

# Arginine: beyond protein<sup>1–4</sup>

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## ABSTRACT

Arginine, a semiessential or conditionally essential amino acid in humans, is one of the most metabolically versatile amino acids and serves as a precursor for the synthesis of urea, nitric oxide, polyamines, proline, glutamate, creatine, and agmatine. Arginine is metabolized through a complex and highly regulated set of pathways that remain incompletely understood at both the whole-body and the cellular levels. Adding to the metabolic complexity is the fact that limited arginine availability can selectively affect the expression of specific genes, most of which are themselves involved in some aspect of arginine metabolism. This overview highlights selected aspects of arginine metabolism, including areas in which our knowledge remains fragmentary and incomplete. *Am J Clin Nutr* 2006;83(suppl):508S–12S.

**KEY WORDS** Arginase, nitric oxide, gene expression, metabolism, ornithine, citrulline

## INTRODUCTION

Interest in arginine metabolism is no longer restricted to biochemists and nutritionists, as evidenced by the many websites and articles in health and fitness magazines touting the benefits of arginine supplementation, usually in regard to enhanced sexual function but also in regard to improvements in immune function and overall health. Claims of beneficial effects (often extrapolated far beyond what can be concluded from published scientific studies) are usually based on the fact that arginine is the precursor for nitric oxide (NO), and it sometimes seems that this is virtually the only aspect of arginine metabolism of which many biomedical researchers are aware. However, this NO-centric view is gradually being replaced by a broader perspective as more investigators come to appreciate the complexity and importance of arginine metabolism. In addition to its role in the synthesis of proteins and NO, arginine is a precursor to at least 6 biologically important compounds in mammals (**Figure 1**) and thus is one of the most metabolically versatile amino acids. Although this versatility has provided fertile ground for investigators in many areas of biomedical research, it also poses considerable challenges for developing an integrated view of arginine metabolism *in vivo*.

Before proceeding further, a brief word about the title of this article is in order. It was assigned for this presentation by Vernon Young, but regrettably I did not have the opportunity to learn what he may have intended by it. I have therefore taken the liberty of interpreting it to mean something along the lines of “What do we know about the roles of arginine apart from its involvement in protein structure and function, and how well do we know it?”

Accordingly, this article will briefly review the status of our knowledge regarding selected aspects of arginine metabolism and highlight areas in which our knowledge remains fragmentary and incomplete. Owing to the multiplicity of enzymes involved in arginine metabolism and the many different combinations that are expressed in various cell types, the regulation of arginine metabolism—although extremely important—is a topic too vast to be discussed in this review. The reader is referred to previous reviews for extensive listings of references and for features of arginine metabolism that are not considered here (1–10).

## SOURCES OF ARGININE

The sources of free arginine within the body are dietary protein, endogenous synthesis, and turnover of body proteins (**Figure 1**). About 40% of dietary arginine is catabolized by the intestine before it can enter the circulation (8). During the fasting state, ≈85% of the arginine entering the circulation is derived from protein turnover, and the remainder originates from *de novo* synthesis (8). At the whole-body level, most *de novo* arginine synthesis occurs in a metabolic collaboration between the small intestine and kidney in what is known as the intestinal-renal axis of arginine synthesis (8, 9). The magnitude of endogenous synthesis is sufficiently great that arginine is not an essential dietary amino acid for healthy adults. However, endogenous arginine synthesis cannot fully meet the needs of infants and growing children or of adults under catabolic stress or with dysfunction of the small intestine or kidney; thus, arginine is classified as a semiessential or conditionally essential amino acid (5, 11).

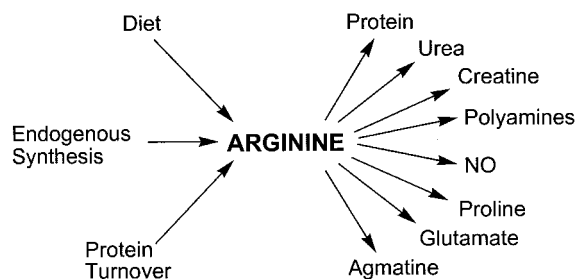
Using stable isotopes, Vernon Young and his colleagues showed that the rate of endogenous synthesis is essentially independent of arginine intake (12), which indicates that arginine homeostasis is achieved principally through regulation of arginine catabolism. This conclusion is supported by the observation that circulating concentrations of arginine are significantly elevated in mice in which expression of type II arginase is ablated (13).

