Arginine Metabolism Revisited¹,²
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Abstract
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Keywords: ADMA, arginase, argininosuccinate, homoarginine, GPCR, mTORC1, nitric oxide

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Figure 1 depicts the major arginine metabolites that are ultimately produced by the action of 4 sets of enzymes that use arginine as substrate: arginine:glycine amidinotransferase (AGAT)³, NO synthases (NOS; 3 isoforms), arginases (2 isoforms), and arginine decarboxylase. A listing of common arginine metabolites and the enzymes that produce them is provided in Table 1 (note that this table should not be considered to be exhaustive). It is important to recognize not only that some metabolites (e.g., ornithine and citrulline) can be produced by >1 enzyme but also that some metabolites (e.g., NO and glutamate) can also be produced by enzymatic reactions that do not involve arginine or arginine metabolites. Essential for understanding the roles and regulation of arginine and its metabolites are sensitive and accurate methods for their measurement. Because discussion of this topic is beyond the scope of this article, the reader is referred to reviews of the methodology (2–5).

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One metabolite not usually associated with arginine metabolism is γ-aminobutyric acid (GABA). Although it is theoretically possible that GABA can be produced from glutamate that is derived from arginine, it is unknown whether the glutamate actually used for GABA synthesis is indeed derived from arginine. It is more likely that the putrescine used for GABA synthesis is produced from arginine via arginase and ornithine decarboxylase. We cannot exclude the possibility that some putrescine for GABA synthesis also may be generated via arginine decarboxylase and agmatinase (10).

Arginine is also involved in 3 types of post-translational modification of proteins: 1) specific arginine residues in proteins can be methylated by a family of protein arginine methyltransferases (11–13) and the methylated arginines (discussed below) are released during protein degradation; 2) arginine can be incorporated into protein by arginylation, a mechanism whereby arginine is covalently attached to specific amino acid residues (Figure 1) (14–16); and 3) specific arginine residues within certain proteins can be post-translationally modified to form ornithine or citrulline (17, 18). These latter amino acids are released upon degradation of the modified proteins, but the amounts are tiny compared with their production by the enzymes listed in Table 1.

The following sections of this review will comment on some emerging areas of interest as well as focus on topics not covered in previous reviews (1, 19). Nutritional aspects of arginine metabolism and arginine supplementation are discussed elsewhere (20–22) and thus will not be reviewed here. The reader is referred to current reviews on arginine metabolism for more detailed discussion and for topics not considered here (23–34).

**Methylated Arginines**

The methylated arginines [asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and N⁴-monomethyl-L-arginine (NMMA)] are released when proteins containing methylated arginine residues are degraded. Although there is evidence suggesting that a small percentage of ADMA may be produced directly from free arginine (35), additional experimental studies are needed to rigorously evaluate this hypothesis. ADMA and NMMA, but not SDMA, are competitive inhibitors of NOS (36). Because elevated plasma concentrations of ADMA are a risk factor for cardiovascular disease and associated with hypertension and renal disease [reviewed in (37–40)], it has been speculated that this reflects its activity as an NOS inhibitor. In this regard, the plasma arginine-to-ADMA ratio may be a better marker for disease than plasma concentrations of either metabolite alone [e.g., (41, 42)].

