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Indian Women of Childbearing Age Do Not Metabolically Conserve Arginine as Do American and Jamaican Women[1,2]

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Abstract

Background: In a previous study in pregnant American women, we reported that arginine flux and nitric oxide synthesis increased in trimester 2. More recently, we reported that Indian women do not increase arginine flux during pregnancy as their American or Jamaican counterparts do.

Objective: The purpose of this study was to determine whether Indian women of childbearing age are producing less arginine and/or catabolizing more arginine and therefore have less available for anabolic pathways than do Jamaican and American women.

Methods: Thirty healthy women aged 28.3 ± 0.8 y from the United States, India, and Jamaica (n = 10/group) were given 6 h primed, constant intravenous infusions of guanidino-$^{15}N_2$-arginine, 5,5-$^{2}H_2$-citrulline, $^{15}N_2$-ornithine, and ring-$^{2}H_5$-phenylalanine, in addition to primed, oral doses of U-$^{13}C_6$-arginine in both the fasting and postprandial states. An oral dose of deuterium oxide was also given to determine fat-free mass (FFM).

Results: Compared with American women, Indian and Jamaican women had greater ornithine fluxes (μmol · kg fat FFM^{-1} · h^{-1}) in the fasting and postprandial states (27.3 ± 2.5 vs. 39.6 ± 3.7 and 37.2 ± 2.0, respectively, P = 0.01), indicating greater arginine catabolism. However, Jamaican women had a higher endogenous arginine flux than did Indian and American women in the fasting (66.1 ± 3.1 vs. 54.2 ± 3.1 and 56.1 ± 2.1, respectively, P = 0.01) and postprandial (53.8 ± 2.2 vs. 43.7 ± 4.9 and 42.8 ± 3.1, respectively, P = 0.06) states. As a consequence, Indian women had lower arginine bioavailability (μmol · kg FFM^{-1} · h^{-1}) in the fasting state (42.0 ± 2.6) than did American (49.9 ± 1.3, P = 0.045) and Jamaican (55.5 ± 3.5, P = 0.004) women, as well as in the postprandial state (40.7 ± 3.5 vs. 51.8 ± 1.2 and 57.5 ± 3.2, respectively, P = 0.001).

Conclusion: Compared with American and Jamaican women, Indian women of childbearing age have a decreased arginine supply because of increased arginine catabolism without an increase in arginine flux. *J Nutr* 2015;145:884–92.

Keywords: arginine, arginase, American, Jamaican, Indian women

Introduction

Of the >20 million infants born with low birth weight in 2000, India accounted for 38% (1). Although the etiology is multifactorial, several studies have reported that low maternal BMI and poor nutritional status are primary risk factors in both Indian (2–4) and non-Indian women (5, 6). During pregnancy, changes in macronutrient metabolism occur to ensure a continuous supply of glucose and amino acids to the growing fetus despite intermittent maternal food intake. Maternal BMI may influence fetal growth by affecting the delivery of nutrients to the fetus when the mother is in the fasting state. Duggleby and Jackson (7) reported that women with greater compared with lower BMIs had higher rates of protein turnover in mid- to late pregnancy. Because protein breakdown is the major source of maternal amino acid supply, a slower protein breakdown may lead to an inadequate supply of amino acids for fetal growth. In particular, an adequate supply of arginine, a nonessential amino acid that becomes conditionally essential during periods of rapid growth

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and wound healing, may be a requirement for normal fetal development.

Arginine is not only used to synthesize proteins, it is also a substrate for the synthesis of numerous biochemical compounds necessary to maintain biological homeostasis (8–10) (**Figure 1**). These include creatine, needed for ATP production in muscle cells, the polyamines, e.g., putrescine, spermidine, and spermine, used to regulate cell proliferation and differentiation, and NO, an important mediator of vascular tone and blood flow (9) and a major contributor to the maternal vascular expansion of pregnancy and regulation of placental blood flow (11, 12). Arginine is also a precursor of proline, an amino acid needed to synthesize fetal cartilage.

Stable isotope tracer studies in pregnant American women have demonstrated that in normal pregnancy, arginine flux and NO synthesis are higher in trimester 2 than in trimester 3 and postpartum (13), indicating an increased supply of arginine to meet the demands of pregnancy, including the faster synthesis of NO. Because of this increased demand for NO production, as well as increased maternal and fetal tissue deposition, it is likely that arginine becomes a conditionally essential amino acid in pregnant woman. In general, arginine availability for reaction with nitric oxide synthase (NOS)[9] and other synthetic enzymes is determined by the balance between its production and its catabolism by arginase. Maternal BMI may be one factor affecting amino acid and, hence, arginine release from protein breakdown (7). However, in pregnant Indian women, there was unexpectedly a negative correlation between maternal BMI and arginine flux (14). Furthermore, Indian women had arginine fluxes in early and late pregnancy that were ~50% lower than in American or Jamaican pregnant adults (14), suggesting that Indian women are unable to increase arginine production to meet the demands of pregnancy independently of BMI. One possible mechanism for decreased arginine production in Indian women may be upregulation of arginases, which convert arginine to ornithine and urea and compete with NOS for arginine (10, 15). Therefore, the purpose of this study was to determine whether Indian women are producing less arginine and/or catabolizing more arginine to ornithine and therefore have less arginine available for anabolic pathways than their Jamaican and American counterparts by conducting a comprehensive study of arginine metabolism in nonpregnant adult Indian, Jamaican, and American women.