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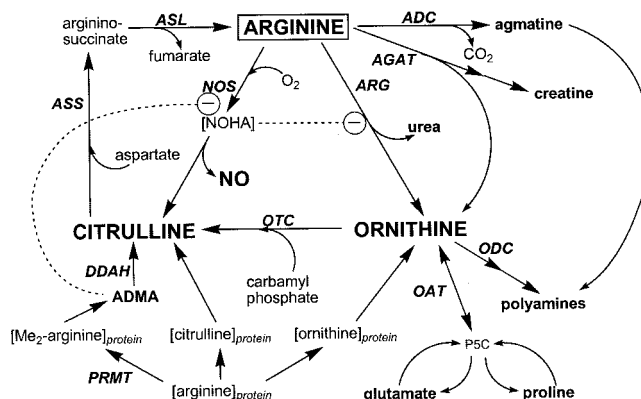
**FIGURE 1.** Sources and metabolic fates of arginine. Putrescine, spermine, and spermidine are the polyamines referred to in this figure. NO, nitric oxide.

De novo synthesis of arginine from citrulline occurs primarily in the proximal tubules of the kidney (8). However, not only is this capability found in many other cell types to varying degrees (14–16) but it also can be highly induced by cytokines and other agents, usually in conjunction with elevated expression of the inducible isoform of nitric oxide synthase (iNOS) (16–18). In such cases, a portion of the citrulline produced by NOS can be recycled to arginine in a pathway termed the citrulline-NO cycle (8, 17).

Although it is essential for a complete understanding of arginine metabolism, we know little of the preferred sources (ie, endogenous synthesis, protein turnover, or uptake from extracellular fluid) of arginine (or of the other substrates) used by the various arginine metabolic enzymes within any particular cell type. Good evidence exists that the arginine synthesized within the urea cycle is not available for hepatic NO synthesis (19) and that arginine imported from outside the cell appears to be the preferred substrate for NO synthesis by endothelial cells [a phenomenon known as the arginine paradox (20, 21)], but endogenously synthesized arginine may be more important for high-output NO synthesis by activated smooth muscle cells (22). Further complicating the picture is recent evidence that multiple intracellular arginine pools exist in endothelial cells but not in some other cell types (23).

**METABOLIC FATES OF ARGININE**

In contrast with the single enzyme that produces arginine, 4 enzymes use arginine as a substrate: arginine decarboxylase, arginine:glycine amidinotransferase, arginase, and NOS (Figure 2). (Although arginyl-tRNA synthetase also uses arginine as a substrate, it is not considered a metabolic enzyme and thus is not discussed here.) All the enzymes listed in Figure 2 have been cloned and at least partially characterized. Arginase and NOS are expressed as 2 or 3 isozymes, respectively, that are the products of distinct genes (3). The action of the 4 sets of enzymes ultimately results in production of the 7 low-molecular weight products depicted in Figure 1. In addition to arginine, ornithine and citrulline are emphasized in Figure 2 not only because they represent key branch points in arginine metabolism but also because they are commonly quantified in clinical analyses of biological fluids and thus can provide clues about disruptions of arginine metabolism in disease. The term *clues* is used advisedly because it should be apparent from the figure that it is not possible to conclusively infer changes in activity of any one of the enzymes listed here solely on the basis of changes in, eg, plasma concentrations of arginine, ornithine, or citrulline.



**FIGURE 2.** Overview of arginine metabolic pathways. Only enzymes that directly use or produce arginine, ornithine, or citrulline are indicated. For the sake of clarity, not all reactants and products are shown. Inhibition of nitric oxide synthase (NOS) and arginase by asymmetric dimethylarginine (ADMA) and N<sup>G</sup>-hydroxy-L-arginine (NOHA), respectively, are indicated by the dashed lines and the dash within a circle. Amino acid residues within proteins are indicated by brackets. ADC, arginine decarboxylase; AGAT, arginine:glycine amidinotransferase; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; DDAH, dimethylarginine dimethylaminohydrolase; Me<sub>2</sub>, dimethyl; NO, nitric oxide; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; P5C, L-Δ<sup>1</sup>-pyrroline-5-carboxylate; PRMT, protein-arginine methyltransferase. Note: P5C is in chemical equilibrium with L-glutamate-γ-semialdehyde via a spontaneous nonenzymatic reaction (not shown). Modified from Figure 1 of reference 24 with permission of the American Society for Nutritional Sciences.