**TABLE 1** Arginine, its common metabolites in mammals, and the enzymes that produce them

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Produced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Argininosuccinate lyase</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Arginase, ornithine aminotransferase, arginine:glycine amidinotransferase</td>
</tr>
<tr>
<td>Citrulline</td>
<td>Ornithine transcarbamylase, NOSs (NOS-1, NOS-2, NOS-3), dimethylarginine dimethylaminohydrolase</td>
</tr>
<tr>
<td>Urea</td>
<td>Arginases I and II, agmatinase</td>
</tr>
<tr>
<td>NO</td>
<td>NOSs (NOS-1, NOS-2, NOS-3), heme-associated globins (e.g., deoxyhemoglobin, myoglobin, various molybdenum metalloenzymes (e.g., sulfate oxidase, mARC proteins))</td>
</tr>
<tr>
<td>Guanidinoacetate</td>
<td>Arginine:glycine amidinotransferase</td>
</tr>
<tr>
<td>Guanidinocetate</td>
<td>Arginine:glycine amidinotransferase</td>
</tr>
<tr>
<td>Agmatine</td>
<td>Arginine decarboxylase</td>
</tr>
<tr>
<td>Proline</td>
<td>Pyrroline-5-carboxylate reductase</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Pyrroline-5-carboxylate dehydrogenase, glutaminase, amino acid transaminases</td>
</tr>
<tr>
<td>Putrescine</td>
<td>Ornithine decarboxylase, agmatinase</td>
</tr>
<tr>
<td>GABA</td>
<td>Glutamate decarboxylase, aldehyde dehydrogenase, &quot;GABA deacetylating enzyme&quot;</td>
</tr>
</tbody>
</table>

1 GABA, γ-aminobutyric acid; mARC, mitochondrial amidoxime reducing component; NOS, NO synthase.
2 Enzymes that can generate the indicated metabolites independently of arginine metabolism.
studies that used cultured endothelial cells have provided strong evidence for a role of ADMA in inhibiting NO-dependent function (43), it is not entirely clear that endogenous ADMA or NMMA exert significant physiologic or pathophysiologic effects as competitive inhibitors of NOS in vivo because their plasma concentrations are low, particularly relative to concentrations of arginine. However, it should be recognized that plasma ADMA concentrations do not necessarily reflect intracellular or tissue concentrations [e.g. (44, 45)]. Because intracellular arginine can be present in distinct intracellular pools (discussed below), the arginine-to-ADMA ratio in specific intracellular pools or compartments is likely to be an important determinant of cellular response to ADMA.

Clearance of NMMA and ADMA occurs via renal excretion and also via metabolism by dimethylarginine dimethylaminohydrolase (DDAH) to form monomethylamine plus citrulline or dimethylamine plus citrulline, respectively (46). ADMA is metabolized also to a minor degree by the mitochondrial enzyme alanine-glyoxylate aminotransferase 2 (AGXT2) to form α-keto-δ-(N⁵,N⁵-dimethylguanidino)valeric acid plus glycine (47). Although 2 DDAH isozymes have been identified, studies in cultured cells and in mice have indicated that only the DDAH1 isozyme metabolizes ADMA (48, 49). SDMA is eliminated primarily via renal excretion, but it can be metabolized to a variable but limited extent by AGXT2 to form symmetric α-keto-δ-(dimethylguanidino)valeric acid plus glycine and also acetylated to form symmetric N⁶-acetyldimethylarginine (50).

**De Novo Synthesis of Arginine**

As indicated in Figure 1, free arginine in the body is provided by the diet, de novo synthesis, and turnover of proteins. The magnitude of de novo synthesis is sufficiently great that arginine is not considered to be an essential dietary amino acid for healthy adult humans. However, de novo synthesis is not sufficient to meet metabolic needs during early development, during infection or inflammation, or under conditions in which renal and/or intestinal metabolic functions are impaired; thus, arginine is classified as a semi-essential or conditionally essential amino acid with regard to dietary requirement. With regard to experimental studies in animal models, it is important to note that the capacity for de novo arginine synthesis can vary among mouse strains, resulting in significant differences in the amount of dietary arginine required for growth (51). However, the arginine content in standard feed pellet diets was sufficiently high to mask the
differences in arginine requirement in the mouse strains used in this study (51).

At the whole-body level, arginine in adults is synthesized by collaboration between the small intestine and the kidney (the “intestinal-renal axis” of arginine synthesis) (6). In adults, enterocytes of the small intestine express carbamyl phosphate synthetase I and ornithine transcarbamylase, thus providing the capacity to produce citrulline from glutamine, proline, or from ornithine produced from arginine by arginase. Citrulline, produced primarily by the small intestine, is taken up by proximal tubules of the kidney and efficiently converted to arginine by the sequential action of argininosuccinate synthetase and argininosuccinate lyase (ASL). Although renal metabolism represents the major fraction of citrulline-to-arginine conversion in the body, up to 40% of citrulline conversion to arginine occurs in extrarenal tissue (52).

