Interaction between murine spf-ash mutation and genetic background yields different metabolic phenotypes

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Submitted 13 August 2007; accepted in final form 8 October 2007.

Marini JC, Erez A, Castillo L, Lee B. Interaction between murine spf-ash mutation and genetic background yields different metabolic phenotypes. Am J Physiol Endocrinol Metab 293: E1764–E1771, 2007. First published October 9, 2007; doi:10.1152/ajpendo.00525.2007.—The spf-ash mutation in mice results in reduced hepatic and intestinal ornithine transcarbamylase. However, a reduction in enzyme activity only translates in reduced ureagenesis and hyperammonemia when an unbalanced nitrogen load is imposed. Six-week-old wild-type control and spf-ash mutant male mice from different genetic backgrounds (B6 and ICR) were infused intravenously with [13C15N2]urea, l-[15N]arginine, l-[5,5-2H2]ornithine, l-[6-3H]citrulline, and l-[ring-D5]phenylalanine to investigate the interaction between genetic background and spf-ash mutation on ureagenesis, arginine metabolism, and nitric oxide production. ICRspf-ash mice maintained ureagenesis (5.5 ± 0.3 mmol·kg⁻¹·h⁻¹) and developed mild hyperammonemia (145 ± 19 μmol/l) when an unbalanced nitrogen load was imposed; however, B6spf-ash mice became hyperammonemic (671 ± 15 μmol/l) due to compromised ureagenesis (3.4 ± 0.1 mmol·kg⁻¹·h⁻¹). Ornithine supplementation restored ureagenesis and mitigated hyperammonemia. A reduction in citrulline entry rate was observed due to the mutation in both genetic backgrounds (wild-type: 128, spf-ash: 60; SE 4.0 μmol·kg⁻¹·h⁻¹). Arginine entry rate was only reduced in B6spf-ash mice (B6spf-ash: 332, ICRspf-ash: 453; SE 20.6 μmol·kg⁻¹·h⁻¹). Genetic background and mutation had an effect on nitric oxide production (B6: 3.4, B6spf-ash: 2.8, ICR: 9.0, ICRspf-ash: 4.6, SE 0.7 μmol·kg⁻¹·h⁻¹). Protein breakdown was the main source of arginine during the postabsorptive state and was higher in ICRspf-ash than in B6spf-ash mice (phenylalanine entry rate 479 and 327, respectively; SE 18 μmol·kg⁻¹·h⁻¹). Our results highlight the importance of the interaction between mutation and genetic background on ureagenesis, arginine metabolism, and nitric oxide production. These observations help explain the wide phenotypic variation of ornithine transcarbamylase deficiency in the human population.

arginine; nitric oxide; urea cycle

ORNITHINE TRANSCARbamylase (OTC) deficiency is the most common urea cycle disorder in humans (3). The reduction in ureagenesis capacity results in high plasma ammonia concentrations and frequent hyperammonemic crises that can result in coma and even death (1). OTC is also expressed in enterocytes where it functions in the synthesis of citrulline, which is then exported into the blood, serving as the precursor for arginine synthesis by the kidney (7). The metabolism of arginine (Fig. 1) is highly compartmentalized, not only involving different organs, but different intracellular compartments and subcompartments, such as mitochondria, caveolae, and cytosol (8, 33). Furthermore, different isoforms of some of the enzymes involved [e.g., arginase, nitric oxide (NO) synthase] exist or different tissue-specific subcellular localization of the same isoform may occur (6). This compartmentalization of arginine metabolism reflects a complex regulation due to the multiple functions of this amino acid. It functions as a precursor for NO, creatine, and agmatine synthesis, in addition to its role in ureagenesis and protein synthesis. Thus, the reduction in OTC activity may not only reduce ureagenesis, but also impair the functional role of arginine in OTC-deficient patients thereby compromising NO synthesis.

Moreover, these complex biochemical and cellular interactions offer opportunities on a genetic level to modify the metabolic consequences of specific mutations. The ultimate effects on metabolite flux contribute to variable expressivity and susceptibility (15). Metabolic analysis of mutations on cogenic mouse strains offers a powerful approach to identify these genetic determinants as well as the associated range of phenotypic expression.

