Differential utilization of systemic and enteral ammonia for urea synthesis in control subjects and ornithine transcarbamylase deficiency carriers1–4

Fernando Scaglia, Juan Marini, Judy Rosenberger, Joseph Henry, Peter Garlick, Brendan Lee, and Peter Reeds

ABSTRACT
Background: Female carriers of ornithine transcarbamylase deficiency (OTCD) have nearly normal rates of total urea synthesis, but they derive less urea from systemic glutamine amide nitrogen than do healthy persons.

Objective: The objective of the study was to investigate whether females with symptomatic OTCD rely on alternative pathways to compensate for the reduced urea synthesis activity observed in this disorder.

Design: The 5-d study involved 6 control subjects (3 males, 3 females) and 6 female OTCD carriers who had a fixed energy intake of 133 kJ · kg⁻¹ · d⁻¹ and a protein intake of 0.8 g · kg⁻¹ · d⁻¹. They underwent two 12-h periods of isotopic tracer administration, separated by 2 d. On both occasions, [¹⁸O] or [¹³C]urea was infused intravenously, and the subjects consumed hourly meals. During the first period, [¹⁵N]NH₄Cl was given intravenously; during the second period, the tracer was given as hourly oral doses.

Results: OTCD carriers produced less urea (P < 0.05) but had a higher (P < 0.05) mean ammonia appearance rate and plasma ammonia concentration than did control subjects. OTCD carriers incorporated a lower (P < 0.001) mean (±SE) proportion of the intravenous [¹⁵N]NH₄Cl dose into circulating urea than did control subjects (16 ± 1% compared with 36 ± 2%), but there was no genotypic difference in the incorporation of orally administered tracer (81 ± 4% compared with 72 ± 4%, respectively).

Conclusion: A good degree of dietary protein tolerance seemed to be retained in OTCD carriers by the maintenance of higher ammonia appearance rates, expansion of the plasma ammonia pool, and reliance on the ability of the perivenous hepatocytes to clear excess ammonia via glutamine synthesis. Am J Clin Nutr 2003;78:749–55.

KEY WORDS Urea synthesis, humans, ammonia flux, ornithine transcarbamylase deficiency, stable isotopes

INTRODUCTION
The most common (≈1:75 000 births) disorder of the urea cycle is ornithine transcarbamylase deficiency (OTCD; 1–3). Persons with OTCD have less ability to synthesize citrulline from ornithine and carbamoyl phosphate than do persons without OTCD. Because OTCD is an X-linked condition, the random nature of X chromosome inactivation results in a wide variation in the capacity for urea synthesis among female OTCD carriers. It is of particular interest that, whereas many OTCD carriers exhibit little dietary protein sensitivity (4, 5) and are not chronically hyperammonemic, the stability of their nitrogen metabolism is sensitive to stresses such as fasting and infection (6, 7) that accelerate the mobilization of protein stores. For example, clinically significant hyperammonemia can occur in otherwise asymptomatic female OTCD carriers during childbirth (5). These clinical observations imply that apparently asymptomatic OTCD carriers may harbor more subtle defects in nitrogen metabolism. This possibility is supported by our findings, which show that fed female asymptomatic OTCD carriers derive considerably less urea from glutamine amide-N than do control subjects, even though the OTCD carriers continue to have apparently adequate rates of urea synthesis (8). The 2 nitrogen atoms of urea, the final compound of mammalian nitrogen metabolism, are derived from separate intracellular precursors: one from free ammonia, incorporated via mitochondrial carbamoyl phosphate, and the other from aspartate-N, incorporated via the synthesis of cytosolic argininosuccinate (1). Although the intermediate extracellular precursors for hepatic ureagenesis are ammonia, glutamine, alanine, and glutamate (9–11), their relative contributions to urea synthesis remain incompletely characterized. Initial precursors also include other amino acids that are deaminated and transaminated in the liver. From a physiologic perspective, it is important to note that different extracellular precursors derive from different anatomical sites. Thus, peripheral (eg, muscle) amino acid metabolism generates glutamine and alanine (12), whereas intestinal amino acid metabolism generates ammonia and alanine (12, 13).

