Clinical Consequences of Urea Cycle Enzyme Deficiencies and Potential Links to Arginine and Nitric Oxide Metabolism

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ABSTRACT Urea cycle disorders (UCD) are human conditions caused by the dysregulation of nitrogen transfer from ammonia nitrogen into urea. The biochemistry and the genetics of these disorders were well elucidated. Earlier diagnosis and improved treatments led to an emerging, longer-lived cohort of patients. The natural history of some of these disorders began to point to pathophysiological processes that may be unrelated to the primary cause of acute morbidity and mortality, i.e., hyperammonemia. Carbamyl phosphate synthetase I deficiency single nucleotide polymorphisms may be associated with altered vascular resistance that becomes clinically relevant when specific environmental stressors are present. Patients with arginosuccinic aciduria due to a deficiency of argininosuccinic acid lyase are uniquely prone to chronic hepatitis, potentially leading to cirrhosis. Moreover, our recent observations suggest that there may be an increased prevalence of essential hypertension. In contrast, hyperargininemia found in patients with arginase 1 deficiency is associated with pyramidal tract findings and spasticity, without significant hyperammonemia. An intriguing potential pathophysiological link is the dysregulation of intracellular arginine availability and its potential effect on nitric oxide (NO) metabolism. By combining detailed natural history studies with the development of tissue-specific null mouse models for urea cycle enzymes and measurement of nitrogen flux through the cycle to urea and NO in UCD patients, we may begin to dissect the contribution of different sources of arginine to NO production and the consequences on both rare genetic and common multifactorial diseases. J. Nutr. 134: 2775S–2782S, 2004.

KEY WORDS: • urea cycle • arginine • nitric oxide

The enzymes of the urea cycle (UC) serve 2 purposes: the detoxification of ammonia into urea and the de novo biosynthesis of arginine (1). The UC disorders (UCD) are a group of inborn errors of metabolism that affect the transfer of nitrogen into urea. Deficiencies of all of the enzymes of the cycle were identified, and, although each specific disorder results in the accumulation of different precursors, hyperammonemia and hyperglutaminemia are common biochemical hallmarks of these disorders (2,3). Most of these disorders [N-acetyl glutamate synthase (NAGS) deficiency, carbamyl phosphate synthetase I deficiency (CPSID), argininosuccinate synthetase (ASS) deficiency, argininosuccinic acid lyase (ASL) deficiency, and arginase 1 (ARG1) deficiency] are inherited in an autosomal recessive fashion, whereas ornithine transcarbamylase deficiency (OTCD) is X-linked (4). These conditions are estimated to have an overall prevalence of 1:8200 in the United States (5). Infants with null activity of a UC enzyme, other than an ARG1 deficiency, usually present with hyperammonemic encephalopathy in the neonatal period (6). Patients with partial activity show late-onset presentation at any age, with hyperammonemic crises that are precipitated by catabolic stress and that carry a significant risk of developmental disabilities (7). These patients may manifest with cerebral palsy, developmental delay, psychiatric illness, hyperammonemia associated with valproate therapy, seizure activity, and neurodevelopmental disabilities (3,8). Variable expressivity is

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Abbreviations used: ALI, acute lung injury; ARG, arginase; ASA, arginosuccinate aciduria; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; BMT, bone marrow transplantation; CPS, carbamyl phosphate synthetase; CPSID, carbamylphosphate synthetase I deficiency; GABA, gamma-amino butyric acid; HA, homoarginine; HTN, hypertension; HVOD, hepatic veno-occlusive disease; NAA, N-acetylaspartate; NAGS, N-acetylglutamate synthase; NMDA, N-methyl-D-aspartate; NOS, neuronal nitric oxide synthase; NO, nitric oxide; NO2, nitrite; NO3, nitrate; NOS, NO synthase; NOx, total NO metabolites; OLT, orthotopic liver transplantation; OTCD, ornithine transcarbamylase deficiency; PVT, pulmonary vascular tone; UC, urea cycle; UCD, urea cycle disorder.
the rule in OTCD females because of a random X-inactivation pattern in the liver (9). Morbidity and mortality in these disorders correlate with the duration and severity of hyperammonemic episodes (2).