We showed previously that ornithine supplementation was able to restore ureagenesis and mitigate hyperammonemia in a hypomorphic mouse model for OTC deficiency (OTCspf-ash) (21). Thus, it seems that increasing the supply of ornithine was able to compensate for the reduced enzyme activity and maintain ureagenesis. Because the de novo production of ornithine is not affected by the reduction in OTC activity caused by the spf-ash mutation, we hypothesized that mouse genetic background might interact with the mutation to produce different phenotypical presentations of the disorder. Thus, the present study was designed to investigate whether the spf-ash mutation interacts with the genetic background of mice to affect the metabolic phenotype of the disorder in relationship to ureagenesis, arginine metabolism, and NO production.

MATERIALS AND METHODS

Animals and Treatments

B6EiC3Sn a/a-Otcspf-ash/J mice (B6spf-ash) were originally obtained from The Jackson Laboratory (Bar Harbor, ME). The mutation was bred into ICR mice (ICRspf-ash) and backcrossed for at least 10 generations. Mice were housed in a SPF facility and had access to a 20% crude protein autoclaved pelleted feed (LabDiet, PicoLab Rodent Diet 20). Dietary proximate analysis was as follows: protein (200 g/kg), gross energy (16.9 MJ/kg), fat (45 g/kg), fiber (60 g/kg), and ash (70 g/kg). Autoclaved reverse osmosis water was available at all times. Mice were under a 12-h light cycle (0600 to 1800) in a temperature (22 ± 2°C) and humidity (55 ± 5%)-controlled environment. All animal procedures were authorized by the Baylor College of Medicine Institutional Animal Care and Use Committee.

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Arginine metabolism and NO production. To study the effect of genetic background (B6 or ICR), mutation (wild-type or spf-ash mutant), and nitrogen load (saline or alanine-glycine at a rate of 6.06 mmol·kg⁻¹·h⁻¹) on arginine metabolism and NO production, 6-wk-old mice (10 per group) were utilized in a 2 × 2 × 2 factorial arrangement of treatments. A schedule similar to the one outlined previously was followed. Approximately 3 h after feed removal, a priming dose of [13C18O]urea (66 μmol/kg), L-[15N2]arginine (23.2 μmol/kg), L-[5,5-D2]ornithine (7.7 μmol/kg), L-[ureido-13C]4,4,5,5,D₄-citrulline (7 μmol/kg), and L-[ring-D₅]phenylalanine (10 μmol/kg) was given to the mice. The priming dose was followed immediately by a continuous infusion of [13C18O]urea (100 μmol·kg⁻¹·h⁻¹), L-[guanido-15N₂]arginine (23.2 μmol·kg⁻¹·h⁻¹), L-[5,5-D₂]ornithine (7.7 μmol·kg⁻¹·h⁻¹), L-[ureido-13C]4,4,5,5,D₄-citrulline (7 μmol·kg⁻¹·h⁻¹), and L-[ring-D₅]phenylalanine (10 μmol·kg⁻¹·h⁻¹) for 4 h. Our preliminary studies and published literature (10) showed that isotopic steady state is reached for these amino acids within 30 min of a primed continuous infusion. At the end of the infusion, mice were euthanized by decapitation and blood, small intestine, and liver were collected. Tissue samples were immediately frozen in liquid nitrogen. Plasma and tissue samples were stored at −80°C. Plasma samples were deproteinized and derivatized within 10 days of collection.

Sample Analysis

Plasma urea isotopic enrichment was determined by EI GCMS after the urea was derivatized to the tert-Butyldimethylsilyl (t-BDMS) derivative. Plasma (20 μl) protein was precipitated with ice-cold acetone (100 μl) and the supernatant containing the urea was obtained after centrifugation at 1,500 × g for 15 min at 4°C. The supernatant was evaporated under a gentle stream of nitrogen gas at 80°C, and the sample was derivatized with 25 μl of a 1:1 mixture of N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSFA; Sigma) and acetonitrile, at 80°C for 20 min in the tightly capped vials. The analysis was performed in a 5973 Agilent GC MSD (Agilent Technologies, Santa Clara, CA) in SIM mode, monitoring m/z ions 231, 232, and 234.