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The present study was designed to test the following 3 hypotheses: 1) enterally generated ammonia and peripherally generated ammonia are metabolized differently in OTCD carriers than in control subjects, 2) ammonia is cleared into urea less effectively in female OTCD carriers than in control subjects, and 3) glutamine is produced at a greater rate in females with OTCD than in control subjects to compensate for the OTCD carriers’ reduced ureagenic capacity. We compared the transfer of the nitrogen from intravenously and orally administered \[^{15}N\]ammonium chloride to urea and simultaneously measured the total rate of urea synthesis with either \[^{18}O\] or \[^{13}C\]urea. This combination in the route of tracer administration allows for the quantification of the \[^{15}N\]ammonia incorporated into urea, as well as the first-pass extraction (FPE) of the tracer by the liver.

**SUBJECTS AND METHODS**

**Subjects**

The study was carried out in the General Clinical Research Center of Texas Children’s Hospital, and it received prior approval from the Institutional Review Board for Investigations in Human Subjects of the Baylor College of Medicine. Both control subjects and the OTCD carriers received monetary compensation for participation in the investigation. All of the OTCD carriers were relatives of patients whose clinical care was supervised by faculty members of the Department of Molecular and Human Genetics. None of the OTCD carriers required pharmacotherapy for their condition, and they were healthy at the time of the investigation. Plasma ammonia concentrations were monitored throughout the study and remained within the clinically normal range. However, according to a retrospective analysis of the relation between plasma ammonia and the rate of urea production, the phenotypic expression of the disorder differed among the OTCD carriers. Six control subjects (3 male, 3 female) and 6 female untreated asymptomatic OTCD carriers were recruited. The isotopic tracers were purchased from Cambridge Isotopes (Woburn, MA), and the isotopic purity of each tracer was measured. The isotopic enrichment of \[^{15}N\]NH₄Cl was 98%.

**Protocol**

On admission, the subjects gave written informed consent, and blood was drawn for baseline clinical measurement of differential blood count, circulating hepatic enzymes, plasma amino acids, and plasma ammonia. The subjects were begun on a baseline conservative diet, and this prompted a change in the analytic approach. Thus, in 2 control subjects and 3 patients, the appearance rate of urea (URa) was measured with \[^{13}C\]urea as the tracer. In these studies, the enrichment of urea was assessed, first, by using selective ion-monitoring GC-MS. From a separate plasma aliquot, in NaCl (0.072 mol/L, 1 mL · kg⁻¹ · h⁻¹) was started and continued for 12 h. Blood samples (10 mL) were taken from the second catheter at 8, 10, and 11.5 h of the infusion. The plasma was isolated for isotopic studies and immediately frozen. During the infusion, the subjects ate 50% of the daily prescribed protein intake in 12 equally divided portions starting at time 0 of infusion and at hourly intervals thereafter until the 11th hour. After the completion of the infusion, the subjects ate the remainder of their diet.

On day 5, after another overnight fast and the removal of baseline samples, the subjects received a primed urea infusion as on day 3 and ingested a single oral dose of \[^{15}N\]ammonium chloride (3 μmol/kg). Thereafter, the subjects ingested an oral dose of \[^{15}N\]ammonium chloride (3.24 μmol · kg⁻¹ · h⁻¹) with each hourly meal starting at time 0.

**Analysis**

Plasma ammonia was measured with the use of a glutamate dehydrogenase enzyme assay on a clinical analyzer (Cobas Fara; Roche Products, Indianapolis). Plasma amino acids were measured by reversed-phase HPLC (Waters Technologies, Milford, MA) with the use of the phenylisothiocyanate derivative (13). An aliquot of plasma was mixed either with 4 volumes of acetone or with an equal volume of acetic acid (1 mol/L) for the determination of the isotopic enrichment of urea and glutamine, respectively. The enrichment of the plasma ammonia was determined on untreated plasma samples.