While the neonatal presentation of UCD is relatively uniform, late-onset presentations are due to hypomorphic mutations whose clinical presentation can be quite varied. The different UCDs have unique clinical features that are only now becoming recognized as the natural history of each disease becomes apparent with a longer-lived patient population. Moreover, the clinical and biochemical consequences of our therapeutic interventions themselves lent insight into unique gene–nutrient–environment interactions related to nitrogen transfer. Much of these emerging data, however, are retrospective and/or anecdotal, and there is a dire need for coordinated natural history studies. In spite of this, the clinical and laboratory observations provide important clues that led to emerging hypotheses that can be tested in appropriate animal models and in human subjects themselves. Similar observations were derived from our diagnostic and therapeutic approaches to these patients.

Clinical and laboratory diagnoses of UCDs may be difficult. Metabolic parameters such as hyperammonemia, plasma amino acid profile, or orotic aciduria are late indicators of metabolic derangement and may be inadequate for predicting clinical severity (10). It is not known whether circulating glutamine correlates well with brain glutamine (11). Although the molecular basis for each of the UCDs was determined, a particular genotype and/or in vitro enzyme activity does not necessarily predict clinical severity (10). Moreover, the detection of OTCD carriers can represent a diagnostic dilemma. Although DNA testing is recommended, in ~20% of the cases, no mutations can be found (12), and the allopurinol loading test is not completely specific or sensitive (13).

We previously developed stable isotope approaches to determine in vivo rates of total body urea synthesis and UC-specific nitrogen transfer to evaluate phenotypic severity in patients with a variety of UCDs (14). The ratio of isotopic enrichments of $^{15}$N-urea/$^{15}$N-glutamine allowed us to distinguish normal control subjects from patients with neonatal and late presentations. As expected, patients demonstrated a relatively greater glutamine flux in association with decreased urea synthesis. This study demonstrated that the $^{15}$N-urea/$^{15}$N-glutamine ratio, i.e., fractional transfer to urea, is a sensitive index of in vivo UC activity and correlates with clinical severity (14). Moreover, we used this approach in an integrated manner to aid in the diagnosis and the prospective treatment of an OTCD female (15). Potentially, this stable isotope protocol could provide a sensitive tool to aid in the diagnosis and the management of UCD patients and, more specifically, OTCD females. These same studies may also lend insight into the transfer of $^{15}$N via the UC into arginine and ultimately nitric oxide (NO) as explored in a previous study (16).

Using a stable isotope approach and by comparing the transfer of $^{15}$N from intravenous vs. oral $^{15}$NH$_4$Cl into urea, we investigated whether OTCD females rely on alternative pathways to compensate for the reduced urea synthetic activity observed in this disorder to maintain good dietary protein tolerance (17). OTCD females seemed to retain good dietary protein tolerance by maintaining higher ammonia appearance rates, expanding the plasma ammonia pool, and clearing excess ammonia via glutamine synthesis in the perivenous hepatocytes (17). The conclusions of the study showed the importance of preventing sudden changes in nitrogen metabolism in UCD patients by minimizing their exposure to conditions that increase peripheral protein catabolism.

**Neurotoxicity in UCDs**

The mechanism of neurotoxicity in UCDs was not completely elucidated. The neurotoxic effect of ammonia was well recognized, although the manner by which it exerts its effects upon the central nervous system is not very well known (18). Its acute effects include increased blood–brain barrier permeability, depletion of intermediates of cell energy metabolism, and disaggregation of microtubules (18). The effects of chronic, mildly elevated ammonia may include alterations of axonal development and alterations in brain amino acid and neurotransmitter levels (19,20). In models of brain edema, where lethal doses of ammonia are administered, gial fibrillary acidic protein is reduced (21) and glutamine is increased (22), preceded by an increase in blood flow (23). It is not known whether NO production plays a role in such an increase. The arginine recycling enzymes are induced in astrocytes by ammonium, possibly originating NO via inducible NO synthase or neuronal NO synthesis (nNOS) (24). The stimulated nNOS might mainly produce $\mathrm{O}_2^-$, which combines with NO to form the highly toxic peroxynitrites (25).