Although it has generally been considered that glutamine is the predominant precursor for endogenous arginine synthesis, experiments to quantify the contributions of various precursors to arginine have yielded varying results, thus engendering controversy in this field [e.g., (53–57)]. Whereas some studies in adult humans reported that ~50% of the carbon for arginine synthesis is derived from enteral glutamine (58) and that proline provides ~40% of the carbon for arginine synthesis (59), other reports have noted higher contributions of glutamine to endogenous arginine synthesis in humans (60). In mice, the principal precursors for citrulline synthesis are arginine and ornithine (61). In at least some cases, differences in results arise from differences in metabolic fates of the labeled atom in the precursors used (i.e., 15N-labeled compared with 13C-labeled or 2H-labeled precursors) (54, 58, 61). Other differences may reflect differences in the species studied, developmental stage, health of the subjects in human studies, or route of administration of the labeled precursor (enteral compared with parenteral) [recently reviewed in (62)].

Intracellular Arginine Pools and the Citrulline-NO Cycle

Intracellular arginine can be derived from 3 possible sources: uptake of extracellular arginine, de novo synthesis, and arginine released by degradation of cellular proteins. However, it should not be assumed that all cell types are capable of de novo arginine synthesis. Multiple arginine pools can exist within cells, depending on how rapidly arginine exchanges between different cellular compartments (e.g., mitochondria, cytosol, lysosomes). Different intracellular pools can apparently exist even within the cytosol compartment. One of the earliest demonstrations of this compartmentation was a study that used permeabilized hepatocytes, in which it was shown that metabolites within the urea cycle are tightly channeled from one reaction to the next (63); thus, synthesis of arginine within the urea cycle is tightly coupled to its hydrolysis by arginase that it does not exhibit any significant exchange with free arginine within the cytosol. Most subsequent investigations on intracellular arginine pools have focused on the availability of arginine for NO synthesis. Consistent with the earlier study that showed tight channeling of arginine within the urea cycle, it was shown that arginine synthesized within the urea cycle did not provide substrate for hepatic NO synthesis, whereas a distinct intracellular arginine pool and exogenously provided arginine could support hepatic NO synthesis (64).

Endothelial cells have multiple intracellular arginine pools available for NO synthesis by endothelial NO synthase (eNOS); arginine derived from turnover of intracellular protein, arginine transported into the cell, and arginine synthesized endogenously from citrulline via the successive action of argininosuccinate synthetase and ASL in the citrulline-NO cycle (65–68). However, different NOS isoforms expressed within the same cell may not rely equally on the same intracellular arginine pool. In endothelial cells stimulated to also express inducible NO synthase (iNOS), arginine transported into the cells was the preferred substrate for iNOS, whereas endogenously synthesized arginine was the preferred substrate for eNOS (69). As reviewed elsewhere (70–72), the expression of the transporters involved in cellular arginine uptake varies according to cell type and also is subject to regulation. A role for ASL in endothelial NO synthesis that is independent of its catalytic function will be discussed in more detail below.

Sources of arginine for NO synthesis have also been evaluated in other cell types. In human neuronal cells, arginine transported into cells was the preferred substrate for neuronal NO synthase (nNOS), although some arginine was available from protein degradation (73). Interestingly, citrulline to arginine conversion supported NO synthesis in one neuronal cell line tested but not in another (73). In activated murine macrophages during mycobacterial infection, arginine synthesis from citrulline is required to sustain NO synthesis when extracellular arginine becomes depleted (74). In infected macrophages that express both arginase and iNOS, arginine transported into the cell was the preferred substrate for arginase, whereas arginine synthesized from citrulline was the preferred substrate for iNOS (75). The latter observation may be explained by the “nonclassical” action of ASL described below.