The isotopic enrichments of glutamine, ammonia, and \[^{15}N\]urea were determined by selective ion-monitoring gas chromatography–mass spectrometry (GC-MS; Hewlett Packard, Palo Alto, CA). Glutamine was measured as the \(n\)-propyl ester of the heptafluorobutyramide derivative (14). Separation of the glutamine derivative was on an HP5 capillary column (Hewlett-Packard), and the labeling was assessed by scanning mass-to-charge ratios (m/z) of 346–348 after negative methane chemical ionization. The labeling of plasma ammonia was measured as described by Nieto et al (15). The method involves the synthesis of norvaline by reacting plasma ammonia with 2oxopentanoic acid in the presence of glutamate dehydrogenase. The resulting norvaline was then measured as the tertiary butyldimethylsilyl derivative, and its enrichment was assessed by scanning m/z ratios of 186–188 after electron impact ionization. Separation was effected on a model 1701 capillary column (J & W Scientific, Folsom, CA). Urea labeling was measured as the 2-pyrimidinol-tertiary butyl dimethylsilyl derivative of urea. The derivative was prepared by incubating the sample for 2 h at 25°C with 250 μL of an aqueous solution of malondialdehyde (700 mmol/L or 50 g/L) together with 25 μL HCl (11 mol/L). At the end of the incubation, the samples were dried under vacuum and mixed with 50 μL of a solution of tertiary butyldimethylsilane (800 mmol/L or 100 g/L) in ethyl acetate. The mixture was placed in a securely sealed vial and incubated at room temperature for 24 h. Separation of the derivative was effected on a 1701 capillary column (J & W Scientific), and labeling was assessed by scanning m/z 153–156 after electron impact ionization.

During the course of the study, \[^{15}N\]urea became unavailable, and this prompted a change in the analytic approach. Thus, in 2 control subjects and 3 patients, the appearance rate of urea (URa) was measured with \[^{13}C\]urea as the tracer. In these studies, the enrichment of urea was assessed, first, by using selective ion-monitoring GC-MS.
The transfer of 15N from infused or ingested ammonia to urea was significantly different: URas determined with [18O]urea and with [13C]urea were 208 ± 33 and 212 ± 33 μmol ⋅ kg⁻¹ ⋅ h⁻¹, respectively (P > 0.7). Likewise, the isotopic enrichment of [15N]urea determined with GC-MS [0.63 ± 0.11 mol percent excess (MPE)] was not significantly different (P > 0.5) from that determined with GC-CIRMS (0.56 ± 0.12 MPE).

Calculations

The URas and the appearance rates of ammonia (ARas) were calculated from the enrichment (E) resulting from intravenous infusion of [15N]NH₄Cl. URas and the appearance rates of ammonia (ARas) were calculated from the enrichment (E) resulting from intravenous infusion of [15N]NH₄Cl:

\[
\text{Ra} = \text{rate of tracer infusion} \times [\text{E}] - \text{Infusion rate (μmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})
\]  

The transfer of 15N from infused or ingested ammonia to urea was expressed as:

\[
\text{Transfer of } 15\text{N to urea} = 2 \times \text{URas} \times \frac{[\text{E}]\text{Urea} (\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})}{100}
\]

The fractional transfer of infused or ingested [15N]ammonia to urea was expressed as:

\[
\text{Fractional transfer of } 15\text{N to urea} = \frac{\text{Transfer of } 15\text{N} \times \text{[15N]}\text{NH}_4\text{Cl infusion rate} (\%)}{100}
\]

In addition, the amount of tracer that escapes FPE by the liver can be calculated as follows:

\[
\text{Tracer escaping FPE} = \text{ARa} \times \left(\frac{[\text{E}]\text{Ammonia}_{oral} - [\text{E}]\text{Ammonia}_{oral}}{[\text{E}]\text{Ammonia}_{oral}}\right)
\]

where \([\text{E}]\text{Ammonia}_{oral}\) is the enrichment of ammonia when the tracer is administered orally.