Although chronic hyperammonemia impairs the activation of N-methyl-D-aspartate (NMDA) receptors and leads to reduced re-uptake of extracellular glutamate (26), acute hyperammonemia leads to excessive activation of NMDA receptors and increased intracellular calcium, which bounds to calmodulin and stimulates NOS. The difference between acute and chronic hyperammonemia might be explained by the neuro-modulation of glutamate receptors by glutathione (27). There is substantial evidence that creatine is essential for axonal elongation (20,23). The exposure of brain cells to ammonia may alter the recycling of arginine, which plays a key role in the central nervous system, not only as a NO precursor but also as a substrate for creatine synthesis. Exposure of brain-cell aggregates to NH$_4$Cl leads to a decrease in intracellular creatine and phosphocreatine concentrations (20). When creatine is added to the aggregate cultures during NH$_4$Cl exposure, it seems to protect the axons from growth impairment (20).

**Management of UCDs**

The management in UCDs is achieved by restricting dietary protein, providing supportive management of catabolic stress, and the use of compounds that remove excess nitrogen compounds by alternative pathways (28,29). Alternative pathway therapy includes the use of sodium phenylbutyrate or sodium benzoate to stimulate the excretion of nitrogen in the form of phenylacetylglutamine and hippuric acid (30). Arginine becomes an essential amino acid in UCDs (29), except in OTCD females. Other groups (11) had a similar experience that use of the recommended dose of citrulline (170 mg · kg$^{-1}$ · d$^{-1}$) in several patients with OTCD resulted in a low serum arginine level, prompting us to use additional supplementation with arginine to ensure optimal metabolic control. Other groups (11) had a similar experience. Further evaluations need to be performed to determine whether there is any advantage in giving arginine for some OTCD patients. Furthermore, there is evidence suggesting branched chain amino acid depletion with the use of sodium phenylbutyrate in UCD patients (32).

In the longer term, advances in liver transplantation and
that besides low plasma citrulline concentrations, arginine deficiency was not corrected in the gut by OLT (35,36). We also observed that besides low plasma citrulline concentrations, arginine concentrations are significantly low (P < 0.05) in patients with OTCD and CPS deficiency who were transplanted and who were not receiving L-arginine supplementation when compared with the pre-OLT levels observed in the same patients (Table 1). Here, correction of the hepatic deficiency does not relieve the net arginine requirement, because de novo arginine synthesis is initiated in the gut with the synthesis of citrulline and is completed in the kidney with the conversion of citrulline into arginine. However, most patients do not have problems with a deficiency as long as they maintain their dietary intake of arginine. Gene therapy trials in OTCD with an adenoviral vector did not result in a useful increase in the enzyme activity (37), and the studies now were discontinued because of severe complications resulting in the death of a patient (38). Hepatocytes transplantation may provide an alternative therapeutic approach (39).

In clinical practice, the first goal when treating hyperammonemic crises is to abate the nitrogen load and subsequent hyperammonemia. For the outpatient management of UCD patients, a depletion of arginine and other essential amino acids can be prevented using a special mixture of essential amino acids to improve the quality of the limited natural protein ingested. In summary, several of the findings described above argue in favor of L-arginine supplementation in sufficient amounts to provide for adequate brain creatine, protein, and NO synthesis in UCD patients.

Observations on arginine metabolism from the study and from the natural history of UCDs

CPSID. CPSID (MIM 237300) is a rare UCD that results from the deficiency of CPSI (EC 6.3.4.16). CPSI is thought to be the rate-limiting enzyme catalyzing the first step of the UC. Expression of CPSI is limited to high levels in the liver and smaller amounts in the intestinal mucosa. This enzymatic step is part of the intramitochondrial component of the UC. CPSID directly affects the removal of waste nitrogen compounds, as well as the production of citrulline, arginine, and urea. Classic CPSID with null activity usually presents in the neonatal period with hyperammonemia, with little effect from the environment. A delayed onset form also was described (40,41). Diagnosis is based on the plasma amino acid profile with elevated glutamine and low or absent citrulline and uric orotic acid. These findings cannot discriminate this disorder from a NAGS deficiency (MIM 237310). To establish a correct diagnosis, enzymatic analysis of the liver tissue or identification of mutations in the CPSI gene is required. Prenatal diagnosis in affected families is feasible, based on mutation analysis or identification of disease-related CPSI haplotypes (42).