For the determination of ornithine enrichments, 5 μl of plasma were deproteinized with ice-cold sulfosalicylic acid (10% wt/vol solution), centrifuged, and the supernatant was passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H⁺ form; Bio-Rad Laboratories, Richmond, CA). Ornithine was eluted with 2 M NH₄OH and the eluate dried under vacuum at 80°C. The eluate was reconstituted in fresh plasma samples, by reductive amination of 2-oxoglutarate and oxidation of NADPH, employing a commercial kit (Sigma).

Plasma citrulline, arginine, and phenylalanine enrichments were determined as their dansyl derivatives by LCMS utilizing a TSQ Quantum Ultra System (Thermo Finnigan, San Jose, CA) and monitoring m/z ions 408, 409, 410, and 413 for arginine, 409, 410, 411, and 414 for citrulline, and 399 and 404 for phenylalanine.

Ammonia was determined in fresh plasma samples, by reductive amination of 2-oxoglutarate and oxidation of NADPH, employing a commercial kit (Sigma).

Tissue samples were homogenized in lysis buffer (0.0625 M Tris-HCl, pH 7.4, 2% SDS) for 1 min, followed by incubation at 95°C for 30 min. The protein concentration of the lysate was measured with Micro BCA reagent (Pierce, Rockford, IL). After treatment with 5% β-mercaptoethanol, 10 μg liver protein extracts were loaded for Western blot assay. Total protein was fractionated on a 12% polyacrylamide gel (ISC BioExpress, Kaysville, UT), transferred to Immun-Blot PVDF membrane (Bio-Rad, Hercules, CA), probed with a nonspecific polyclonal antibody against iNOS (provided by Mendel Tuchman, Children’s National Medical Center, Washington, DC) and immunoreactive OTC was detected by enhanced chemiluminescence.
(Amersham Biosciences, Piscataway, NJ) (23). The density of each band was normalized utilizing α-tubulin (Sigma 1:10,000) and quantified using an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA).

Calculations

The entry rate of urea, ornithine, arginine, citrulline, and phenylalanine was calculated from the isotopic dilution of the infused tracer at plateau enrichment, as

\[ Ra_M = i_M \cdot \left( \frac{100}{E_M} - 1 \right) \]

where \( Ra_M \) is the plasma entry rate (flux) of the metabolite \( M \) (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)), \( i_M \) is the infusion rate (\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)), and \( E_M \) is the enrichment of \( M \) at plateau (mpe).

The rate of conversion of arginine to citrulline (\( RC_{\text{Cit} \rightarrow \text{Arg}} \)), citrulline to ornithine (\( RC_{\text{Cit} \rightarrow \text{Orn}} \)), and ornithine to citrulline (\( RC_{\text{Orn} \rightarrow \text{Cit}} \)) were determined as follows (30a)

\[ RC_{\text{xy}} = Ra_y \cdot \frac{E_y}{E_x} \cdot \frac{Ra_x}{i_x + Ra_x} \]

where \( Ra_x \) and \( Ra_y \) are the plasma fluxes of the precursor and product, respectively, determined from the steady-state enrichments of the infused tracers \( [1^{15}\text{N}_2] \text{arginine}, [5,5 D_2] \text{ornithine}, [1^{15}\text{C},4,4,5,5,D_4] \text{citrulline} \); \( E_x \) and \( E_y \), the respective plasma enrichments of the precursors and products \( i_x \) and \( i_y \) is the rate of infusion of the labeled precursor. Because the conversion of arginine to ornithine implies the loss of the guanido group, and hence the isotopic label, the conversion of arginine to ornithine was estimated by the ratio between \( [6,1^{15}\text{C},5,4,4,D_4] \text{arginine} \) (from the infused citrulline) and \( [5,5 D_4] \text{ornithine} \).