The very complexity of Figure 2 may give the impression that we know more about arginine metabolism *in vivo* than is actually true. In fact, the figure is oversimplified because it does not indicate which enzymes are expressed as isozymes, patterns of tissue-specific expression, subcellular localization, the presence of various inter- and intracellular transport systems, which enzymes are regulated, or the flux of substrates. Because it is a composite of the reactions present in the body as a whole, Figure 2 should not be taken to represent arginine metabolism occurring within any single organ or cell type. For example, the cyclic conversion of ornithine to citrulline to arginine to ornithine represents the core reactions of the urea cycle that is highly expressed almost exclusively in periportal hepatocytes. However, the conversion of ornithine to citrulline to arginine also represents the pathway for net arginine synthesis that occurs primarily in the intestinal-renal axis noted previously. Thus, the tissue-specific expression of different subsets or isoforms of the arginine metabolic enzymes that results in a variety of distinct metabolic outcomes is not apparent from the figure alone.

Interactions among some of the enzymes and metabolites are known. Owing to their common use of arginine as a substrate, NOS and arginase can mutually limit substrate availability (8). Arginase-dependent limitation of substrate availability for NOS has been shown (25), but the converse has not. Instead, N<sup>G</sup>-hydroxy-L-arginine, the intermediate in NO synthesis, is also a potent arginase inhibitor (26), and during high-output NO synthesis it can accumulate sufficiently to significantly inhibit arginase (27). Finally, turnover of proteins containing methylated arginine residues releases asymmetric dimethylarginine (ADMA), which is a potent inhibitor of the NOS enzymes (28). Although these interactions can readily be shown with cultured

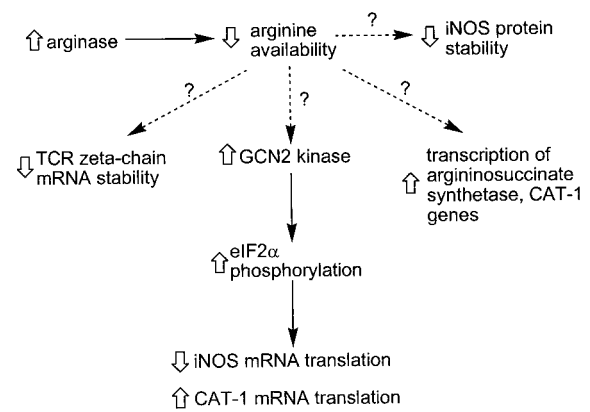
cell models, the evaluation of such effects *in vivo*, particularly at specific anatomic sites, poses considerable technical challenges.

Much of what is known regarding the dynamics of arginine metabolism *in vivo*—particularly in humans—comes from the elegant tracer studies carried out by Vernon Young and his colleagues. Because much of this information has been presented elsewhere (8), only a few points will be noted here. Rates of production of arginine, its precursors, and derivatives vary greatly. For example, the rates of urea and creatine synthesis in healthy fasted adults are, respectively,  $\approx 180$  and 8 times that of NO, which is  $1 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{kg body wt}^{-1}$  (8). Rates of arginine metabolism among the various pathways vary significantly between infants and adults (8) and also during injury and disease (29–32). No comparable data are available regarding rates of agmatine and polyamine synthesis.

The branch of mammalian arginine metabolism about which we know the least is that involving agmatine, the decarboxylation product of arginine. Although its properties as a cell-signaling agent have been studied by pharmacologists for some time (33), the proposed physiologic roles of agmatine or its derivatives in, eg, regulation of NO or polyamine synthesis (6, 34–36) have yet to be firmly established. Cloning of arginine decarboxylase was recently reported (37), but controversy exists as to whether it is enzymatically active in mammalian tissues (38), despite reports to the contrary (39, 40). Agmatinase, the enzyme that converts agmatine to putrescine, has also been cloned (41, 42), but sequence analysis suggests that it may not be functional in all animals, including mice (43). Taken together, arginine decarboxylase and agmatinase constitute a route for synthesis of polyamines that is an alternative to the ornithine decarboxylase-dependent pathway, but virtually no data exist regarding the quantitative significance of this alternative pathway.

Because agmatine is a product of microbial metabolism, it also is not clear how much of the agmatine found in tissues (44) is the product of mammalian cells or of enteric bacteria. Agmatine can be found in some foods, particularly those that are products of fermentation, such as cheeses and some spirits (45, 46), but it is unlikely that the small amounts found in such foods contribute significantly to the total agmatine present in the body.