NO was identified as the active factor in the endothelial-derived relaxing factor in the 1980s (43,44), and it was the focus of hundreds of investigations regarding its role in the regulation of systemic and pulmonary vascular resistance. There are increasing data showing that NOS may play a role in oxidative injury and that NOS may generate free radicals that can result in oxidative damage. Therefore, the relationship between NO and L-arginine, an intermediate of the UC, as well as a precursor of NO, raised the question as to whether polymorphisms in the UC genes may play a role in the pathophysiology of neonatal pulmonary hypertension, postcardiac surgery pulmonary hypertension, and bone marrow transplantation (BMT) complications, and whether patients with a classic UCD may have abnormal blood pressure regulation. It was hypothesized (45,46) that CPSI polymorphisms may affect the function of the enzyme in conjunction with certain environmental stressors such that the availability of the NO substrates may become clinically relevant. Summar et al. (47) evaluated a known CPSI polymorphism to determine if it influences NO metabolite concentrations or NO dependent vasodilatation in adults. A C > A (cytosine > adenosine) transversion at base 4340 in exon 36 of the CPSI gene results in the substitution of asparagine for threonine in the critical region for N-acetylglutamate binding (T1405N). The Thr1405 variant is known to be associated with lower enzymatic activity (30–40% lower activity), when compared with the Asn1405 variant (45). This CPSI polymorphism was shown to significantly influence venous NO concentrations as well as NO dependent and independent vasodilatation (47). These results support the hypothesis that the CPSI genotype affects the L-citrulline/L-arginine cycle and subsequent NO production. Also, this hypothesis was compatible with the fact that CPSI genotype did not affect the response to the NO-independent tissue-type plasminogen activator response to bradykinin (47).

It was hypothesized that this functional polymorphism in the CPSI gene might limit the availability of precursors for NO synthesis and consequently might influence pulmonary vascular resistance, resulting in persistent pulmonary hypertension of the newborn (48). When compared with the general population, the infants in the study (65 near-term neonates with respiratory distress) had a significantly skewed distribution of the genotypes for the CPSI variants at the amino acid position 1405 (P < 0.005). None of the infants with pulmonary hypertension were homozygous for the T1405N polymorphism. When compared with infants without pulmonary hypertension, infants with pulmonary hypertension had lower mean plasma concentrations of arginine (P < 0.001) and NO metabolites (P = 0.05). These data, when combined with previous observations (49), suggest that arginine deficiency might precede and lead to impaired NO synthesis in infants with persistent pulmonary hypertension.

Regarding postcardiac-surgery–related pulmonary hypertension, arginine levels in patients with evidence of increased pulmonary vascular tone (PVT+) were compared with all

| Table 1 |

| Amino acid levels pre- and post-OLT in UCD subjects |

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diagnosis</th>
<th>Pre-OLT arginine</th>
<th>Post-OLT arginine</th>
<th>Post-OLT citrulline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1♂</td>
<td>CPSID</td>
<td>63 ± 9</td>
<td>33 ± 6</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>2♂</td>
<td>OTCD</td>
<td>49 ± 6</td>
<td>20 ± 3</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>3♂</td>
<td>CPSID</td>
<td>101 ± 19</td>
<td>32 ± 7</td>
<td>8 ± 4</td>
</tr>
</tbody>
</table>

1 Pre-OLT subjects were supplemented with citrulline (200 mg/kg/d). Posttransplantation subjects were not supplemented.
2 Means with standard deviations are shown. N for each mean = 5.
3 Pre- and post-OLT arginine levels are significantly different (P < 0.03), normal range for arginine (0–1 y range): 42–132 μM.
4 Citrulline levels are low normal, even post-transplantation; normal range for citrulline (0–1 y range): 2–41 μM.
other patients (PVT−) to determine if the development of PVT+ was associated with a decrease in substrate for NO production (45,50). Arginine levels were significantly lower in the PVT+ patients (P < 0.05). Patients with the AA genotype were less likely to develop increased postoperative PVT. The overrepresentation of CCs and underrepresentation of AAs in the PVT+ patients made this group significantly different from the general population (P < 0.022). Similar observations were noted in hepatic veno-occlusive disease (HVOD) after BMT (45). The presence of post-BMT complications [acute lung injury (ALI), and HVOD] was correlated with the T1405N genotypes. All patients with the CPSI T1405N genotype, even those who developed ALI, had longer survival and did not develop HVOD (P < 0.05). In this study, suboptimal production of arginine and NO correlated with increased morbidity and mortality after BMT (45).