During the deamination of ornithine to L-glutamate–γ-semialdehyde by Ornithine AminoTransferase (OAT), one deuterium is lost from ornithine to citrulline (\( [13\text{C},4,4,5,5,D_4] \text{citrulline}/[13\text{C},4,4,5,5,D_4] \text{arginine}, [13\text{C},4,4,5,5,D_4] \text{citrulline}/[5,5 D_2] \text{ornithine}, and \( [5,5 D_3] \text{ornithine}/[5,5 D_3] \text{citrulline} \)) and \( i_x \) is the rate of infusion of the labeled precursor. The conversion of ornithine to citrulline implies the loss of the guanido group, and hence the isotopic label, the conversion of arginine to ornithine was estimated by the ratio between \( [6,1^{15}\text{C},5,4,4,D_4] \text{arginine} \) (from the infused citrulline) and \( [5,5 D_4] \text{ornithine} \).

The net rate of arginine released from body protein breakdown \( (R_{\text{Prot} \rightarrow \text{Arg}}) \) was calculated based on phenylalanine entry rate \( (Ra_{\text{Phe}}) \) and by assuming an arginine/phenylalanine molar ratio of 1.29 (12).

\[ R_{\text{Prot} \rightarrow \text{Arg}} = 1.29 \cdot Ra_{\text{Phe}} \]

During feed deprivation, arginine entering the plasma either originates from citrulline or protein degradation. The net rate of arginine originating from protein degradation and metabolized intracellularly \( (R_{\text{Arg} \rightarrow \text{Mt}}) \) was calculated as follows

\[ R_{\text{Arg} \rightarrow \text{Mt}} = R_{\text{Prot} \rightarrow \text{Arg}} - (R_{\text{Arg} \rightarrow \text{Cit}} + R_{\text{Cit} \rightarrow \text{Arg}}) \]

Data Analysis

The following broken-line model was fitted to urea production and plasma ammonia data from the ornithine titration study utilizing Nonlinear Regression Analysis Program (NLREG; Sherrod 2003)

\[ Y = a + b \cdot O, \quad \text{for } O < O_{\text{breakpoint}} \]

\[ Y = a + b \cdot O_{\text{breakpoint}}, \quad \text{for } O > O_{\text{breakpoint}} \]

where \( Y \) is the observed urea production or plasma ammonia concentration at a given ornithine supplementation rate \( O \); \( a \), the intercept; \( b \), the slope of the function; \( O_{\text{breakpoint}} \), is the breakpoint of the function (minimal supplementation rate of ornithine to achieve plateau urea production or plasma ammonia concentration).

The experimental design for the arginine metabolism and NO production study was a completely randomized design with a 2 × 2 factorial arrangement of treatments. Data were analyzed statistically with the proc mixed procedure of SAS (v. 9.1, SAS, Cary, NC). Fixed effects were genetic background (B6 or ICR), mutation (wild-type or mutant), and nitrogen load (saline or alanine-glycine) and their interactions. If a significant interaction was obtained (\( P \leq 0.05 \)), the post hoc Tukey procedure for multiple pairwise comparisons was also applied. Preplanned orthogonal contrasts were conducted to compare the effect of genetic background on the \( spf-ash \) mutation.

RESULTS

Ornithine Titration

The addition of ornithine to the alanine-glycine mixture infused increased \( (P < 0.001) \) urea production and reduced \( (P < 0.001) \) plasma ammonia in B6\( spf-ash \) mice. Breakpoint analysis showed that supplementation with ~100 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) was needed to prevent the reduction in ureagenesis and to mitigate hyperammonemia caused by the nitrogen load imposed (Figs. 2 and 3). However, the infusion of alanine-glycine did not cause hyperammonemia nor reduce ureagenesis in ICR\( spf-ash \) mice and thus ornithine supplementation had no effect in these animals.

Arginine Metabolism and NO Production

In the arginine metabolism and NO production study, the entry rate of urea was affected by genetic background [B6 and
ICR, 3.55 and 4.06 (SE 0.08) mmol·kg⁻¹·h⁻¹, respectively; 
\( P < 0.001 \), mutation [control wild-type and mutant spf-ash, 
3.66 and 3.96 (SE 0.08) mmol·kg⁻¹·h⁻¹, respectively; 
\( P < 0.016 \)], and nitrogen load (\( P < 0.001 \)). As expected, urea production was higher in the alanine-glycine treatment than in the 
saline control (\( P < 0.001 \)), and the difference in the amount of urea produced represented 73% of the nitrogen load infused. However, urea 
genesis was not depressed in B6\textsuperscript{spf-ash} mice, although two 
mice developed signs of hyperammonemia. No effect of nitrogen load was detected for any of the other entry rates measured, and thus results were pooled to show the effect of genetic background and spf-ash mutation.

