

R Is for Arginine: Metabolism of Arginine Takes off Again, in New Directions Thomas Michel

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R Is for Arginine Metabolism of Arginine Takes off Again, in New Directions

Thomas Michel, MD, PhD

First published in 1962, Ray Bradbury's short story collection *R is for Rocket* provided a view of space travel with a prescience and sophistication that transformed the simpler notions of the time. In a simpler era dating back only to the mid-1980s, L-arginine (abbreviated R in the single-letter amino acid code) seemed to be just another amino acid, notable principally for its guanido group, which conferred arginine with a high pK_a and provided its distinction as 1 of the 3 basic amino acids. Abundant in many types of food, synthesized from simple precursors, or recycled in different tissues in the body, arginine is classified as a nonessential amino acid; except under extreme conditions, nutritional states of arginine deficiency are rare. Yet how much more complicated and interesting has arginine become in the past 3 decades! The role of arginine as a humdrum amino acid was forever transformed by the discovery that this amino acid is a substrate for the formation of nitric oxide by all mammalian nitric oxide synthases.^{1,2} The metabolic transformations of arginine and their roles in cardiovascular (patho)physiology have since been explored in many hundreds of articles.²⁻⁷ The current issue of *Circulation* reports a new and intriguing aspect of arginine metabolism involving the compound homoarginine,⁸ with even broader implications for our understanding of the roles of metabolic systems in vascular biology and disease.

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Homoarginine looks a lot like arginine except that it has an extra carbon (methylene group) in its side chain (Table 1). Because of the involvement of other arginine and arginine metabolites in vascular homeostasis,²⁻⁷ this structural similarity has recently inspired numerous clinical studies exploring the relationship between plasma homoarginine levels and human disease states.⁸⁻¹² Unlike the methylated arginine metabolites, which are competitive inhibitors of nitric oxide (NO) synthases (NOS),⁶ homoarginine can serve as an NOS substrate (albeit not a very good one).^{13,14} Because NO is generally viewed as a biologically salutary molecule, it seemed like a plausible hypothesis that low plasma homoarginine levels would be associated with

cardiovascular disease states and that higher homoarginine levels might be good for you. This hypothesis appears to be largely correct; however, in 1 clinical study with somewhat contrary findings,¹⁰ low plasma homoarginine levels have been found to be associated with increased morbidity or mortality in patients with coronary artery disease, cerebrovascular disease, diabetes mellitus, chronic kidney disease, and other diseases.^{8,11,12} What is less clear is the molecular mechanism for these associations.

The study reported by Choe et al⁸ provides important new clues about homoarginine while raising interesting new questions. Consistent with previous papers reporting an association between higher plasma homoarginine levels and reduced cardiovascular morbidity or mortality, Choe et al show that higher homoarginine was independently associated with a reduction in mortality in stroke patients. The authors speculate that their data "suggest that homoarginine may positively influence the beneficial pathways of NO,"⁸ although no direct measurements of the activity of the NO pathway were reported in this study. This still seems like a plausible hypothesis, yet a direct effect of homoarginine on endothelial NOS catalysis may be less likely; homoarginine appears to be a relatively poor NOS substrate in vivo¹³ and in vitro¹⁴ and may even lead to NOS uncoupling.¹⁴ But the Cho et al article goes well beyond prior clinical reports on homoarginine by performing genome-wide association studies analyzing single-nucleotide polymorphisms in stroke patients stratified by plasma homoarginine levels. These analyses led to an intriguing and novel discovery that low plasma homoarginine levels are significantly associated with polymorphisms in the gene encoding arginine:glycine amidinotransferase (AGAT; Figure). AGAT encodes an enzyme known to be expressed in the kidney and is mostly studied for its role in creatine metabolism, but more recently, AGAT was shown to be involved in the synthesis of homoarginine.¹⁵ Another significant genome-wide association study finding in the Choe et al study⁸ was in the ornithine transcarbamoylase gene, but this was a weaker association and was not followed up in detail. It is interesting to note that polymorphisms in ornithine transcarbamoylase (a key enzyme in the urea cycle) have previously been implicated in genome-wide association studies of hypertension and stroke.¹⁶ Focusing in detail on the AGAT polymorphisms that they had discovered, the authors⁸ found that most of these polymorphisms were located in intronic regions of the AGAT gene, documenting just 1 missense mutation (exchange of a glutamine residue for histidine). The effect of this amino acid change on AGAT enzyme activity was not explored in this study, nor were levels of AGAT protein measured. Yet the authors still provide several clear and compelling lines