The data from CPSI polymorphisms suggest that subtle functional polymorphisms could play a major role in common diseases, with a potential role of intracellular deficiency. However, if this is the case, it is still not clear why similar anecdotal observations were not reported for upstream deficiency. Hence, it is possible that morbidity may also be associated with ammonia, although the degree of impairment appears out of proportion to the degree of recorded hyperammonemia. Hence, it is possible that morbidity may also be associated with such as ASA deficiency, which is a cell autonomous deficiency of ASA, leading to impaired NO production. The most compelling clinical evidence is the more complex clinical phenotype in ASA. Patients with ASA can suffer from progressive hepatic disease, with hypertension and potential downregulation of endothelial NO production secondary to intracellular deficiency of i-arginine is intriguing. The more mundane explanation is that ASA may be toxic to hepatocytes. Interestingly, NO was associated with the pathogenesis of liver cirrhosis (56), and this may potentially contribute to liver dysfunction. Specifically, it was proposed that reduced NO production may lead to impaired blood pressure regulation in the liver, resulting in hypertension (56). A low intracellular arginine flux may contribute to alternative generation of free radicals, leading to cellular damage. These mechanisms may contribute to the significant liver dysfunction observed in ASA. However, the contribution of plasma arginine to the intracellular arginine pool leading to NO production may confound this mechanism. On the other hand, the contribution of de novo, intracellular arginine production via ASA to NO production in different cell types is unknown. In all cases, these patients offer a unique opportunity to study the in vivo human consequences when intracellular contributions of de novo arginine production via ASA is clearly abolished due to the underlying genetic defect.

Using our cohort of UCD and control subjects studied via our 15N glutamine/16O/13C urea primed constant infusion protocol (14), we further measured the 15N enrichment in total NO metabolites or NOx [nitrite (NO2−) plus nitrate (NO3−)] and steady-state NOx levels. Interestingly, no remarkable differences were noted in the plasma concentrations of NOx, NO2−, NO3−, arginine, and ASA in 2 ASL deficient subjects, one of whom had had essential hypertension (Table 2). However, both were being treated with arginine (500 mg · kg−1 · d−1) at the time. In an unrelated male subject with ASA, we were able to perform a stable isotopic infusion before and after OLT. As expected, the subject had a markedly increased urea flux (140 μmol · kg−1 · h−1) pre-OLT vs. 294 μmol · kg−1 · h−1 post-OLT.

**Table 2**

<table>
<thead>
<tr>
<th>Subject</th>
<th>NOx2,3</th>
<th>NO2−</th>
<th>NO3−</th>
<th>Arginine4</th>
<th>ASA5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: w/HTN</td>
<td>48</td>
<td>13</td>
<td>35</td>
<td>81</td>
<td>127</td>
</tr>
<tr>
<td>2: w/o HTN</td>
<td>41</td>
<td>10</td>
<td>31</td>
<td>78</td>
<td>106</td>
</tr>
</tbody>
</table>

1. Subject 1 is a 9-y-old male with ASA and essential hypertension, and subject 2 is a 32-year-old female with ASA, who is normotensive.
2. NO2−, NO3−, and NOx values are shown. Comparable levels can be seen for all metabolites.
3. NOx was measured as previously described (16).
4. Normal range for arginine (2–18 y range) is 18–127 μmol/L.
5. Normal range for ASA: 0 μmol/L.
urea cycle enzyme deficiencies

μmol · kg⁻¹ · h⁻¹ post-OLT) after liver transplantation, in spite of being on the same protein intake (1.1 g/kg/d). However, the glutamine flux was not markedly different (388 μmol · kg⁻¹ · h⁻¹ vs. 410 μmol · kg⁻¹ · h⁻¹). Interestingly, the fractional transfer of ¹⁵N from arginine into NOx at 7.5 h of infusion was not dramatically affected by liver transplantation (Table 3). In both experiments, ¹⁵N content of NOx and arginine was lower in the ASL deficiency subject compared with control subjects, even though the controls were on the lower protein intake (0.4 g · kg⁻¹ · d⁻¹), indicating impaired transfer of glutamine-amide nitrogen to NO. This suggests that hepatic arginine via nitrogen transfer through ASL and the UC is not a major contributor to total body NO production. In fact, the fractional transfer of ¹⁵N from arginine into NOx was identical pre- vs. post-OLT. As expected, citrulline and ASA levels were still elevated after liver transplantation (Table 3). These data support the hypothesis that de novo hepatic sources of arginine synthesis do not contribute significantly to NOx production. These data are limited by the fact that citrulline flux or NOx flux were not independently measured in these studies. Hence, the actual flux of the NO pathway could not be derived. In the future, the measurement of citrulline and/or NOx flux, in conjunction with an independent measure of total plasma arginine flux, would be informative in dissecting the contributions of de novo production of arginine from the UC vs. exogenous contribution of arginine into total body NO flux.