Western blot analysis showed a comparable reduction in OTC abundance in mutant mice, from both backgrounds, in hepatic and intestinal tissue (Fig. 4). Citrulline entry rate was reduced (\( P < 0.001 \)) in mutant animals, but a greater reduction (\( P < 0.001 \)) was observed in B6\textsuperscript{spf-ash} mice (Table 1). The entry rate of arginine, however, was only reduced in B6\textsuperscript{spf-ash} mice. A significant reduction (\( P < 0.001 \)) in ornithine entry rate due to genetic background was detected in B6 mice compared with ICR mice. The plasma entry rate of urea cycle intermediates (UCI) was greater in ICR mice (\( P < 0.001 \)) and in wild-type animals (\( P < 0.029 \)). Similarly, a higher entry rate of phenylalanine was found in ICR mice (\( P < 0.001 \)) and in animals carrying the spf-ash mutation (\( P < 0.003 \)).

The conversion of arginine to citrulline, a proxy for NO production, was higher in ICR wild-type control mice (9 \( \mu \text{mol·kg}^{-1}·\text{h}^{-1} \)) representing 1.94% of the total plasma arginine entry rate of these animals, whereas in B6 and ICR\textsuperscript{spf-ash} mice it was \( \approx 0.9 \% \) (Table 1). The conversion of citrulline into arginine was not only quantitatively higher in ICR wild-type control mice (126 \( \mu \text{mol·kg}^{-1}·\text{h}^{-1} \)), but it represented a higher percentage (89%) of the citrulline entry rate. Likewise, a larger quantity (23.4 \( \mu \text{mol·kg}^{-1}·\text{h}^{-1} \)) and greater proportion (16.5%) of
the plasmatic citrulline was converted into ornithine in ICR wild-type control mice than in mutant mice from both backgrounds and B6 wild-type controls. Plasmatic ornithine also made a greater contribution (112.7 mol·kg⁻¹·h⁻¹; 24% of the ornithine entry rate) to the synthesis of citrulline in ICR wild-type control mice.

The entry rates and interconversion of the UCI in mutant spf-ash mice from both genetic backgrounds, as well as the arginine originating from protein breakdown, are shown in Table 2. Most of the circulating arginine was derived from protein breakdown in all the mice studied, but spf-ash mutant mice derived a larger proportion from this source (Table 2). However, not all the arginine released from protein breakdown entered the circulation; an important proportion (up to ~37%) was metabolized intracellularly.

**DISCUSSION**

The combination of mouse genetic models together with stable isotope multitracer protocols allows for the dissection of complex metabolic pathways in vivo in conscious animals. The present study shows that the spf-ash mutation interacted with the genetic background of mice in most of the variables analyzed. A similar reduction in OTC enzyme abundance in both B6 and ICR resulted in reduced citrulline entry rate. ICR mice, however, were able to compensate for the reduced OTC enzyme activity and displayed arginine entry rates similar to control animals.

**Ornithine Titration**

In agreement with our previous report, supplementation with ornithine prevented hyperammonemia and restored ureagenesis in B6 spf-ash mice (21). However, the alanine-glycine protocol failed to reduce ureagenesis and to cause hyperammonemia in ICR spf-ash mice and thus ornithine supplementation had no effect in these animals. The reduction in ureagenesis, however, was not observed when the multitracer protocol was followed and it could be explained in part by the “supplementation” due to the infusion of tracers (38 μmol UCI·kg⁻¹·h⁻¹). Nevertheless, two B6spf-ash mice developed clear signs of hyperammonemia, but without a reduction in ureagenesis when infused with the alanine-glycine mixture.