Methods

The study was conducted at Baylor College of Medicine in Houston, Texas, St. John's Academy of Health Sciences in Bangalore, India, and the Tropical Metabolism Research Unit at the University of the West Indies in Kingston, Jamaica. Ten healthy women of childbearing age were recruited from the general public at each site. The race and ethnic makeup of each group was similar to that of the local population (**Table 1**). All participants provided written, informed consent, and the study protocol and consents were approved by the University Hospital of the West Indies Ethics Committee, the St. John's Medical College Hospital Institutional Ethics Review Board, and the Baylor College of Medicine Institutional Review Board. All participants were in good health as established by medical history, physical examination, and blood chemistry measurements and were between 20 and 35 y of age. A pregnancy test was performed to ensure that no participants were pregnant.

[9] Abbreviations used: FFM, fat-free mass; FM, fat mass; LC-MS/MS, LC tandem MS; NOS, nitric oxide synthase.

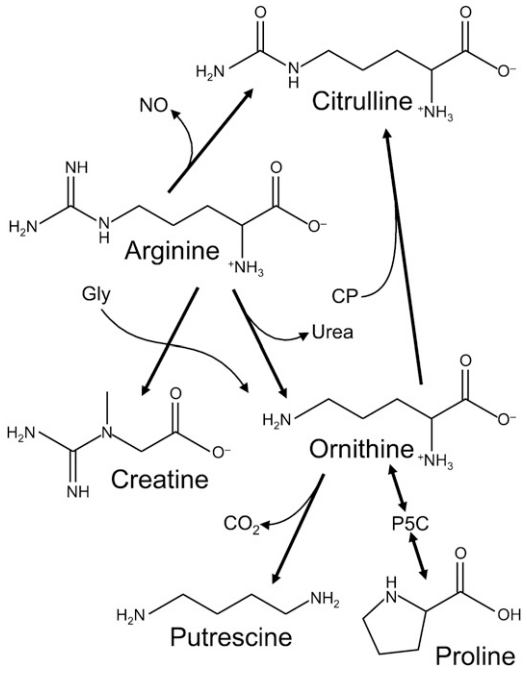

FIGURE 1 Relevant pathways of arginine metabolism. CP, carbamoyl phosphate; P5C, pyrroline-5-carboxylate.

Isotope infusions. Tracer infusions were performed in all subjects at the Children's Nutrition Research Center Metabolic Research Unit, St. John's Research Institute at St. John's Academy of Health Sciences, and the Tropical Metabolism Research Unit at the University of the West Indies. Sterile solutions of guanidino-$^{15}N_2$-arginine, U-$^{13}C_6$-arginine, 5,5-$^2H_2$-citrulline, $^{15}N_2$-ornithine, ring-$^2H_5$-phenylalanine, ^{13}C-bicarbonate, and ureido-^{15}N-citrulline (Cambridge Isotope Laboratories) were prepared with the use of strict aseptic techniques and tested for sterility and lack of pyrogens before infusion.

All participants were studied in both the postprandial and fasting states. They were randomly assigned to the order of the infusion protocols (fasting vs. postprandial study first), and the 2 infusion protocols were conducted 4–7 d apart. Studies were scheduled to coincide with the luteal phase of the menstrual cycle. For the fasting state study, the participants were instructed to stop eating at 2200 the night before the infusion. The next morning, participants were admitted to the research unit at each institution and an intravenous catheter was placed in the antecubital vein for isotope infusions and in a hand vein of the contralateral arm for blood sampling. The hand was heated to arterialize blood samples. After baseline blood and breath samples were obtained, primed, continuous intravenous infusions of $^{15}N_2$-arginine (prime = 4 μmol · kg^{-1}; infusion = 4 μmol · kg^{-1} · h^{-1}), $^2H_2$-citrulline (prime = 1.5 μmol · kg^{-1}; infusion = 1 μmol · kg^{-1} · h^{-1}), $^{15}N_2$-ornithine (prime = 2 μmol · kg^{-1}; infusion = 2 μmol · kg^{-1} · h^{-1}), and $^2H_5$-phenylalanine (prime = 3 μmol · kg^{-1}; infusion = 3 μmol · kg^{-1} · h^{-1}) were started and maintained for 6 h. Simultaneous with the 6 h intravenous infusion, primed oral doses of U-$^{13}C_6$-arginine were administered, with the total dose divided into equal aliquots administered every 30 min (prime = 6 μmol · kg^{-1}; cumulative oral dose = 4 μmol · kg^{-1} · h^{-1}). An oral dose of deuterium oxide (diluted 1:10 with H_2O, 0.1 g · kg^{-1}) was also given at the start of the tracer infusion protocol for measurement of total body water (TBW), from which fat-free mass (FFM) was calculated. Prime doses of ^{13}C-bicarbonate (4 μmol · kg^{-1}) and ^{15}N-citrulline (prime = 0.08 μmol · kg^{-1}) were also given to prime the secondary pools of bicarbonate and citrulline with labels derived from the U-$^{13}C_6$-arginine and $^{15}N_2$-arginine, respectively. At hour 4 of the infusion, indirect calorimetry was performed for 30 min to measure CO_2 excretion rate. Blood and breath samples were obtained every 20 min between hours 5 and 6 of the infusion protocol.

For the postprandial state study, participants again fasted from 2200 the night before admission to the research unit at each institution. The participants consumed 16 isocaloric and isonitrogenous meals prepared

TABLE 1 Characteristics of women of childbearing age from the United States, India, and Jamaica[1]

	United States	India	Jamaica	*P*
Race/ethnicity	8 W (4 H, 4 E), 1 A, 1 C	10 I	10 A	
Age, y	26.3 ± 1.1	30.0 ± 1.1	28.6 ± 1.5	0.12
Height, cm	164 ± 1.7[a]	152 ± 1.6[b]	160 ± 1.6[a]	<0.0001
Weight, kg	59.7 ± 1.4[a]	46.5 ± 1.2[b]	60.0 ± 2.0[a]	<0.0001
Fat-free mass, kg	42.3 ± 0.7[a]	32.9 ± 0.8[c]	37.6 ± 0.8[b]	<0.0001
Fat mass, kg	17.4 ± 0.9[ab]	13.6 ± 1.0[b]	22.4 ± 2.1[a]	<0.001
BMI, kg/m^2	22.2 ± 0.3[ab]	20.3 ± 0.6[b]	23.5 ± 0.9[a]	<0.01
Systolic blood pressure, mm Hg	106 ± 1.7	103 ± 3.7	108 ± 2.4	0.44
Diastolic blood pressure, mm Hg	65.1 ± 3.5	71.5 ± 1.5	67.3 ± 3.6	0.33
Mean arterial pressure, mm Hg	78.7 ± 2.6	82.0 ± 1.5	80.8 ± 3.1	0.60