The proportion of tracer escaping FPE is calculated as follows:

\[
\text{Fraction of tracer escaping FPE} = \frac{\text{Tracer escaping FPE} \times 100}{[\text{15N}]\text{NH}_4\text{Cl oral administration rate} (\%)}
\]

When [15N]NH₄Cl was infused intravenously, plasma glutamine reached an isotopic enrichment proportional to that of its precursor (plasma ammonia). According to this reasoning, the oral tracer that escapes the FPE and reaches the peripheral circulation will result in a similar proportional peripheral glutamine enrichment; thus:

\[
\frac{[\text{E}]\text{Glutamine due to tracer escaping FPE}}{[\text{E}]\text{Glutamine}_{oral} \times [\text{E}]\text{Glutamine}_{oral}} = \frac{[\text{E}]\text{Glutamine}_{oral} \times [\text{E}]\text{Glutamine}_{oral}}{[\text{E}]\text{Glutamine}_{oral}}
\]

where \([\text{E}]\text{Ammonia}_{oral}\) and \([\text{E}]\text{Glutamine}_{oral}\) are the enrichments of plasma ammonia and glutamine, respectively, when the tracer is administered intravenously.

When the [15N]NH₄Cl was given orally, a higher peripheral [E]Glutamine was observed because of hepatic glutamine synthesis before [15N]NH₄Cl reached the peripheral circulation. Although some glutamine might have been synthesized in the liver when [15N]NH₄Cl was infused peripherally, a larger proportion of the dose was available for hepatic glutamine synthesis when the dose was given orally. This can be calculated as follows:

\[
\frac{[\text{E}]\text{Glutamine due to liver synthesis}}{[\text{E}]\text{Glutamine due to} \times \text{FPE (MPE)}} = \frac{[\text{E}]\text{Glutamine due to tracer escaping FPE}}{[\text{E}]\text{Glutamine due to}}
\]
Although the appearance rate of glutamine (GRa) was not determined in this study, the values reported by Lee et al (8) for control subjects (391 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) and asymptomatic OTCD subjects (379 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)) on an identical protein level can be used to estimate the actual mass transfers of the ingested tracer:

\[
\text{Oral} \ [^{15}N]\text{NH}_4\text{Cl} \text{ incorporated into glutamine } = E\text{Glutamine}_{\text{oral}} \times \text{GRa} \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (8)
\]

and

\[
\text{Oral} \ [^{15}N]\text{NH}_4\text{Cl} \text{ escaping FPE converted to glutamine in peripheral tissues } = \ ^{15}\text{Glutamine due to tracer escaping FPE} \times \text{GRa} \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (9)
\]

and

\[
\text{Oral} \ [^{15}N]\text{NH}_4\text{Cl} \text{ incorporated into glutamine by the liver (FPE) } = \text{Equation 8 } - \text{ Equation 9} \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (10)
\]

Because the tracer can be incorporated into other products in the liver besides glutamine, the exact amount of tracer incorporated into urea in FPE cannot be calculated, but the maximal amount can be estimated:

Maximal amount of tracer converted into urea in FPE = Dose - Eq 4 - Eq10 \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (11)

Finally, both oral \([^{15}N]\text{NH}_4\text{Cl}\) incorporated into glutamine by the liver and the maximal amount of tracer converted into urea in FPE can be calculated as a percentage of the dose by dividing Equation 10 and Equation 11 by the amount of tracer ingested, respectively.

**Statistical analysis**

The data were analyzed by one-way analysis of variance, with the route of ammonia administration and the genotype as independent variables. Post hoc pairwise comparisons, using the Tukey adjustment for multiple comparisons, were conducted. Values reported are means ± SEs (n = 6).