![Fig. 5. Arginine originating from protein breakdown and from de novo synthesis from citrulline in spf-ash mice from 2 different genetic backgrounds (B6 and ICR).](http://ajpendo.physiology.org/)
**OTC Abundance and Its Effect on Citrulline and Arginine Entry Rates**

A reduction in OTC enzyme abundance was observed in mutant mice, from both backgrounds, which is consistent with the lower OTC activity described previously in *spf-ash* mice (27). This decrease in activity has been shown from a reduction in enzyme abundance, and not in enzyme-specific activity (26). The reduction in OTC abundance in mutant mice resulted in a decrease in the entry rate of citrulline, which was more pronounced in B6 *spf-ash* mice. This was not reproduced for arginine entry rate for which ICR *spf-ash* mice had rates similar to wild-type control animals; in contrast, arginine entry rate was diminished by ~22% in B6 *spf-ash* mice. The entry rates for arginine and citrulline in the present experiment were similar to the ones reported by others utilizing more invasive procedures in an unconscious mouse model (2, 10).

Between 75 and 89% of the citrulline flux was accounted for in rodents that the liver takes very little, if any, of the circulating citrulline (34), although this concept has been recently questioned in humans (32). In their seminal work, Windmueller and Spaeth (34) showed that 83% of circulating citrulline was taken up by the kidney, which exported 75% of this citrulline as arginine. Their results offer a glimpse of two little known processes; the utilization of citrulline for arginine synthesis by peripheral tissues, and the possible channeling of arginine synthesized in the kidney into other products, such as guanidinoacetate. Our data support the idea that some of the citrulline produced by the small intestine is utilized by peripheral tissues for the local synthesis of arginine.

The utilization of citrulline by endothelial cells and macrophages to generate arginine and support the local production of NO synthesis has been shown in the past (11, 35). This implies that the conversion of [15N2]arginine to [15N]citrulline fails to take this source of arginine into account, thereby providing a minimal estimate for NO synthesis.

**NO Production**

NO production, determined by the conversion of [15N2]arginine to [15N]citrulline, represented a small proportion (up to 1.9%) of the total arginine flux. These findings agree with previous reports utilizing an identical protocol conducted both in humans (4) and mice (10). Despite the reduced amount of arginine utilized for the synthesis of NO, it seems that NO production might be responsive to arginine supplementation, a phenomenon which has been dubbed “arginine paradox” (14).

**ICR**

ICR *spf-ash* mice had a higher phenylalanine entry rate, indicating greater protein breakdown, and thus more arginine was released from protein in these animals which contributed to the higher arginine entry rate observed. Protein breakdown was the main source of circulating arginine during the postabsorptive state in wild-type control and *spf-ash* mice of both backgrounds. However, a large proportion (up to 37%) of the arginine released by endogenous protein is further metabolized intracellularly before it reaches the general circulation. Liver releases very little, if any, arginine into the general circulation and as a consequence it does not contribute to the plasma arginine entry rate (5, 34). However, it is clear from the high urea entry rate that a very active arginine synthesis takes place in the hepatocytes. In the present experiment, the urea entry rate (~4.9 mmol) was ~10-fold higher than the arginine entry rate showing the high compartmentalization of urea metabolism.

**Ornithine Entry Rate and Conversion to Citrulline**

Ornithine rate of appearance, the third UCI studied, was reduced only in B6 wild-type control mice. Ornithine entry rate should not be affected, at least directly, by the reduction in OTC activity due to the *spf-ash* mutation. Ornithine has been shown in mice to be the first UCI to increase in the liver after a sudden nitrogen load, expanding the amount of intermediates and supporting the need for ammonia detoxification through urea synthesis (17). Similarly, in sheep, the portal infusion of ammonia increased the extraction rate of ornithine, but not of the other UCI (24). Thus, it seems that ornithine is crucial in the expansion of hepatic UCI to sustain ureagenesis and 2) that it originates in extrahepatic tissue. However, the infusion and sampling protocol employed in the present report measured whole body entry rates and were unable to determine the intestinal release and liver uptake of ornithine.

