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

Juan C. Marini,1 Ayelet Erez,2 Leticia Castillo,1 and Brendan Lee2,3

1USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics and 2Department of Molecular and Human Genetics, Baylor College of Medicine, and 3the Howard Hughes Medical Institute, Houston, Texas

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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 [13C6]urea, [15N2]arginine, [1-5,5-D2]ornithine, [1-6-13C]citrulline, and [1-ring-D3]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 metabolic 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/1 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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Address for reprint requests and other correspondence: J. C. Marini, 1100 Bates St., Mail Stop BCM320, Houston, TX 77030 (e-mail: marini@bcm.edu).
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-[5,5,5,5,5, D₅]orotic acid (7.7 μmol/kg), and l-[urodeido-¹³C, 4,4,5,5,5, D₅]citrulline (7 μmol/kg), and l-[ring-D₃]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-[5,5,5,5,5, D₅]orotic acid (7.7 μmol·kg⁻¹·h⁻¹), l-[ureido-¹³C,4,4,5,5, D₅]citrulline (7 μmol·kg⁻¹·h⁻¹), and l-[ring-D₃]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 (MTBSTFA; Sigma) and acetonitrile, at 80°C for 20 min in the tightly capped V-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% w/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 with a low vacuum Technologies, Santa Clara, CA) in SIM mode, monitoring m/z ions 546, 548, and 550.

Plasma citrulline, arginine, and phenylalanine enrichments were determined as their dansyl derivatives by LCMS utilizing a TQS 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-mecaptoethanol, 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.

Ornithine titration. To determine the supplementation rate of ornithine needed to restore ureagenesis and reduce hyperammonemia in spf-ash mutant mice of two different backgrounds, 6-wk-old mice were utilized in a completed randomized experimental design. At 0700 the day of the infusion, feed was removed and mice were transferred to a new cage with paper towel bedding. Mice were weighed at 0930 and infusions started at 1030. The lateral tail vein catheterization procedure has been described in detail elsewhere (20).

Mice were restrained by adhesive tape across the base of the tail during the infusion.

Mice were continuously infused for 4 h with an equimolar glycine-alanine mixture (Sigma, St. Louis, MO) at a rate of 6.06 mmol·kg⁻¹·h⁻¹, resulting in a nitrogen load of 85 mg N·kg⁻¹·h⁻¹. We previously showed that mutant B6 spf-ash mice developed hyperammonemia and a reduced rate of ureagenesis with this protocol (21); however, there were no differences between control wild-type and mutant spf-ash mice if the nitrogen load was provided by a complete mixture of amino acids (22). Mutant mice (B6 spf-ash and ICR spf-ash, 5 per group) were also infused with increasing rates of ornithine (0, 80, 160, 240, and 320 μmol·kg⁻¹·h⁻¹). Wild-type control mice (B6 and ICR, 5 per group) were also infused with the glycine-alanine mixture but received no ornithine supplementation and served as controls. A priming dose of [¹³C¹⁸O]urea (60 μmol/kg) followed immediately by a continuous infusion of [¹³C¹⁸O]urea (90 μmol·kg⁻¹·h⁻¹) was conducted to determine the entry rate of urea. Preliminary work showed that urea isotopic plateau enrichment is reached within 1 h utilizing this infusion protocol (19). After a 4-h infusion, mice were euthanized by decapitation and blood was collected. Because of the concern of incomplete mixing of the tracer in blood, which would lead to a higher enrichment when blood is collected by decapitation, a short interval (15–20 s) between the end of the infusion and decapitation was allowed. Plasma was obtained after centrifugation at 1,500 g for 15 min at 4°C; ammonia was measured in fresh plasma, and the rest of the plasma was frozen at −20°C until analysis.

INTERACTION BETWEEN spf-ash MUTATION AND GENETIC BACKGROUND
(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

\[ Ra_M = \frac{100}{E_M} \frac{i_M}{i_M + R_M} - 1 \]

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 (\( Rc_{Cit \rightarrow Arg} \)), citrulline to ornithine (\( Rc_{Cit \rightarrow Orn} \)), and ornithine to citrulline (\( Rc_{Orn \rightarrow Cit} \)) were determined as follows (30a)

\[ R_{c_{x \rightarrow y}} = \frac{E_x}{E_y} \left( \frac{Ra_x}{i_x + Ra_x} \right) - 1 \]