**RESULTS**

The characteristics of the subjects involved in the study are shown in Table 1. Although the plasma ammonia concentrations of the OTCD carrier group were within clinically acceptable limits, the mean concentration was higher (42 \( \mu \text{mol} \cdot \text{L}^{-1} \)) than that in control subjects (26 \( \mu \text{mol} \cdot \text{L}^{-1} \)) and asymptomatic OTCD subjects (25 \( \mu \text{mol} \cdot \text{L}^{-1} \)) on an identical protein level. The plasma concentrations of glutamine and alanine showed no significant genotypic difference (results not shown).

The \(^{18}\text{O}\) or \(^{13}\text{C}\) and \(^{15}\text{N}\) isotopic enrichments of urea, ammonia, and \(^{15}\text{N}\)urea (n = 6 per genotypic group). ○, intragastric study in control subjects; ●, intravenous study in control subjects; Δ, intragastric study in ornithine transcarbamylase deficiency (OTCD) carriers; and ▲, intravenous study in OTCD carriers. The plasma ammonia pool was at isotopic steady state by 8 h of infusion. The isotopic enrichment of both \(^{18}\text{O}\) or \(^{13}\text{C}\)urea and \(^{15}\text{N}\)urea rose at a nonsignificant rate of 2%/h from 0800 to 1200. MPE, mol percent excess.

**Figure 1.** Mean (±SD) ratios of tracer to tracee during the time course of isotopic enrichments of \(^{18}\text{O}\) or \(^{13}\text{C}\) urea, \(^{15}\text{N}\)ammonia, and \(^{15}\text{N}\)urea on an identical protein level can be used to estimate the actual mass transfers of the ingested tracer:

\[
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\]

and

\[
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\]

and

\[
\text{Oral} \ [^{15}N]\text{NH}_4\text{Cl} \text{ incorporated into glutamine by the liver (FPE) = Equation 8 } - \text{ Equation 9} \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (10)
\]

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Maximal amount of tracer converted into urea in FPE = Dose - Eq 4 - Eq10 \ (\mu\text{mol} \ ^{15}\text{N} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \quad (11)

Finally, both oral \([^{15}N]\text{NH}_4\text{Cl}\) incorporated into glutamine by the liver and the maximal amount of tracer converted into urea in FPE can be calculated as a percentage of the dose by dividing Equation 10 and Equation 11 by the amount of tracer ingested, respectively.

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**Figure 1.** Mean (±SD) ratios of tracer to tracee during the time course of isotopic enrichments of \(^{18}\text{O}\) or \(^{13}\text{C}\) urea, \(^{15}\text{N}\)ammonia, and \(^{15}\text{N}\)urea (n = 6 per genotypic group). ○, intragastric study in control subjects; ●, intravenous study in control subjects; Δ, intragastric study in ornithine transcarbamylase deficiency (OTCD) carriers; and ▲, intravenous study in OTCD carriers. The plasma ammonia pool was at isotopic steady state by 8 h of infusion. The isotopic enrichment of both \(^{18}\text{O}\) or \(^{13}\text{C}\)urea and \(^{15}\text{N}\)urea rose at a nonsignificant rate of 2%/h from 0800 to 1200. MPE, mol percent excess.

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TABLE 2
Mean plasma isotopic enrichment of administered tracers and 15N-labeled products of [15N]NH4Cl given intravenously or orally to control subjects and OTCD carriers.

<table>
<thead>
<tr>
<th>Primary tracers</th>
<th>15N-labeled products</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPE</td>
<td>MPE</td>
</tr>
<tr>
<td>Intravenous administration</td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.751</td>
</tr>
<tr>
<td>OTCD carriers</td>
<td>0.988</td>
</tr>
<tr>
<td>Oral administration</td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.778</td>
</tr>
<tr>
<td>OTCD carriers</td>
<td>0.965</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.056</td>
</tr>
<tr>
<td>Effect of route</td>
<td>0.973</td>
</tr>
<tr>
<td>Effect of genotype</td>
<td>0.001</td>
</tr>
<tr>
<td>Route x genotype</td>
<td>0.663</td>
</tr>
</tbody>
</table>

1 n = 6 per group. OTCD, ornithine transcarbamylase deficiency; MPE, mol percent excess. Means in a column with different letters are significantly different, P < 0.05.