Unfortunately, the mouse mutant for ASL was not helpful with these studies. Asl null mice die in the perinatal period secondary to hyperammonemia. There do not appear to be differences in NOx level, muscle and liver creatine, and urinary cyclic guanosine monophosphate (cGMP) levels (57). Ultimately, the generation of tissue-specific null mutants that can bypass the neonatal lethality attributable to hyperammonemia may enable better physiological studies of nitrogen transfer into arginine in different tissues.

**ARG1 deficiency.** Argininemia (MIM 207800) is a rare autosomal recessive UC deficiency caused by a defect in the final step of the UC, the hydrolysis of arginine to urea and ornithine by the enzyme ARG1 (EC 3.5.3.1). This is a rare disorder, for example, with an estimated incidence of 1 in 1,000,000 live births in Quebec (58). This condition results in elevated plasma arginine and ammonia levels, with elevated tissue levels of arginine and other guanidino compounds (1). Patients affected with this condition usually present with a neurological syndrome that consists of a variable degree of cognitive deficits, epilepsy, and progressive spastic diplegia (59), whose pathogenesis is not very well understood. Those patients who were ascertained and treated at birth with protein restriction and essential amino acid supplementation seem to remain asymptomatic, with the oldest patients being >35 yr of age (60). This suggests that chronically elevated levels of arginine directly contribute to the neuropathology. In asymptomatic patients whose treatment was begun at diagnosis, there is ample evidence that effective therapy stops the progressive neurological degeneration. While the lower-extremity spasticity may progress slightly, it can be effectively treated with botox or tendon-release procedures.

It is unlikely that elevated plasma ammonia is the main neuropathogenic agent in argininemia, because hyperammonemia seldom occurs in this condition, and the severe spasticity observed in this disease does not commonly occur in other UC defects (61). Guanidino compounds accumulate in other conditions, such as uremia and epilepsy, and there is evidence suggesting that these compounds might contribute to the neurological dysfunction observed in these disorders (62,63). It was postulated that these compounds might be involved as well in the neuropathology of argininemia (64–66). The guanidino compounds that accumulate in hyperargininemic patients [N-acetylarginine (NAA), homoarginine (HA), and arginic acid] were found to have epileptogenic properties (67–69). These guanidino compounds may affect neurotransmission or membrane fluidity (70,71). Certain guanidino compounds accumulating in argininemia might affect (GABA)-ergic neurotransmission, by inhibiting gamma-aminobutyric acid (GABA) and glycine responses in cultured neurons (67). Regarding the potential for guanidino compounds to modulate membrane fluidity, it was shown that methylguanidine inhibits brain Na+, K+, ATP-ase (72), an enzyme embedded in the cell membrane. NAA, HA, and AA significantly inhibit Na+, K+, ATP-ase activity in the cerebral cortex of rats (73).

Arginine is known to modulate neuronal survival through its ability to serve as a substrate for NOS (74). Subsequent studies showed that the protective effects of arginase were related to depletion of arginine. In one study, it was demonstrated that arginine, HA, NAA, and AA induced free-radical production and decreased cellular antioxidant defenses in vitro (75). The guanidino compounds were found to inhibit the activities of catalase, superoxide dismutase, and glutathione peroxidase, which are considered to be the main enzymatic defenses in the brain against free radicals. Although the concentration of guanidino compounds used in the previously described animal experiments were similar to those observed