[1] Values are means ± SEMs, $n = 10$ for each group. Means in a row without a common superscript letter are different, $P < 0.05$ (post hoc Tukey's multiple comparison test). A, African ancestry; C, Chinese ancestry; E, European ancestry; H, Hispanic ancestry; I, Indian; W, white.

from Ensure Powder (Abbott Nutrition) every 0.5 h starting 2 h before the isotope infusions. All Ensure meals were prepared exactly the same way as per manufacturer instructions at all 3 sites from the same batch of Ensure powder purchased in the United States. Because the participants were resting in bed, the experimental meal was designed to provide energy at 24 kcal · kg^{-1} · d^{-1} and protein at 0.8 g · kg^{-1} · d^{-1}. Because meals are normally consumed within 12 h each day, the subjects consumed 2.0 kcal · kg^{-1} · h^{-1} energy and 0.066 g · kg^{-1} · h^{-1} protein. By the end of the experiment, each subject consumed two-thirds of the energy required for the day during the 8 h period. Other than the meals, the tracer infusion protocol was identical to the one performed in the fasting state.

Sample analyses. The blood samples were drawn into prechilled tubes containing sodium heparin or EDTA. The tubes were centrifuged immediately at 1000 *g* for 15 min at 4°C, and the plasma was separated and stored immediately at −70°C for later analysis.

The plasma isotope enrichments of arginine, citrulline, ornithine, phenylalanine, proline and putrescine were measured by LC tandem MS (LC-MS/MS). The amino acids were converted into their 5-dimethylamino-1-napthalene sulfonamide derivatives, and ions were analyzed by selected reaction monitoring on a triple quadrapole mass spectrometer as previously described (12). The transitions monitored were precursor ion *m/z* 410 to product ion 392 for $^{15}N_2$-arginine, precursor ion *m/z* 414 to product ion 397 for U-$^{13}C_6$-arginine, precursor ion *m/z* 410 to product ion 72 for $^2H_2$-arginine derived from $^2H_2$-citrulline, precursor ion *m/z* 411 to product ion 72 for $^2H_2$-citrulline, precursor ion *m/z* 601 to product ion 170 for $^{15}N_2$-ornithine, precursor ion *m/z* 604 to product ion 170 for $^{13}C_5$-ornithine derived from $^{13}C_6$-arginine, precursor ion *m/z* 354 to product ion 170 for $^{13}C_5$-proline derived from $^{13}C_6$-arginine, precursor ion *m/z* 559 to product ion *m/z* 170 for $^{13}C_4$-putrescine derived from $^{13}C_6$-arginine, and precursor ion *m/z* 404 to product ion 170 for $^2H_5$-phenylalanine. Plasma isotope enrichments and concentration of creatine were measured by LC-MS/MS after creatine was converted into its butylated derivative (16). The transitions monitored was precursor ion *m/z* 189 to product ion 90 for ^{13}C-creatine derived from ^{13}C-arginine. Plasma concentrations of putrescine were measured by isotope dilution with the use of 1,4-$^{15}N_2$-putrescine dihydrochloride (Sigma-Aldrich) as an internal standard. The 5-dimethylamino-1-napthalene sulfonamide derivative was analyzed by LC-MS/MS monitoring precursor ion *m/z* 557 to product ion *m/z* 170.

Plasma amino acid concentrations were measured by ultraperformance LC with the use of precolumn derivitization with 6-amino-quinolyl-N-hydroxysuccinimidyl carbamate. Plasma concentrations of C-reactive protein and TNF-α were measured by ELISA with the use of commercially available kits (EMD Millipore).

Plasma arginase activity was measured by the conversion of $^{15}N_2$-arginine to $^{15}N_2$-urea on 50 μL of plasma with the use of a modification of the radioisotope method (17). Concentration of $^{15}N_2$-urea was measured with the use of isotope dilution with $^{13}C,^{15}N_2$-urea as an internal standard. The isotope enrichments of urea were measured by GC-MS of its *n*-propyl ester heptafluorobutyramide derivative. The unit of arginase activity is defined as μmol urea formed · min^{-1}.

Calculations. To standardize the kinetic data, all measurements are expressed per kilogram of FFM. The total flux (Q) of arginine, citrulline, ornithine, and phenylalanine were calculated from the following standard steady-state equation:

$$Q(\mu mol \times kg\ FFM^{-1} \times h^{-1}) = (E_{inf}/E_{plat}) \times i \quad (1)$$

where E_{inf} is the tracer-to-tracee ratio of the tracer infusate, E_{plat} is the tracer-to-tracee ratio of the tracee in plasma at isotopic steady state, and *i* is the infusion rate of the tracer (μmol · kg FFM^{-1} · h^{-1}). Endogenous flux is equal to total flux minus intake, which includes the infusion plus dietary intake in the postprandial state. The dietary intake of arginine and phenylalanine was calculated from the composition of the Ensure meal that was provided by the manufacturer (Abbott Nutrition).