“Nonclassical” Actions of Arginine

Metabolic Enzymes

In addition to hyperammonemia due to impairment of the urea cycle, hypertension has been reported in individuals with ASL deficiency (76, 77). This was not unexpected due to the essential role of ASL in the conversion of citrulline to arginine, which can provide substrate to eNOS in the citrulline-NO cycle, as noted above. Surprisingly, however, it was discovered that the role of ASL in supporting NO synthesis goes beyond its catalytic activity. ASL has a structural role independent of its enzymatic activity; it is directly associated with eNOS, nNOS, and iNOS in endothelial, neuronal, and macrophage cells, respectively, and this association is independent of its enzymatic activity (78). It was already appreciated that the activity of eNOS is regulated by interactions with a variety of proteins (79), but these studies showed that ASL is required for the assembly of multiprotein complexes containing NO-S; in the absence of complex formation, NO production is greatly reduced.

Arginase II also has been found to have regulatory properties independent of its enzymatic activity that are important in arterogenesis. Both wild-type and enzymatically inactive arginase II had similar effects on mitochondrial dysfunction in cultured human vascular smooth muscle cells, thus promoting cellular senescence and apoptosis (80). In endothelial cells, overexpression of either wild-type or enzymatically inactive arginase II suppressed autophagy (81). Consistent with these results, silencing arginase II expression in senescent endothelial cells enhanced autophagy, but treatment of cells with the arginase inhibitor S-(2-boronoethyl)-L-cysteine (BEC) did not (81). Although both studies identified the signaling pathways involved in the responses to arginase II, additional experiments...
are needed to identify the cellular component or components that directly interact with arginase II to effect these responses.

**Homoarginine**

The enzyme AGAT catalyzes the first step in creatine biosynthesis, utilizing arginine plus glycine to produce guanidinoacetate plus ornithine (Table 1, Figure 2). However, it can also utilize lysine instead of glycine, thus producing homoarginine plus ornithine (82, 83) (Figure 2). It has been speculated that homoarginine also may be synthesized from lysine via the sequential action of ornithine transcarbamylase, argininosuccinate synthetase, and ASL (84), but there is no firm experimental support for this notion, in contrast to multiple lines of evidence supporting the role of AGAT in homoarginine synthesis. Plasma concentrations of homoarginine in healthy adults are low [1.4–2.5 μM (85–88)]. There had been relatively little interest in this metabolite until current reports that its plasma concentrations inversely correlated with the risk of cardiovascular disease and overall mortality (84, 89–91) and, conversely, that increased plasma concentrations were independently associated with reduced mortality in stroke patients (83). Consequently, there has been a considerable uptick in the number of publications on homoarginine, primarily involving the association of its plasma concentrations with various diseases [reviewed in (92–94)]. Because homoarginine is a weak substrate for NOS (95, 96) and arginase (97), it could also serve as an inhibitor of these enzymes. A weak correlation between plasma homoarginine and the plasma arginine-to-ornithine ratio suggests a possible role for homoarginine as an arginase inhibitor (86). At present, however, there is no solid evidence that the role of homoarginine in vivo involves direct interaction with NOS or arginase.

**Arginine Sensors**

Several cellular components have been shown to be capable of responding to changes in arginine concentration, including the protein kinase general control nonrepressible 2 (GCN2), which is activated in response to amino acid starvation; the master growth regulator mechanistic target of rapamycin complex 1 (mTORC1), which is activated in response to amino acid sufficiency as well as by other nutrients; and certain G-protein–coupled receptors (GPCRs) that are activated by arginine binding. It should be emphasized, however, that responses of these signaling pathways are not exclusive to arginine because they can respond to changes in concentrations of other amino acids as well as of a variety of other compounds (98). Amino acid deficiency results in increased levels of uncharged transfer RNAs that activate GCN2, which results in a global decrease in protein synthesis (99). Interestingly, the activation of GCN2 by stringent arginine deficiency can result in divergent and selective effects on the expression of specific proteins involved in arginine metabolism. For example, GCN2 activation results in increased phosphorylation of the eukaryotic initiation factor 2α (eIF2α), which can lead to preferential inhibition of translation of mRNAs encoding iNOS (100), although it also can preferentially enhance translation of other mRNAs, including that of the cationic amino acid transporter-1 (CAT-1) (101, 102).

