28 and from L -[1- ^{13}C]leucine tracer on days 4 and 25 for the APF group at 1 week adequate intake and 4 weeks APF.

Figure 5.

Schematic representation of the contribution of proline, glutamate and aspartate to arginine synthesis. Alpha-ketoglutarate and oxaloacetate donate carbon skeleton for synthesis of glutamate and aspartate, respectively. L-Glutamic γ semialdehyde forms L- Δ' -pyrroline-5-carboxylic acid to yield L-proline and ornithine. Ornithine and carbamyl phosphate will form citrulline, and aspartate will yield arginino succinate, an intermediate precursor of arginine.

Table 1

Composition of L-amino acid mixtures used for the control and the arginine- and precursor-free (APF) diet given to healthy subjects

Amino acid	Control (mg kg ⁻¹ day ⁻¹)	APF (mg kg ⁻¹ day ⁻¹)
Tryptophan	18.6	18.6
Threonine	56.1	56.1
Isoleucine	74.8	74.8
Leucine	99.1	99.1
Lysine-HCl	90.1	90.1
Methionine	35.3	35.3
Cystine	26.3	26.3
Phenylalanine	65.1	65.1
Tyrosine	48.4	48.4
Valine	83.6	83.6
Histidine-HCl·H ₂ O	36.5	36.5
Arginine-HCl	89.4	0
Aspartic acid	78.9	0
Glutamic acid	134.9	0
Proline	49.9	0
Alanine	73.2	176.8
Glycine	41.8	149.0
Serine	99.9	208.6
Total nitrogen	157.7	157.7

Table 2

Amino acid	Control group (<i>N</i> = 4)		APF group (<i>N</i> = 6)					
	1 week adequate		4 week adequate		1 week adequate		4 week APF	
	Fed	Fast	Fed	Fast	Fed	Fast	Fed	Fast
Arginine	135 ± 17 ^a	127 ± 11	138 ± 18 ^a	126 ± 11	124 ± 29 ^a	96 ± 25	60 ± 7 ^{a,b}	86 ± 12 ^b
Citrulline	30 ± 4 ^a	36 ± 3	29 ± 5 ^a	36 ± 3	28 ± 3 ^a	32 ± 3	26 ± 2 ^a	34 ± 3
Ornithine	74 ± 5 ^a	65 ± 9	69 ± 7 ^a	60 ± 2	70 ± 6 ^a	60 ± 3	40 ± 7 ^b	43 ± 9 ^b
Proline	234 ± 8 ^a	189 ± 14	251 ± 15	185 ± 11a	238 ± 13 ^a	196 ± 12	152 ± 11 ^b	149 ± 9 ^b
Glutamate	48 ± 7 ^a	32 ± 9	46 ± 7 ^a	30 ± 8	51 ± 6 ^a	34 ± 5	22 ± 3 ^b	23 ± 3 ^b
Aspartate	20 ± 2 ^a	16 ± 2	19 ± 2 ^a	17 ± 1	19 ± 3 ^a	17 ± 2	6 ± 1 ^b	4 ± 0.5 ^b
Leucine	155 ± 29 ^a	124 ± 20	155 ± 29 ^a	122 ± 20	190 ± 24 ^a	145 ± 11	189 ± 21 ^a	144 ± 11
Ammonia		27 ± 1		29 ± 2		27 ± 4		30 ± 4

All values are mean $\pm$ SD and expressed as μM , except for ammonia ($\text{mg day}^{-1} \text{ l}^{-1}$).

^a $P < 0.05$; mixed model ANOVA, fed vs. fast at 1 or 4 weeks for control or APF group.^b $p < 0.05$; mixed model ANOVA, change between week 1 and week 4 of APF group for a given state (fed or fasted).