The percentage of administered U-$^{13}C_6$-arginine that was oxidized is calculated from the following equation:

$$Arg\ Oxd\ (\%) = [(Ra\,CO_2 \times E\,CO_2)/(i_{Arg} \times 6)] \times 100 \quad (2)$$

where Ra CO_2 = VCO_2 /0.78; that is, the rate of CO_2 excretion divided by 0.78 to correct for the incomplete excretion of CO_2 produced in the fasting state (0.82 in the postprandial state) (18), E CO_2 is the plateau isotopic enrichment of breath CO_2, i_{Arg} is the rate of administration of the oral U-$^{13}C_6$-arginine tracer, and 6 accounts for the labeled carbons in the U-$^{13}C_6$-arginine tracer. Total arginine oxidized was calculated from the following equation:

$$\text{Total Arg Oxid}(\mu mol \times kg\,FFM^{-1} \times h^{-1}) = \text{Arg flux} \times \%\ \text{arginine oxidized} \quad (3)$$

De novo synthesis of arginine was calculated from the conversion of $^2H_2$-citrulline to $^2H_2$-arginine from the following equation:

$$\text{Arg Synthesis}\,(\mu mol \times kg^{-1} \times h^{-1}) = Q_{Cit\rightarrow Arg} = Q_{Arg} \times E_{Arg}/E_{Cit} \quad (4)$$

where Q_{Arg} is the flux of arginine, E_{Cit} is the plasma isotopic enrichment of the M+2 citrulline infused, and E_{Arg} is the isotopic enrichment of the M+2 isotopomer of arginine derived from citrulline.

Enteral arginine tracer hydrolyzed to ornithine was calculated from the conversion of enterally administered U-$^{13}C_6$-arginine to $^{13}C_5$-ornithine as follows:

$$Q_{Arg\rightarrow Orn}(\mu mol \times kg^{-1} \times h^{-1}) = Q_{Orn} \times (E_{Orn}/i\ {}^{13}C_6\text{–Arg} \times 0.99) \times I\ IG\ Arg \quad (5)$$

where Q_{Orn} is the flux of ornithine, E_{Orn} is the plasma isotopic enrichment of M+5 ornithine derived from arginine, i is the infusion rate

of $^{13}C_6$-Arg, I is the IG intake of arginine, and 0.99 is the tracer enrichment.

Ornithine flux is a surrogate for arginine catabolism and turnover (19); as such, it is an index of arginine hydrolysis. Arginine bioavailability, for protein and NO syntheses, was estimated from the difference between its flux and hydrolysis as follows:

$$\text{Arg Bio}-\text{avail}\,(\mu\text{mol}\times\text{kg FFM}^{-1}\times\text{h}^{-1}) = \text{Arg flux}-\text{Ornithine flux} \quad (6)$$

The nitric oxide synthesis rate was calculated from the rate of conversion of arginine to citrulline via the NOS reaction as previously described by us (20):

$$\text{NO Synthesis}(\mu\text{mol}\times\text{kg}^{-1}\times\text{h}^{-1}) = Q_{\text{Arg}\rightarrow\text{Cit}} = Q_{\text{Cit}}\times E_{\text{Cit}}/E_{\text{Arg}} \quad (7)$$

The percentage of enteral arginine extracted by the splanchnic tissues was calculated from the following equation:

$$\%AA_{\text{splan}} = \left[1-\frac{E_{\text{pEN}}}{E_{\text{pIV}}}\times\frac{i_{\text{IV}}}{i_{\text{EN}}}\right] \quad (8)$$

where E_{pEN} and E_{pIV} are the plateau isotopic enrichments in the plasma of the enteral (U-$^{13}C_6$-arginine) and intravenous ($^{15}N_2$-arginine), and i_{IV} and i_{EN} are the infusion rates of the intravenous and enteral tracers. Absolute splanchnic uptake of enteral arginine (diet + tracer or tracer alone in fasting state) was calculated from the following equation:

$$\textit{Arg Uptake}\,(\mu mol\cdot kg^{-1}\cdot h^{-1}) = \%\,AA_{\text{splan}} \times \text{Rate of intake of enteral Arg} \quad (9)$$

The fractional synthesis rates (FSRs) of putrescine or creatine or proline were calculated according to the precursor-product equation as previously described (21) from the following equation:

$$\text{FSR}(\%\text{pool}\times\text{h}^{-1}) = [(\text{Eprod}_{t_2}-\text{Eprod}_{t_1})/\text{Epre}]\times(24\times100)/(t_2-t_1) \quad (10)$$

where Eprod_{t2} – Eprod_{t1} is the increase in isotopic enrichment of the product (putrescine or creatine or proline) over the time interval (t_2–t_1) of the infusion and Epre is the plateau isotopic enrichment in plasma of the precursor, arginine.

The absolute synthesis rate was calculated as the product of the plasma concentration of putrescine or creatine or proline and their respective FSRs. The units of absolute synthesis rate are expressed as $\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$.

TBW was calculated as follows:

$$\text{TBW(mL)} = E_{\text{D2O}}\times(\text{dose}/E_{\text{pD2O}})\times 1.04 \quad (11)$$

where E_{D2O} is the enrichment of the deuterium oxide dose, E_{pD2O} is the plasma water enrichment, and 1.04 is the factor that converts deuterium dilution space to total water (22). FFM and fat mass (FM) were calculated as follows:

$$\text{FFM(kg)} = \text{TBW}/0.73, \text{ and FM(kg)} = \text{Body weight}-\text{FFM} \quad (12)$$

where 0.73 is the water content or hydration of FFM in adult humans (23).

Statistical analysis. Outcome variables are summarized by group as means ± SEMs. Differences in the physical characteristics and dietary intakes of the 3 groups were determined by 1-factor ANOVA. Similarly, differences in kinetic data between the 3 groups were assessed by 1-factor ANOVA in the fasting and postprandial states. If the ANOVA was significant, post hoc comparisons were performed with the use of Tukey's multiple comparison test. Tests were considered to be statistically significant if $P < 0.05$. Data analyses were performed with the use of GraphPad Prism software (version 5).