Although this article will not discuss the role of arginine and its posttranslational modifications in the function of proteins, it should be appreciated that these modifications include not only methylation of arginine residues (47) but also conversion to citrulline (48) or ornithine (49) (Figure 2). The modified arginine residues are released as free amino acids during protein turnover, and elevated concentrations of at least one of them (ADMA) represent a risk factor for cardiovascular disease (50), probably through its inhibition of the NOS enzymes (27), as indicated in Figure 2. Because homocysteine can inhibit the activity of dimethylarginine dimethylaminohydrolase (51), the enzyme that degrades ADMA (52), a complex interplay exists between metabolism of the sulfur-containing amino acids and arginine metabolism. At the whole-body level, the fraction of free citrulline and ornithine derived from protein turnover is probably quite small. However, because recent studies indicate that multiple intracellular pools of arginine can exist in at least some cell types (23), arginine derived from protein turnover may therefore represent a not insignificant fraction of at least one of these pools within specific cells. Investigators have only recently become



**FIGURE 3.** Effects of limiting arginine availability on gene expression. Block arrows signify increases or decreases in the indicated features. Pathways that have not yet been characterized are indicated by dotted lines and question marks. CAT-1, cationic amino acid transporter 1; eIF2 $\alpha$ , eukaryotic initiation factor 2 $\alpha$ ; GCN2, general control of nutrition 2; iNOS, inducible nitric oxide synthase; mRNA, messenger RNA; TCR, T cell receptor.

aware that protein turnover can release various arginine derivatives; thus, the potential effect of this process on arginine metabolism remains to be evaluated.

### REGULATION OF GENE EXPRESSION BY ARGININE

Reduced arginine availability, as can occur during catabolic stress, can preferentially alter the expression of specific proteins beyond what might be anticipated merely as a consequence of changes in rates of global protein synthesis. Not surprisingly, most of the affected proteins are themselves involved in some aspect of arginine metabolism. In keeping with current nomenclature trends, we may consider the investigation of the selective effects of arginine on gene expression to constitute the field of “argenomics,” a subset of the effects of nutrients on gene expression (ie, nutrigenomics). Argenomics can be considered to have originated in the early 1960s when Robert Schimke showed that activities of argininosuccinate synthetase and argininosuccinate lyase in several mammalian cell lines are repressed by arginine and increase when arginine is replaced by citrulline (53, 54). The mechanisms underlying such changes were not known at the time but were later shown to involve regulation at both the transcriptional and the posttranscriptional level of the argininosuccinate synthetase gene (55, 56). However, the precise mechanisms whereby the changes in transcription occur still remain to be elucidated some 40 y later.

More recently, the expression of several other genes was also shown to be preferentially affected by reductions in arginine availability, which can occur as the result of increased arginase activity. These include iNOS, cationic amino acid transporter 1 (CAT-1), and the zeta-chain of the T cell receptor. Intriguingly, the effects of arginine deprivation are not identical for these genes nor are the mechanisms that are involved. Arginine deprivation results in decreased expression of iNOS (57, 58) and the T cell receptor zeta-chain (59, 60) but results in increased expression of CAT-1 (61–64). These changes occur via reduced translational efficiency of iNOS messenger RNA (mRNA) (57), decreased stability of iNOS protein (58), decreased half-life of the mRNA encoding the T cell receptor zeta-chain (59), and increases both in translational efficiency of CAT-1 mRNA (64)



and in transcription of the CAT-1 gene (62) (Figure 3). The altered translational efficiencies of iNOS and CAT-1 mRNAs are a consequence of increased activity of GCN2 kinase, resulting in phosphorylation (and decreased activity) of eukaryotic initiation factor 2 $\alpha$  (57), but with opposite outcomes. In the case of iNOS mRNA, translation initiation can be entirely ablated under these conditions, but in the case of CAT-1 mRNA, remodeling of the structure of an mRNA leader sequence and increased translation initiation at an internal ribosome entry site result in increased CAT-1 expression. Some of the regulatory effects of arginine deprivation also can be cell-type-specific. For example, arginine deprivation results in reduced iNOS protein stability in macrophages (58) but not in astrocytes (57). Moreover, unpublished studies in the author's laboratory indicate that arginine deprivation also can reduce iNOS expression at a pretranslational level in some cell types. Because the molecules that sense changes in arginine concentration and the signal transduction pathways that link the sensors to the end effects are not known, their identification represents a challenging aspect of ongoing research in arginine metabolism.

**CONCLUDING REMARKS**

It is clear that our knowledge regarding the identities of the arginine metabolites and the enzymes that use or produce arginine has advanced greatly in recent years, and separate chapters could easily be written (or already have been, in some cases) for each of the enzymes and metabolites described here. At the same time, it also is apparent that much remains to be done, including expansion of our knowledge regarding the dynamic state of arginine metabolism in vivo, especially within local anatomic sites, and characterization of the roles and regulation of the many arginine metabolic enzymes and transport systems in health and disease. This is an encouraging state of affairs not only for those of us who already have succumbed to fascination with the metabolic intricacies of this remarkable amino acid but also for those who are seeking new challenges.



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