Table 3
Plasma isotopic enrichment of ι -[guanidino- $^{15}\text{N}_2$ 5,5, $^2\text{H}_2$]arginine, ι -[^{13}C -ureido-5,5, $^2\text{H}_2$]citrulline, ι -[1- ^{13}C]leucine, [^{13}C]urea and derived ι -[guanidino- ^{13}C -5,5, $^2\text{H}_2$]arginine for the fed and fast states

Tracer or derivative	Control group (N = 4)		APF group (N = 6)			
	Diet 1 week adequate		Diet 4 week adequate		Diet 1 week APF	
	Fed	Fast	Fed	Fast	Fed	Fast
ι -[guanidino- $^{15}\text{N}_2$ 5,5, $^2\text{H}_2$]arg	0.060 $\pm$ 0.002	0.084 $\pm$ 0.009	0.057 $\pm$ 0.004	0.086 $\pm$ 0.005	0.062 $\pm$ 0.008	0.090 $\pm$ 0.014
ι -[5- ^{13}C]arg	0.068 $\pm$ 0.006	0.093 $\pm$ 0.003	0.064 $\pm$ 0.003	0.092 $\pm$ 0.004	0.063 $\pm$ 0.011	0.094 $\pm$ 0.012
ι -[^{13}C -ureido-5,5, $^2\text{H}_2$]cit	0.067 $\pm$ 0.008	0.048 $\pm$ 0.006	0.066 $\pm$ 0.004	0.036 $\pm$ 0.006	0.085 $\pm$ 0.033	0.057 $\pm$ 0.019
ι -[1- ^{13}C]leu	0.040 $\pm$ 0.008	0.057 $\pm$ 0.016	0.036 $\pm$ 0.005	0.048 $\pm$ 0.005	0.037 $\pm$ 0.006	0.054 $\pm$ 0.015
[^{13}C]urea	0.038 $\pm$ 0.004	0.042 $\pm$ 0.004	0.041 $\pm$ 0.008	0.045 $\pm$ 0.009	0.038 $\pm$ 0.009	0.040 $\pm$ 0.007
Derived						
ι -[guanidino- ^{13}C 5,5, $^2\text{H}_2$]arg	0.007 $\pm$ 0.001	0.009 $\pm$ 0.0001	0.007 $\pm$ 0.001	0.010 $\pm$ 0.002	0.007 $\pm$ 0.001	0.010 $\pm$ 0.001

All values tracer/tracee ratio and expressed as mean $\pm$ SD.

Table 4
Plasma fluxes of L-[guanidino-¹⁵N₂,5,5,2H₂]arginine, L-[5-¹³C]arginine L-[¹³C-ureido-5,5,2H₂]citrulline, L-[1-¹³C]leucine, [¹³C]urea and rates of *de novo* arginine synthesis for the fed and fast states

Tracer/flux	Control group (N = 4)			APF group (N = 6)		
	1 week adequate		4 week adequate	1 week adequate		4 week APF
	Fed	Fast	Fed	Fed	Fast	Fed
L-[guanidino- ¹⁵ N ₂ ,5,5,2H ₂]arginine	82 ± 3 ^a	60 ± 7	86 ± 6 ^a	80 ± 11 ^a	55 ± 10	45 ± 6 ^b
L-[5- ¹³ C]arginine	72 ± 7 ^a	52 ± 2	77 ± 3 ^a	81 ± 15 ^a	53 ± 8	45 ± 6 ^b
L-[¹³ C-ureido-5,5,2H ₂]citrulline	14 ± 2 ^a	20 ± 3	14 ± 1 ^a	12 ± 4 ^a	18 ± 6	21 ± 6 ^b
L-[1- ¹³ C]leucine	103 ± 19 ^a	74 ± 19	113 ± 17 ^a	114 ± 28 ^a	78 ± 18	117 ± 16 ^a
L-[1- ¹³ C]urea	184 ± 23	168 ± 19	177 ± 35	195 ± 63	179 ± 31	159 ± 61
Arg <i>de novo</i> synthesis	7.7 ± 2	10.4 ± 2	8.4 ± 2	6.9 ± 2	9.5 ± 3	11.0 ± 6
						10.8 ± 3

All values are mean ± SD; μmol kg⁻¹ h⁻¹.