Results

Participant characteristics. Women from the United States, India, and Jamaica were similar in age, but Indian women were substantially shorter, weighed less, and had less FFM than women from Jamaica and the United States (Table 1). They also had a lower BMI than Jamaican women. However, Jamaican women had a lower percentage of FFM and higher percentage of FM than women from India and the United States. There were no differences between the groups in systolic, diastolic, or mean blood pressure.

Habitual dietary intakes. The habitual dietary intake of the participant was estimated with the use of the 3 d dietary record. Total energy intake was not different between the groups, but when expressed per kilogram of body weight, the Indian women had a substantially higher energy intake than the Jamaican women (**Table 2**). Protein intakes were not different, but fat intake was higher in American women and carbohydrate intake higher in Indian women.

Arginine and phenylalanine kinetics. Because study participants had different body compositions, all kinetic data were standardized by expressing per kilogram of FFM.

In the fasting state, arginine flux was significantly higher in Jamaican women than in American and Indian women (**Table 3**). There were no differences in arginine oxidation between the 3 groups. De novo arginine synthesis was significantly higher in Indian women than in Jamaican women but there was no significant difference between Indian and American women. Endogenous phenylalanine flux, which is derived only from

TABLE 2 Habitual dietary intake of women of childbearing age from the United States, India, and Jamaica[1]

	United States	India	Jamaica	*P*
Total energy, kcal/d	1691 ± 104	1581 ± 35	1625 ± 121	0.71
Energy, kcal · kg^{-1} · d^{-1}	28.4 ± 1.7^{a,b}	34.2 ± 1.2^{a}	27.2 ± 2.1^{b}	0.016
Protein intake, g · kg^{-1} · d^{-1}	1.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.2	0.216
Protein intake, g · kg FFM^{-1} · d^{-1}	1.7 ± 0.1	1.4 ± 0.1	1.9 ± 0.3	0.09
Fat intake, g · kg^{-1} · d^{-1}	1.2 ± 0.1^{a}	0.9 ± 0.1^{b}	0.8 ± 0.1^{b}	0. 016
Carbohydrate intake, g · kg^{-1} · d^{-1}	3.3 ± 0.3^{b}	5.7 ± 0.2^{a}	3.8 ± 0.3^{b}	0.03
Arginine intake, mg · kg^{-1} · d^{-1}	64 ± 4	61 ± 3	48 ± 8	0.11

[1] Values are means ± SEMs, $n = 10$ for each group. Means in a row without a common superscript letter are different, $P < 0.05$ (post hoc Tukey's multiple comparison test). FFM, fat-free mass.

TABLE 3 Arginine, citrulline, and phenylalanine kinetics in women of childbearing age from the United States, India, and Jamaica during fasting and postprandial states[1]

	United States	India	Jamaica	*P*
Fasting state, μmol · kg FFM^{-1} · h^{-1}				
Endogenous arginine flux	56.1 ± 2.1[b]	54.2 ± 3.1[b]	66.1 ± 3.1[a]	0.01
Arginine oxidation	13.8 ± 1.0	12.2 ± 0.8	14.3 ± 1.7	0.48
de novo Arginine synthesis	8.5 ± 0.4[a,b]	10.2 ± 0.8[a]	8.3 ± 0.2[b]	0.03
Endogenous phenylalanine flux	39.7 ± 0.9	39.0 ± 2.2	43.8 ± 2.3	0.17
Postprandial state, μmol · kg FFM^{-1} · h^{-1}				
Total arginine intake (diet + tracers)	36.9 ± 0.4[b]	36.6 ± 0.9[b]	41.2 ± 1.6[a]	0.005
Total arginine flux	79.1 ± 3.3[b]	80.3 ± 4.9[b]	95.9 ± 2.9[a]	0.008
Endogenous arginine flux	42.8 ± 3.1	43.7 ± 4.9	53.8 ± 2.2	0.06
Arginine oxidation	14.3 ± 0.7	13.3 ± 1.2	15.0 ± 1.3	0.29
de novo Arginine synthesis	9.1 ± 0.5	9.1 ± 0.7	8.7 ± 0.4	0.80
Total phenylalanine intake (diet + tracer)	36.9 ± 0.5[b]	37.1 ± 0.8[b]	41.8 ± 1.6[a]	0.005
Total phenylalanine flux	58.8 ± 1.6	59.4 ± 3.0	63.3 ± 2.7	0.39
Endogenous phenylalanine flux	21.9 ± 1.5	22.2 ± 2.9	21.5 ± 1.6	0.96

[1] Values are means ± SEMs, $n = 10$ for each group. Means in a row without a common superscript letter are different, $P < 0.05$ (post hoc Tukey's multiple comparison test). FFM, fat-free mass.

protein breakdown in the fasting state, was not different between groups, indicating no differences in the rate of protein breakdown between the 3 groups of women.

The results in the postprandial state parallel those in the fasting state (Table 3). Total arginine flux was significantly higher in Jamaican women than in American and Indian women. Similarly, endogenous arginine flux was ~25% higher in the Jamaican women than in American and Indian women ($P = 0.06$). There were no differences in arginine oxidation or de novo arginine synthesis between the groups. Both phenylalanine intake and endogenous phenylalanine flux were not different between the groups.

Splanchnic uptake of arginine. In the fasting state, there was no difference in the percentage uptake or absolute splanchnic uptake of the $^{13}C_6$-arginine tracer between the 3 groups (**Figure 2**A and B). However, in the postprandial state, the percentage splanchnic uptake of dietary arginine was significantly lower in Jamaican women than in American women, and both the percentage and absolute splanchnic uptake was lower in Jamaican women than in Indian women (Figure 2C and D).