2 Normalized to a 3.24 μmol·kg⁻¹·h⁻¹ dose of [15NH4Cl].

circulating ammonia, and it did not differ significantly (P = 0.56) between control subjects and OTCD carriers. Although the isotopic enrichment of circulating glutamine was lower in the OTCD carriers, the difference between the control subjects and OTCD carriers was not affected by genotype. During the oral administration of [15N]NH4Cl, both plasma urea and glutamine showed a higher (P < 0.05) enrichment than did peripheral plasma ammonia (Table 2). No difference (P > 0.92) in urea production between the 2 infusion days (days 3 and 5) was observed in control subjects and OTCD carriers when the data were calculated to pool the marginal means of days 3 and 5 (Table 3). A significantly higher (P < 0.001) URa was detected in control subjects (218 ± 31 μmol·kg⁻¹·h⁻¹) than in OTCD carriers (177 ± 17 μmol·kg⁻¹·h⁻¹). It was of particular interest that, in OTCD carriers, there was a significant (r = 0.88) negative linear relation between the circulating ammonia concentration and the URA (Figure 2). No relation between these variables was apparent in the control subjects (r = 0.12; data not shown).

During the intravenous infusion of [15N]NH4Cl, the ARa was higher (P < 0.001) in OTCD carriers, but a smaller (P < 0.001) proportion of the dose was incorporated into urea in the OTCD carriers than in control subjects (Table 3). In both groups, the fractional transfer of ammonia-N to urea was higher (P < 0.001) when the ammonia tracer was given orally. Importantly, the difference between the incorporation of intravenous and oral ammonia nitrogen into urea was greater (P < 0.001) in the OTCD carriers (+ 400%) than in the control subjects (100%). As a consequence, there was a highly significant (P < 0.001) interaction between genotype and route of administration with regard to the utilization of the 2 sources of ammonia for urea synthesis.

The fate of the oral dose of [15N]NH4Cl is shown in Figure 3. Because the ARa into the splanchnic circulation was not determined, we were unable to measure the actual mass transfer of tracer. A significantly (P < 0.001) larger proportion of the tracer escaped the FPE by liver either as ammonia or glutamine in OTCD carriers than in control subjects, which resulted in a lower (P < 0.001) incorporation into urea in the OTCD carriers.

TABLE 3
Urea and ammonia appearance rates and incorporation of the tracer into urea in control subjects and OTCD carriers.

<table>
<thead>
<tr>
<th></th>
<th>Appearance rate</th>
<th>[15N] incorporation into urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea</td>
<td>Ammonia</td>
</tr>
<tr>
<td></td>
<td>μmol·kg⁻¹·h⁻¹</td>
<td>μmol·kg⁻¹·h⁻¹</td>
</tr>
<tr>
<td>Intravenous administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>221^2</td>
<td>581</td>
</tr>
<tr>
<td>OTCD carriers</td>
<td>175</td>
<td>883</td>
</tr>
<tr>
<td>Oral administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>215</td>
<td>42.7</td>
</tr>
<tr>
<td>OTCD carriers</td>
<td>180</td>
<td>42.7</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>10.5</td>
<td>42.7</td>
</tr>
<tr>
<td>Effect of route</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Effect of genotype</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Route x genotype</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

1 n = 6 per group. OTCD, ornithine transcarbamylase deficiency; NA, not applicable. Values in the same column with different letters are significantly different, P < 0.05.

2 X.
FIGURE 2. Relation between the rate of urea production and the plasma ammonia concentration in 6 individual female ornithine transcarbamylase deficiency (OTCD) carriers. There was a significant negative and linear relation between the circulating ammonia concentration and the rate of urea production.