^a *P* < 0.05; mixed model ANOVA, fed vs. fast within each study period at 1 or 4 weeks for control or APF group.

^b *P* < 0.05; mixed model ANOVA, change between week 1 and week 4 of the Control or APF groups, for a given state (fed or fasted).

Table 5

Isotopic enrichment of ^{13}C in expired air, carbon dioxide production (VCO_2), ^{13}C output (V^{13}CO_2), plasma oxidation rates of arginine and leucine using bicarbonate and glutamate as the precursor pool, and intakes of arginine and leucine following L-[5- ^{13}C] arginine and L-[1- ^{13}C]leucine infusions

Parameter	Control group (N = 4)		APF group (N = 6)			
	1 week adequate		4 week adequate		1 week adequate	
	Fed	Fast	Fed	Fast	Fed	Fast
[^{13}C]arg infusion						
$^{13}\text{CO}_2$ enrichment (APE $\times 10^{-3}$)	7.7 $\pm$ 2.3	9.1 $\pm$ 1.9	5.6 $\pm$ 0.9	7.5 $\pm$ 1.5	6.9 $\pm$ 1.3	9.5 $\pm$ 1.6
VCO_2 (mmol $\text{kg}^{-1} \text{h}^{-1}$)	11.3 $\pm$ 1.0	7.7 $\pm$ 0.3	8.6 $\pm$ 0.8	5.9 $\pm$ 0.2	10.6 $\pm$ 1.0	7.4 $\pm$ 0.2
V^{13}CO_2 ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	1.0 $\pm$ 0.3	1.0 $\pm$ 0.3	1.0 $\pm$ 0.3	0.9 $\pm$ 0.3	0.9 $\pm$ 0.2	1.0 $\pm$ 0.2
Arg ox ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	25.2 $\pm$ 5.4 ^a	16.9 $\pm$ 3.9	24.6 $\pm$ 5.6 ^a	15.6 $\pm$ 3.5	23.3 $\pm$ 7.5 ^a	18.3 $\pm$ 5.3
Dietary Arg. intake ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	35 $\pm$ 1.2	0	35 $\pm$ 1.2	0	37 $\pm$ 15	0
[^{13}C]leu infusion						
$^{13}\text{CO}_2$ enrichment (APE $\times 10^{-3}$)	10.4 $\pm$ 0.8	9.5 $\pm$ 1.2	10.2 $\pm$ 0.5	11.5 $\pm$ 0.5	11.0 $\pm$ 2.3	10.7 $\pm$ 1.9
VCO_2 (mmol $\text{kg}^{-1} \text{h}^{-1}$)	11.3 $\pm$ 0.8	7.2 $\pm$ 0.3	8.7 $\pm$ 1	5.6 $\pm$ 0.5	10.5 $\pm$ 0.9	7.9 $\pm$ 0.8
V^{13}CO_2 ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	1.3 $\pm$ 0.2	1.1 $\pm$ 0.2	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3	1.4 $\pm$ 0.3	1.1 $\pm$ 0.1
Leu ox ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	24 $\pm$ 5.9 ^a	14.0 $\pm$ 4.0	20.3 $\pm$ 9 ^a	12.1 $\pm$ 5.3	35.0 $\pm$ 7.6 ^a	20.6 $\pm$ 4.7
Dietary Leu. intake ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	62.1 $\pm$ 2.1	0	62 $\pm$ 1.8	0	65.8 $\pm$ 14	0

All values are mean $\pm$ SD.

^a $P < 0.05$ by mixed model ANOVA, fed vs. fast within each study period at 1 or 4 weeks for control or APF group.

^b $P < 0.05$ by mixed model ANOVA. Change between week 1 and week 4 of the Control or APF groups, for a given state (fed or fasted).