Arginine bioavailability and hydrolysis to ornithine. Because ornithine and urea are products of arginine hydrolysis by arginases, the rate of production of ornithine reflects arginine hydrolysis by arginase. In both the fasting and postprandial states, ornithine flux was significantly higher in Indian and Jamaican women than in American women (**Table 4**). In the fasting state, enteral arginine converted to ornithine was also higher in Indian women than in American women. Arginine bioavailability for utilization in metabolic pathways other than by arginase was significantly lower in Indian women but not in American women than in Jamaican women in both the postprandial and the fasting states. In the fasting state, plasma arginase activity was not different between the 3 groups of women (Table 4).

The plasma arginine metabolome. In the fasting state, plasma arginine concentration was significantly lower in Jamaican women than in American women (**Table 5**). There were no differences in plasma citrulline or ornithine concentrations. In the postprandial state, there were no significant differences in plasma arginine, citrulline, or ornithine concentrations.

NO, proline, putrescine, and creatine synthesis. In the fasting state, NO synthesis was significantly slower in Indian women than in Jamaican women, and in the postprandial state, both American and Indian women had significantly slower NO synthesis than did Jamaicans (**Figure 3**).

In the fasting state, there were no differences between the groups in the plasma concentration and absolute synthesis rate of proline (**Table 6**). Although the plasma putrescine concentration was higher in American women than in Jamaicans and Indians, there were no differences between the groups in the absolute synthesis rate of putrescine. Similarly, although the plasma creatine concentration was lower in Indian women than in Americans and Jamaicans, there were no differences between the groups in the absolute synthesis rate of creatine.

In the postprandial state, there were no differences between the groups in the plasma concentrations and absolute synthesis rates of proline and putrescine. However, the plasma creatine concentration was higher in Jamaican women than in Indian women and creatine absolute synthesis rate was higher in Jamaican women than in American and Indian women.

Plasma markers of inflammation. There were no differences in the plasma concentrations of C-reactive protein between the groups of women (1.94 ± 0.46, 5.17 ± 3.7, and 3.26 ± 1.14 μg · mL^{-1} in American, Indian, and Jamaican women, respectively). There was also no difference in the plasma concentrations of TNF-α between the 3 groups (8.32 ± 0.20, 8.05 ± 0.13, and 8.27 ± 0.13 pg · mL^{-1} in American, Indian, and Jamaican women, respectively).

Discussion

The primary purpose of this study was to determine whether healthy adult Indian women with childbearing potential produce less arginine and/or catabolize more arginine to ornithine, hence have less arginine available for the synthesis of other key biomolecules such as NO, than do their Jamaican and American counterparts. The results suggest that Indian women had increased arginine hydrolysis without a compensatory increase in arginine flux, resulting in decreased bioavailability of arginine. This reduced arginine bioavailability was associated with a slower NO synthesis compared with Jamaican women in

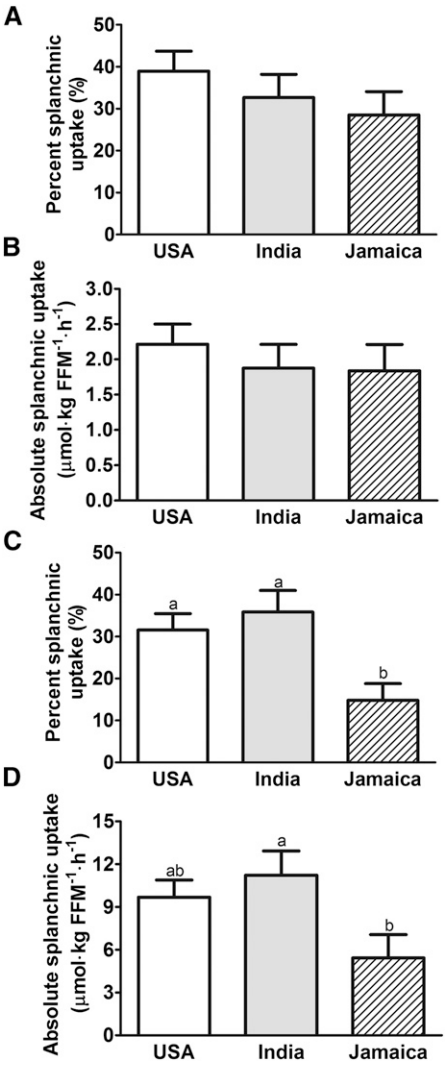

FIGURE 2 Relative (A,C) and absolute (B,D) splanchnic uptake of arginine in women of childbearing age from the United States, Jamaica, and India during fasting (A,B) and postprandial (C,D) states. Values are means ± SEMs, $n = 10$. Labeled means without a common letter differ, $P < 0.05$ (post hoc Tukey's multiple comparison test). FFM, fat-free mass.

both the postprandial and fasting states. Although American women also had slower arginine flux than did Jamaican women, they were able to conserve arginine bioavailability because of decreased arginine hydrolysis. Jamaican women, on the other hand, had higher arginine hydrolysis than did American women, but they had the highest arginine bioavailability because of a markedly higher arginine flux that compensated for the higher rate of arginine hydrolysis. The implication of these findings is that Indian women may have a higher dietary requirement for arginine.

This is the first study, to our knowledge, that used stable isotope tracer techniques to quantitate arginine hydrolysis in vivo in women of childbearing potential. Because plasma arginase activity was not different between the 3 groups, the increased hydrolysis of arginine by Indian women in both the fasting and postprandial states indicate an overall increase in arginase activity probably occurring intracellularly in organs and tissue beds. Arginases exist in 2 isoforms, arginase 1 and 2. Arginase 1 is predominantly expressed in the cytosol of hepatocytes, whereas arginase 2, a mitochondrial enzyme, is widespread in the body. In endothelial cells, arginase 2 activity has been shown to limit NO synthesis and NO-dependent vasodilation (24). Increased arginase 2 activity has been implicated in the pathogenesis of vascular diseases such as pulmonary hypertension (25), and may play a role in placental dysfunction in pregnancies with fetal growth restriction (26). Arginase 2 may also mediate vascular smooth muscle cell dysfunction by directing arginine metabolism toward ornithine and subsequently to the polyamines and proline, which can then stimulate smooth muscle cell proliferation and collagen deposition (27).