DISCUSSION

Although the chemical principles of urea synthesis were postulated many years ago (16), the process is biochemically and physiologically complex, and many aspects still are not well understood. Urea synthesis is compartmentalized within the liver, and the overwhelming majority of the process occurs in the periportal hepatocytes (17). The enzymes of the urea cycle are also compartmentalized between the mitochondria and cytoplasm. In addition, and of particular significance to the present study, different quantities of the main extracellular sources of urea nitrogen (ammonia, glutamine, alanine, and glutamate) derive from different sites within the body. Thus, nitrogen supplied to the liver from the peripheral amino acid metabolism is largely in the form of hepatic arterial glutamine and alanine, whereas the urea nitrogen derived from the direct intestinal catabolism of dietary amino acids (13, 18) is in the form of ammonia and alanine in the hepatic portal circulation.

Animal studies in the past decade (13, 14, 18) and earlier (19) support the idea that at least three-quarters of the dietary amino acids that undergo intestinal catabolism, a process that may account for 35% of whole-body amino acid catabolism, are presented to the liver as hepatic portal venous ammonia. The present study was designed to test the general hypothesis that OTCD carriers handle this enteral ammonia source for urea synthesis differently than do control subjects. An earlier study from our laboratory (8) showed that patients with partial defects in urea synthesis; the results of the present study largely confirmed the hypothesis that there are marked differences between the ways that OTCD carriers and non-OTCD carriers metabolize nitrogen. In this study, we showed that FPE of the tracer was higher for control subjects than for OTCD carriers, OTCD carriers incorporated a larger proportion of the tracer into glutamine in the liver than did control subjects, and, despite the fact that their maximal incorporation of the tracer into urea in FPE was only 11%, OTCD carriers were able to incorporate as much as 81% of the tracer dose into urea.

Our results also showed a reduced FPE of the orally administered tracer in OTCD carriers and incorporation of a low proportion of the intravenous dose into urea. Although there are very few other human studies comparing the in vivo utilization of peripheral and enteral ammonia for urea synthesis (20, 21), particularly with subjects in the fed state (20), this limited literature largely supports the present observations. An almost complete FPE of portal ammonia was previously reported in humans (20), as was a large incorporation of oral ammonia into nonessential amino acids (21). This ability to retain ammonia-N in amino acids could benefit OTCD carriers in subsequently directing the nitrogen into urea, which would account for the incorporation of 81% of tracer into urea that we observed in the OTCD carriers. It seems highly likely that, as expected from the metabolic properties of the periportal hepatocytes (20), the extraction of portal ammonia by the liver in control subjects is primarily, although not exclusively, channeled to urea synthesis, as indicated by a 61% FPE of the oral tracer into urea. In contrast, because of the lesser ability of OTCD carriers than of non-OTCD carriers to detoxify ammonia, more tracer escaped periportal urea synthesis and was converted into glutamine by the perivenous hepatocytes. It was also reported previously that there is a substantial difference in the metabolic fate of ammonia, depending on the route of tracer administration (21). This finding agrees with our observations. The reason for this
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difference is not clear (21); it could derive from a number of mechanisms (eg, clearance of ammonia by the kidney or preferential incorporation of newly synthesized amino acid), and it warrants further investigation.

These results add evidence that asymptomatic OTCD carriers have to rely on alternative pathways to temporarily detoxify ammonia before it is incorporated into urea. Thus, a good degree of dietary protein tolerance appears to be maintained in OTCD carriers through the maintenance of higher ARa, expansion of the plasma ammonia pool to levels closer to the upper normal limit, and reliance on the ability of perivenous hepatocytes to clear excess ammonia via glutamine synthesis. Although these alternative pathways seem to be successful in handling ammonia, they are also very fragile, as indicated by the hyperammonemic episodes that characterize the disorder. This fact reinforces the importance of preventing sudden changes in nitrogen metabolism in patients with defects in the urea cycle, either by minimizing their exposure to conditions, such as infection, that increase peripheral protein catabolism or by avoiding drastic changes in dietary protein intake.

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