Total amino acid starvation in cultured cells results in inactivation of mTORC1 and consequent reduction in phosphorylation of its substrate p70 ribosomal protein S6 kinase (99); the re-addition of specific amino acids results in the activation of mTORC1. Although leucine is the most potent amino acid activator of mTORC1 (99, 103), arginine is also an activator in rodent and porcine cells (104–107). However, the identity of the cellular factor or factors that directly sense arginine or other amino acids for activation of mTORC1 has been elusive. There is strong evidence, however, that the GPCR T1R1/T1R3 signals the availability of amino acids to mTORC1 (108). In more recent studies that tested responses to individual amino acids, arginine was shown to activate mTORC1 via T1R1/T1R3 in the murine pancreatic β cell line MIN6 (107). In earlier studies, cells expressing murine T1R1/T1R3 had little response to arginine unless inosine monophosphate was present (109), indicating that the metabolite milieu can be important in determining response. Regardless of signaling pathway, it is important to consider possible species differences in cellular responses to arginine. For example, human T1R1/T1R3 is less responsive to arginine than is murine T1R1/T1R3 (110). The most recently identified candidate for the amino acid sensor involved in mTORC1 activation is solute carrier 38A9 (SLC38A9), a lysosomal amino acid transporter (111, 112). With regard to arginine sensing, SLC38A9 must be expressed in order for mTORC1 to interact with multiple additional proteins to become activated in response to arginine (111). A sensor that exhibits a preference for basic amino acids, including arginine, is G-protein–coupled receptor, class C, group 6, subtype A (GPCRC6A) (113, 114). Because GPCRC6A is responsive to a variety of metabolites and hormones (115–118), studies to elucidate its physiologic role specifically with regard to arginine sensing are likely to be challenging. Investigations to confirm and extend identification of the proximal sensors of arginine and other amino acids are ongoing, and it is likely that additional candidates for amino acid sensing will be identified.

Because there has been a rapidly growing appreciation of the complex interplay between the host organism and the microbiome over the past several years, it is appropriate to briefly note how arginine sensing may be utilized by intracellular pathogens. An amino acid–sensing pathway, composed of the periplasmic arginine-binding protein Arf1 and the diguanylate cyclase Salmonella transmembrane 1987 (STM1987), that is highly specific for arginine has been identified recently in Salmonella typhimurium (119), a microbe that is a major cause of food poisoning. Activation of this pathway by arginine promotes synthesis of extracellular cellulose, a component of environmental and host-associated biofilms. Thus, the likely consequence would be to promote chronic virulence and bacterial persistence within infected macrophages, which would be detrimental to the host (120). Because this pathway can be activated at arginine concentrations <1 μM (119), it is likely that it could be activated even within infected macrophages that are expressing iNOS or arginase. This example suggests that, in addition to expressing arginine metabolic enzymes, other pathogens or commensals may use arginine-sensing mechanisms that facilitate their survival in the host.

**Concluding Remarks**

Although there have been considerable advances in our understanding of the roles for and regulation of arginine metabolism in health and disease, it should be apparent from the brief discussion here that there is much more to be learned. In particular, elucidation of the dynamics of arginine flux and metabolism in vivo in humans remains an especially challenging area of investigation. In addition, a detailed knowledge of arginine metabolism in the brain is lacking. Investigations of intracellular arginine pools would be greatly enhanced if
there were methods for visualizing intracellular free arginine, analogous to methods for visualizing intracellular calcium. We anticipate that the development and application of new technologies will expand our knowledge and appreciation of the metabolism of this fascinating and versatile amino acid.

**Note added in proof:** Another arginine sensor has been identified since submission of this manuscript (121).

**Acknowledgments**

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