In the present study, compared with American women, Indian women had significantly higher ornithine flux, as well as rate of conversion of enteral arginine to ornithine, indicating that Indian women might have an increase in arginase 2 activity in splanchnic tissues, possibly the gut. Ornithine flux has been previously shown to reflect arginine conversion to ornithine and urea. Castillo et al. (18) demonstrated that healthy subjects had a reduced ornithine flux as well as decreased conversion of arginine to ornithine when consuming an arginine-free vs. an arginine-rich diet, indicating that ornithine flux was responsive to arginine supply. In a subsequent study, Yu et al. (19) found that, despite increased urea flux in burn patients, the ratio of ornithine flux to arginine flux was similar in healthy controls and burn patients. This finding suggested that ornithine flux reflects nonurea-cycle arginine metabolism and that ornithine flux serves as a surrogate for arginine turnover and catabolism. The high arginine hydrolysis of Indian women is accompanied by a decreased global arginine bioavailability compared with Jamaican women in the fasting state, and compared with both American and Jamaican women in the postprandial state. Although Jamaican women also had a high rate of arginine

TABLE 4 Arginine hydrolysis and bioavailability of women of childbearing age from the United States, India, and Jamaica during fasting and postprandial states[1]

	United States	India	Jamaica	*P*
Fasting state				
Ornithine flux, μmol · kg FFM^{-1} · h^{-1}	17.6 ± 1.5^{b}	23.7 ± 1.5^{a}	23.4 ± 1.6^{a}	0.01
Arginine bioavailability, μmol · kg FFM^{-1} · h^{-1}	49.9 ± 1.3^{a,b}	42.0 ± 2.6^{b}	55.5 ± 3.5^{a}	0.004
Enteral arginine tracer hydrolyzed to ornithine, μmol · kg FFM^{-1} · h^{-1}	1.0 ± 0.08^{b}	1.41 ± 0.11^{a}	1.29 ± 0.1^{a,b}	0.04
Plasma arginase activity, μmol urea · min^{-1}	0.21 ± 0.03	0.24 ± 0.03	0.32 ± 0.04	0. 096
Postprandial state				
Ornithine flux, μmol · kg FFM^{-1} · h^{-1}	27.3 ± 2.5^{b}	39.6 ± 3.7^{a}	37.2 ± 2.0^{a}	0.01
Arginine bioavailability, μmol · kg FFM^{-1} · h^{-1}	51.8 ± 1.2^{a}	40.7 ± 3.5^{b}	57.5 ± 3.2^{a}	<0.001
Enteral arginine hydrolyzed to ornithine, μmol · kg FFM^{-1} · h^{-1}	5.7 ± 0.3	7.2 ± 0.9	6.9 ± 0.4	0.19

[1] Values are means ± SEMs, $n = 10$ for each group. Means in a row without a common superscript letter are different, $P < 0.05$ (post hoc Tukey's multiple comparison test). FFM, fat-free mass.

TABLE 5 Arginine metabolome of women of childbearing age from the United States, India, and Jamaica during fasting and postprandial states[1]

	United States	India	Jamaica	*P*
Fasting state, μmol · L^{-1}				
Arginine	79.9 ± 5.8[a]	69.9 ± 5.6[a,b]	56.0 ± 3.1[b]	<0.01
Citrulline	26.6 ± 1.7	28.2 ± 2.4	22.7 ± 1.3	0.11
Ornithine	34.2 ± 3.6	45.7 ± 4.3	43.2 ± 4.5	0.13
Postprandial state, μmol · L^{-1}				
Arginine	83.0 ± 5.6	85.7 ± 6.1	76.8 ± 5.8	0.56
Citrulline	23.0 ± 1.4	25.9 ± 2.2	21.1 ± 1.3	0.15
Ornithine	37.3 ± 3.8	49.3 ± 3.8	50.0 ± 4.6	0.06

[1] Values are means ± SEMs, n = 10 for each group. Means in a row without a common superscript letter are different, $P < 0.05$ (post hoc Tukey's multiple comparison test).

hydrolysis, as evidenced by a faster ornithine flux than American women in both the fasting and postprandial states, they had the highest arginine flux, hence greatest arginine bioavailability, of all the groups in both the fasting and postprandial states. Decreased arginine bioavailability combined with a decreased rate of NO synthesis in fasting Indian women strongly suggest that utilization of arginine by arginase 2 is a key metabolic difference limiting arginine availability for reaction with NOS, and may be an important contributor to the high incidence of underweight births in Indian women (2–4). This is possible because decreased arginine bioavailability impairs fetal growth directly by attenuating fetal protein synthesis and indirectly by reducing maternal NO synthesis. Decreased maternal NO synthesis will, in turn, restrict placental-fetal blood flow and, hence, delivery of nutrients to the fetus.