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**Table 2. Origin of plasma arginine in WT and *spf-ash* mutant mice of 2 different genetic backgrounds (B6 or ICR)**

<table>
<thead>
<tr>
<th>Origin of RaArg, μmol·kg⁻¹·h⁻¹</th>
<th>WT</th>
<th><em>spf-ash</em></th>
<th>WT</th>
<th><em>spf-ash</em></th>
<th>SE</th>
<th>Mut†</th>
<th>Back†</th>
<th>Int†</th>
</tr>
</thead>
<tbody>
<tr>
<td>RaArg</td>
<td>421.0*</td>
<td>332†</td>
<td>455*</td>
<td>453*</td>
<td>20.6</td>
<td>0.032</td>
<td>0.001</td>
<td>0.495</td>
</tr>
<tr>
<td>RaArg</td>
<td>89.7†</td>
<td>53.3§</td>
<td>126.0*</td>
<td>61.6‡</td>
<td>5.8</td>
<td>0.001</td>
<td>0.001</td>
<td>0.497</td>
</tr>
<tr>
<td>RaArg</td>
<td>389¶</td>
<td>506†</td>
<td>421†‡</td>
<td>618§</td>
<td>23.2</td>
<td>0.003</td>
<td>0.001</td>
<td>0.094</td>
</tr>
<tr>
<td>RaArg</td>
<td>332†</td>
<td>298‡</td>
<td>329†</td>
<td>391*</td>
<td>17.1</td>
<td>0.369</td>
<td>0.007</td>
<td>0.004</td>
</tr>
<tr>
<td>RaArg</td>
<td>57§</td>
<td>123‡</td>
<td>177‡</td>
<td>226*</td>
<td>12.7</td>
<td>0.001</td>
<td>0.001</td>
<td>0.495</td>
</tr>
</tbody>
</table>

Results are means ± SE (n = 20). Mut †, effect of the mutation; Back†, effect of the genetic background; Int†, interaction between the 2 main effects. * †, ‡, § Values in the same row with different superscript letters are statistically different at P < 0.05. 1 RaArg, plasma entry rate of arginine; 2 RaArg, contribution of citrulline to the plasma entry rate of arginine; 3 RaArg, arginine released from protein breakdown; RArg–MI, contribution of protein breakdown to the plasma entry rate of arginine; RArg–MI, arginine released from protein breakdown and metabolized intracellularly; RArg–MI (%), percentage of arginine released from protein breakdown that was metabolized intracellularly.
The de novo synthesis of ornithine takes place in mitochondria. The carbon skeleton provided by L-glutamate could be more easily correlated with specific clinical effects. A limitation of studying the contribution of NO dysregulation in situations of the cumulative metabolite flux through a specific biochemical pathway in vivo and is the best correlate with human phenotype (37). Genetic background is an important determinant of the cumulative metabolite flux through a specific biochemical pathway in vivo and is the best correlate with human phenotype (15, 16).

The early diagnosis, improved care, and overall better prognosis of OTC deficiency patients and UCDs in general have underscored clinical findings that point to alternative pathophysiological processes related to dysregulation of arginine metabolism and/or NO production. For example, a reduced NO production (25) and an increased prevalence of essential hypertension (29) have been reported in some patients. The translation of these animal studies to the context of human UCD would allow us to begin to correlate specific clinical consequences with nitrix oxide dysregulation in situations where genetic determinant, i.e., urea cycle mutation, is paramount. A limitation of studying the contribution of NO dysregulation to common disease processes is limited by the major impact of environment in the general population. In urea cycle patients a highly penetrant genetic mutation affecting NO flux could be more easily correlated with specific clinical effects.

The present study highlights the importance of the interaction between mutation and genetic background on ureagenesis and NO production. Supplementation of OTC disorder patients with UCI might prove helpful in not only increasing their ureagogenic potential but in sustaining NO production. The relative efficacy of UCI supplementation to affect NO production could potentially be more easily evaluated in the urea cycle disorder patient.

ACKNOWLEDGMENTS

We thank I. Cajo and M. Wu for technical assistance. The polyclonal hOTC antibody was a gift from H. Morizono and M. Tuchman.

GRANTS

The work was supported by USDA Grant 6250-51000-044 and National Institutes of Health Grants K01-RR-024173 and R01-DK-54450.

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