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 \( [^{15}\text{N}_2] \text{arginine}, [^{5,5}\text{D}_2] \text{ornithine}, [^{13}\text{C},^{4,4,5,5,\text{D}_4}] \text{citrulline} \) and \( [^{13}\text{C},^{4,4,5,5,\text{D}_4}] \text{arginine} \), respectively, determined from the steady-state enrichments of the infused tracers \( [^{15}\text{N}_2] \text{arginine}, [^{5,5}\text{D}_2] \text{ornithine}, [^{13}\text{C},^{4,4,5,5,\text{D}_4}] \text{citrulline} \) and \( [^{13}\text{C},^{4,4,5,5,\text{D}_4}] \text{arginine} \). Thus, 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 \( [^{6,5-13}\text{C},^{5,4,4,4}\text{D}_4] \text{arginine} \) from the infused citrulline and \( [^{5,5,4,4}\text{D}_4] \text{ornithine} \). During the deamination of ornithine to L-glutamate-γ-semialdehyde by Ornithine AminoTransferase (OAT), one deuterium is lost from \( [^{5,5}\text{D}_2] \text{ornithine} \). Because the tracer used cannot be recycled when ornithine is deaminated-reaminated by OAT, the protocol employed measured the direct utilization of plasmatic ornithine for citrulline synthesis.

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

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

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 (\( R_{Arg,M1} \)) was calculated as follows

\[ R_{Arg,M1} = R_{Prot \rightarrow Arg} - (R_{Arg} - Rc_{Cit \rightarrow Arg}) \]

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)

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

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

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; \( O_{breakpoint} \) 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. Breakdown analysis showed that supplementation with \( \sim 100 \mu \text{mol} \text{ ornithine·kg}^{-1} \cdot \text{h}^{-1} \) 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 [4.92 and 2.70 (SE 0.08) mmol·kg$^{-1}$·h$^{-1}$, respectively; $P < 0.016$], and the difference in the amount of urea produced represented 73% of the nitrogen load infused. However, ureagenesis was not depressed in B6$^{spf-ash}$ 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$^{spf-ash}$ mice (Table 1). The entry rate of arginine, however, was only reduced in B6$^{spf-ash}$ 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 μmol·kg$^{-1}$·h$^{-1}$) representing 1.94% of the total plasma arginine entry rate of these animals, whereas in B6 and ICR$^{spf-ash}$ mice it was ~0.9% (Table 1). The conversion of citrulline into arginine was not only quantitatively higher in ICR wild-type control mice (126 μmol·kg$^{-1}$·h$^{-1}$), but it represented a higher percentage (89%) of the citrulline entry rate. Likewise, a larger quantity (23.4 μmol·kg$^{-1}$·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 μmol·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 Fig. 5. Most of the circulating arginine was derived from arginine originating from protein breakdown, are shown in mice from both genetic backgrounds, as well as the spf-ash wild-type control mice. Nevertheless, two B6 spf-ash mice developed clear signs of hyperammonemia, but without a reduction in ureagenesis when infused with the alanine-glycine mixture.

**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 B6 spf-ash mice developed clear signs of hyperammonemia, but without a reduction in ureagenesis when infused with the alanine-glycine mixture.

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**Table 1.** Plasmat entry rate and interconversions of urea cycle intermediates in WT and spf-ash mutant mice of 2 different genetic backgrounds (B6 or ICR)

<table>
<thead>
<tr>
<th></th>
<th>B6</th>
<th>ICR</th>
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<tr>
<td></td>
<td>WT</td>
<td>spf-ash</td>
</tr>
<tr>
<td>Plasma entry rate, μmol·kg(^{-1}·h^{-1})</td>
<td>421*</td>
<td>332†</td>
</tr>
<tr>
<td>RaArg</td>
<td>118*</td>
<td>43†</td>
</tr>
<tr>
<td>RaCit</td>
<td>266†</td>
<td>372*</td>
</tr>
<tr>
<td>RaCit</td>
<td>804†</td>
<td>747†</td>
</tr>
<tr>
<td>RaOrn</td>
<td>301†</td>
<td>327†</td>
</tr>
</tbody>
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Results are means ± SE (n = 20). Mut†, effect of the mutation; Back†, effect of the genetic background; Int†, interaction between the 2 main effects.

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[Fig. 5](#fig5) Arginine originating from protein breakdown and from de novo synthesis from citrulline in spf-ash mice from 2 different genetic backgrounds (B6 and ICR). RaM, denotes the plasma entry rate of metabolite M; X→Y, interconversion of metabolite X into Y; ArgMI, arginine released from protein and metabolized intracellularly. Values are expressed in μmol·kg\(^{-1}·h^{-1}\) unless noted. Significance was calculated using preplanned orthogonal contrasts, means with superscript differ (P < 0.05).
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 to result 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 from protein breakdown that was 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 showing the high compartmentalization of urea metabolism.

Contribution of Protein Arginine to Ra

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.
The de novo synthesis of ornithine takes place in mitochondria. The carbon skeleton provided by L-glutamate can be more easily correlated with specific clinical effects. A limitation of studying the contribution of NO dysregulation to urea cycle disorder patients with UCI might prove helpful in not only increasing their ureogenic 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.

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REFERENCES

17. Li MX, Nakajima T, Fukushima T, Kobayashi K, Seiler N, Sabeki T. Aberrations of ammonia metabolism in ornithine carbamoyltrans-