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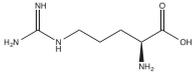
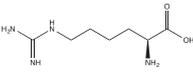
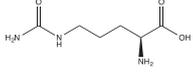
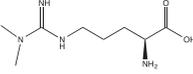
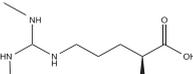
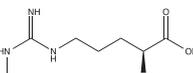
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Table 1. Properties of selected arginine metabolites

Compound	Structure	eNOS K_m or (IC_{50})	Plasma/intracellular levels (μM)	Source or synthetic pathway	Major catabolic pathways	Relation of plasma levels to CV disease
L-Arginine ^{1,2,5,13,14}		$\sim 3 \mu M$	$\sim 60\text{-}100 \gg 200 \mu M$	Proteolysis > diet, recycling via ASS/ASL, de novo synthesis	NOS, Arginases, AGAT	No clear relation. Acute infusion improves vascular function, but long-term supplementation may be harmful.
L-Homoarginine ⁸⁻¹⁴		$\sim 70 \mu M$	$\sim 1 \mu M$ / ?	Diet, recycling, OTC/ASS/ASL or via AGAT	NOS, Arginases?, others?	\downarrow levels seen in stroke, CV disease; \uparrow levels are protective. Supplements \downarrow stroke in mice.
L-Citrulline ^{1,2}		Not a NOS inhibitor/substrate	$\sim 20\text{-}100$ / $\sim 20\text{-}30 \mu M$	NOS product, product of DDAHs, diet	"citrulline cycle" via ASS/ASL	No clear relation to CV disease.
ADMA ²⁻⁷		($\sim 1 \mu M$)	$0.4\text{-}4 \mu M$	PRMTs \rightarrow proteolysis	DDAH-1 and DDAH-2, arginases	\uparrow levels seen in CV disease
SMA ²⁻⁶		Not a NOS inhibitor/substrate	$\sim 1 \mu M$ / ?	PRMTs \rightarrow proteolysis	Renal excretion	No clear relation to CV disease.
NMMA ¹⁻⁶		($\sim 1 \mu M$)	$\sim 0.4\text{-}3 \mu M$	PRMTs \rightarrow proteolysis	DDAH-1 and DDAH-2, arginases	\uparrow levels seen in CV disease

This table summarizes basic features of several major arginine metabolites relevant to cardiovascular (CV) disease states. The first column lists principal arginine metabolites and provides key references (mostly review articles) that support the information provided in this table and in the text; because of space limitations, the numerous relevant original research articles cannot be listed here. The structure of L-arginine (second column) differs from L-homoarginine by the additional methylene group present in the homoarginine side chain. The third column shows the concentration of the compound that yields half-maximal enzyme activity (K_m) for active substrates or (in parentheses) the concentration that yields half-maximal suppression of enzyme activity for inhibitor compounds (IC_{50}). The K_m has been extensively validated for L-arginine in studies of the purified enzyme. In contrast, the endothelial nitric oxide synthase (eNOS) K_m value for L-homoarginine is based either on analyses of crude cell lysates or on studies of other nitric oxide synthase (NOS) isoforms. The concentrations of NOS inhibitors yielding IC_{50} are determined mostly in studies in which asymmetrically *N*^ω-dimethylated arginine (ADMA) or monomethylated arginine (NMMA) is added to cultured endothelial cells and eNOS activity is estimated, and the IC_{50} values for inhibitors depend on the extracellular arginine concentration. ADMA and NMMA appear to be competitive inhibitors of eNOS, whereas symmetrically dimethylated arginine (SMA) serves as neither a substrate nor an inhibitor of NOS isoforms. Plasma levels for these compounds have been reported in many studies, but the intracellular concentrations have been less extensively characterized. The relative roles of different synthetic and catabolic pathways are best known for arginine and less so for the other arginine metabolites. The text and Figure provide further discussion.