<table>
<thead>
<tr>
<th>Subject</th>
<th>NOx² (m + 1)</th>
<th>Arg² (m + 2)</th>
<th>¹⁵NOx/¹⁵NArg</th>
<th>NOx</th>
<th>NO₂</th>
<th>NO₃</th>
<th>Arg³</th>
<th>ASA³</th>
<th>NH₃³</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA ϭ</td>
<td>0.73</td>
<td>0.155</td>
<td>47.1</td>
<td>72</td>
<td>16</td>
<td>57</td>
<td>22 ± 54</td>
<td>231 ± 43</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>pre-OLT w/Arg</td>
<td>0.036</td>
<td>0.076</td>
<td>47.4</td>
<td>64</td>
<td>6</td>
<td>58</td>
<td>22 ± 54</td>
<td>231 ± 43</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>post-OLT</td>
<td>0.13</td>
<td>0.85</td>
<td>15.3</td>
<td>57</td>
<td>8</td>
<td>49</td>
<td>84 ± 4</td>
<td>0</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>Control</td>
<td>0.16</td>
<td>0.50</td>
<td>32</td>
<td>53</td>
<td>23</td>
<td>33</td>
<td>88 ± 34</td>
<td>0</td>
<td>18 ± 4</td>
</tr>
</tbody>
</table>

1 Subjects were admitted and stabilized on a restricted protein intake (0.4 g · kg⁻¹ · d⁻¹). On d 3, primed coinfusions of ¹⁵N-glutamine and ¹⁸O¹³C urea were performed for 8 h as previously described (14). Blood sampling was performed at 0, 4, 6, and 7.5 h.
2 ¹⁵N enrichment shown as molar percent excess is shown at the 7.5-h time point and ¹⁵N Arginine and ¹⁵NOx were measured as previously described (16).
3 ASA normal range: 0 µmol; Arg: arginine, normal range for 2–18 yr: 18–127 µmol; NH₃ normal range: 22–48 µmol.
4 For multiple samples (N = 3), means with standard deviations are shown.
in patients with argininemia (67), it would be difficult to extrapolate these findings to an in vivo condition. However, it is tempting to think that these mechanisms may contribute to the pathogenesis of the neurological dysfunction found in ARGI deficiency. The transfer of nitrogen through the UC into NO was studied in 2 siblings with ARGI deficiency using our standard nitrogen flux protocol (Table 4). Both exhibited comparable glutamine fluxes and urea fluxes, but the latter was markedly low compared with control values (14). As expected, the fractional transfer of $^{15}$N from glutamine to urea was near zero.

However, one of the siblings (subject 145) exhibited better metabolic control when on the protein-restricted study diet (0.4 g · kg$^{-1}$ · d$^{-1}$), with a normal steady-state arginine level, while the other sibling (subject 146) had a significantly higher steady-state arginine level. Interestingly, the enrichment of $^{15}$N in NOx was substantially higher in subject 146 with higher serum arginine levels vs. in the subject with normal serum arginine, supporting a direct correlation between plasma arginine levels and the fractional transfer of $^{15}$N into NOx was substantially higher in subject 146.

Conclusions

Human deficiencies of UC enzymes offer unique clinical and experimental opportunities to study and to understand the effects of intracellular dysregulation of arginine production, as well as consequences of exogenous elevations of arginine. Unique genotype-phenotype correlations are emerging in both these rare diseases and in more common pathophysiologic processes. The clinical observations raise important questions and hypotheses about the contribution of dysregulated NO metabolism to the pathogenesis of these conditions. At the same time, UCD patients constitute a unique resource for answering these same questions.

Specifically, human and animal models of UCDs are unique examples of gene-nutrient interactions centered on nitrogen transfer between dietary vs. peripheral sources. They offer potentially important tools for dissecting extracellular vs. intracellular contributions of arginine to the production of NO, free radicals, polyamines, and creatine. Genetic polymorphisms in CPSI, the gene encoding the rate-limiting enzyme of the UC, may correlate with altered arginine availability and endothelial dysfunction during specific times of environmental stress. ASL deficiency is a window into the consequences of deficient de novo production of intracellular arginine. In contrast, ARGI deficiency will provide insight into the consequences of chronic elevations of extracellular arginine as well as the role of ARGI as a direct intracellular regulator of NO flux. The therapeutic approaches to UCD patients may further offer models for understanding the effects of exogenous arginine supplementation, the contribution of liver to nitrogen transfer in patients who have undergone liver transplantation, and the effects of diverting flux away from UC nitrogen transfer using alternative route therapy, i.e., sodium phenylbutyrate.

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