Further investigation is needed to better understand the mechanisms underlying increased arginase 2 activity in Indian and Jamaican women. In vitro, multiple agents can induce arginase expression, including lipopolysaccharide and TNF-α (24). Increased systemic inflammation could be a potential source of arginase activation in Indian women. Although Indian women did not have significantly higher plasma markers of inflammation in the present study, this may be because of the relatively small sample size. Furthermore, these measurements do not measure gut-specific inflammation, and intestinal arginase 2 activity may be a major contributor to arginine catabolism, as evidenced by the hydrolysis of enteral arginine in both the postprandial and fasting states in the present study. However, one cannot rule out the possible effect of diet on gut arginase 2 activity and of race and ethnicity on arginase 2 polymorphisms. With respect to diet, habitual protein intake, which potentially can influence arginase activity, was not different between the groups, and although all participants were receiving the same amount of protein and arginine during the postprandial state experiment, the Indian women had a higher rate of arginine hydrolysis than did their American and Jamaican counterparts. Furthermore, in the fasting state, Indian women hydrolyzed substantially more of the enterally administered arginine tracer, suggesting that their gut arginase 2 was elevated even in the absence of food. Although there is one arginase 2 polymorphism, arg2 rs3742879 single nucleotide polymorphism, that has been shown to be associated with lower exhaled NO in children with allergy or asthma, indicating competition and/or inhibition of NO synthesis (11), there are no data in the literature indicating that race or ethnicity has an effect on its distribution.

Indian and American women consistently had slower arginine flux than Jamaican women. The reason for the slower arginine flux, however, was not obvious. It was not from slower de novo arginine synthesis, because there was very little difference between the 3 groups. Furthermore, arginine derived from whole-body proteolysis was not different between the groups, because protein breakdown, estimated by endogenous phenylalanine flux, was similar between the 3 groups. A possible explanation is that it was due to intracellular hydrolysis of arginine by arginase immediately after its release from proteolysis and before it entered the arginine free pool being traced by the labeled arginine tracer.

Creatine synthesis consumes 20–30% of arginine's amidino groups, and therefore imposes an appreciable burden on the metabolism of arginine (28). In adults, approximately one-half of the daily requirement for creatine comes from meat, fish, and other animal products in the diet; the remainder is derived from endogenous synthesis from arginine, glycine, and methionine (28). Creatine is involved in cellular energy production, but it also has neuroprotective effects (29). Thus, creatine may play an important role in fetal development. Jamaican women had the highest absolute synthesis rate of creatine in the postprandial state, and a significantly higher plasma concentration of creatine than did Indian women in both the postprandial and fasting states. Because of the mostly vegetarian diets of the Indian women in this study, the availability of arginine to synthesize creatine may be particularly important and, thus, lower arginine availability for this metabolic pathway in Indian women on the whole may be an important contributor to fetal outcomes.

In summary, significant differences were found in the flux, hydrolysis, bioavailability and utilization of arginine between American, Jamaican, and Indian women. Most importantly, Indian women of childbearing potential may have insufficient arginine for biosynthetic pathways because of increased arginine catabolism by arginase without increased arginine flux, implying that they may need a higher dietary arginine intake to compensate. The implication of these findings is that Indian women may have a higher dietary requirement for arginine.

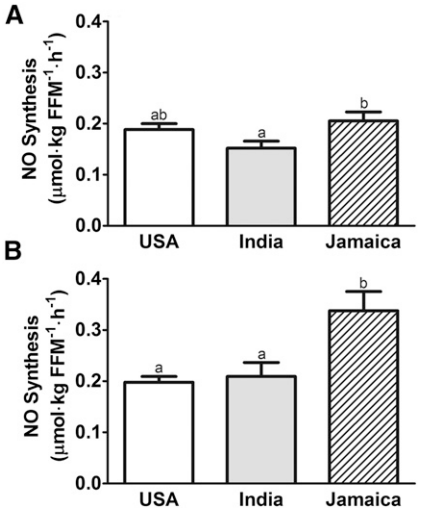

FIGURE 3 NO synthesis in women of childbearing age from the United States, Jamaica, and India during fasting (A) and postprandial (B) states. Values are means ± SEMs, n = 10. Labeled means without a common letter differ, $P < 0.05$ (post hoc Tukey's multiple comparison test).

TABLE 6 Plasma proline, putrescine, and creatine concentrations and absolute synthesis rates of women of childbearing age from the United States, India, and Jamaica during fasting and postprandial states[1]

	United States	India	Jamaica	*P*
Fasting state				
Proline concentration, μmol · L^{-1}	180 ± 21.8	137 ± 11.1	137 ± 12.0	0.10
Proline ASR, μmol · L^{-1} · h^{-1}	0.59 ± 0.13	0.83 ± 0.33	0.39 ± 0.09	0.36
Putrescine concentration, μmol · L^{-1}	1.39 ± 0.13[a]	0.80 ± 0.07[b]	0.94 ± 0.13[b]	<0.01
Putrescine ASR, μmol · L^{-1} · h^{-1}	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.70
Creatine concentration, nmol · L^{-1}	33.2 ± 3.5[a]	19.5 ± 2.1[b]	34.6 ± 3.3[a]	<0.01
Creatine ASR, μmol · L^{-1} · h^{-1}	0.99 ± 0.31	1.36 ± 0.32	1.64 ± 0.39	0.42
Postprandial state				
Proline concentration, μmol · L^{-1}	217 ± 18.4	202 ± 11.7	194 ± 9.6	0.49
Proline ASR, μmol · L^{-1} · h^{-1}	0.36 ± 0.08	0.47 ± 0.14	0.24 ± 0.06	0.26
Putrescine concentration, μmol · L^{-1}	0.97 ± 0.11	0.81 ± 0.11	0.75 ± 0.14	0.42
Putrescine ASR, μmol · L^{-1} · h^{-1}	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.39
Creatine concentration, nmol · L^{-1}	31.3 ± 3.0[a]	20.0 ± 1.8[b]	41.9 ± 4.1[a]	<0.001
Creatine ASR, μmol · L^{-1} · h^{-1}	1.04 ± 0.17[b]	1.03 ± 0.19[b]	1.96 ± 0.29[a]	0.009

[1] Values are means ± SEMs, $n = 10$ for each group. Means in a row without a common superscript letter differ, $P < 0.05$ (post hoc Tukey's multiple comparison test). ASR, absolute synthesis rate.

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