AGAT indicates arginine:glycine amidinotransferase; ASL, arginosuccinate lyase; ASS, arginosuccinate synthase; DDAH, dimethyl diaminoarginine hydrolase; OTC, ornithine transcarbamoylase; and PRMT, protein arginine methyltransferases.

of evidence suggesting that AGAT has a key role in homoarginine metabolism. They showed that overexpression of AGAT increased homoarginine levels in a cell model and analyzed AGAT^{null} knockout mice. They found that AGAT^{null} knockout mice had low plasma homoarginine levels and showed that these mice had increased morbidity in an experimental stroke model that could be ameliorated by homoarginine feeding. Conversely, in a distinct knockout mouse model in which the gene for a different arginine-metabolizing enzyme called guanidinoacetate methyltransferase (GAMT) was deleted, the mice had higher homoarginine levels and smaller experimental strokes. The authors conclude, "These results provide evidence that higher homoarginine levels attenuate stroke severity and improve outcome in mice and suggest that homoarginine is causally involved in the (patho)physiology of ischemic stroke." This seems like a reasonable inference, yet the molecular mechanisms remain incompletely defined. Moreover, this study did not show that plasma homoarginine levels improved risk

prediction over conventional risk factors. So before we start crop-dusting the population with homoarginine, let us take a closer look at how homoarginine fits into a complicated network that involves not only AGAT and GAMT but also many other complexly modulated enzymes and pathways that regulate arginine and its metabolites.

Let us start with arginine, which has been the most extensively studied of these compounds. The Figure presents a simplified (but still complicated) scheme showing many of the intersecting cycles of arginine metabolism that appear to be most clearly related to cardiovascular (patho)physiology. Table 1 gives the molecular structures and the references supporting the summary presented below. L-Arginine levels in the plasma are in the $\approx 100\text{-}\mu\text{mol/L}$ range, and intracellular arginine levels appear to be even higher. The NOS half-maximal enzyme activity (K_m) for L-arginine is much lower than its overall intracellular concentration, but it appears that arginine and arginine-metabolizing enzymes may have differential subcellular distributions, raising the possibility that arginine

substrate, the direct consequences of homoarginine-dependent arginase inhibition on NOS activity are less obvious. Clinical studies have reported that long-term arginine supplementation may have deleterious effects, raising caveats about the possible long-term consequences of homoarginine supplementation despite the clearly beneficial effects of short-term homoarginine supplementation in mouse stroke models, as reported by Choe et al.⁸

The AGAT^{null} and GAMT^{null} mouse knockout models that were carefully studied by Choe et al provide additional intriguing clues to possible mechanisms whereby homoarginine may modulate vascular (patho)physiology. Homoarginine can be formed from L-arginine and the amino acid L-lysine by the actions of our now-familiar AGAT enzyme, as shown in the Figure. AGAT is a mitochondrial enzyme that is expressed most abundantly in the kidney and is critically involved in the synthesis of creatine, a vital metabolite in energy metabolism. Marked decreases in both homoarginine and creatine were documented in plasma from AGAT^{null} mice, associated with significant increases in the size of experimental stroke.⁸ Yet previous studies of AGAT^{null} mice documented multiple other metabolic changes in these knockout mice.¹⁸ The metabolic phenotype of AGAT^{null} mice is quite complex, and the link between homoarginine and stroke needs to be viewed in the context of numerous biochemical derangements in this knockout mouse model. However, the fact that homoarginine but not creatine supplementation improved the stroke phenotype provides strong suggestive evidence for a central role for homoarginine in attenuating the severity of stroke in this mouse model. But despite the intriguing associations between homoarginine levels and stroke protection in these informative mouse models, the links between AGAT polymorphisms, homoarginine, and vascular disease in humans likely reflect a greater complexity.

In humans, AGAT expression appears to be highest in kidney and liver. Yet AGAT is also expressed in the myocardium, where levels of AGAT appear to be dynamically regulated in heart failure.¹⁹ Although arginine is clearly required for AGAT catalysis, the enzyme is somewhat promiscuous in its choice of cosubstrates: AGAT generates homoarginine when lysine is used as the cosubstrate or instead promotes creatine synthesis when glycine is used (Figure). This enzymatic promiscuity may also depend on the specific tissue in which AGAT is expressed and the relative availability of lysine versus glycine for catalysis. Because creatine synthesis can lead to the generation of the proatherogenic metabolite homocysteine,²⁰ subtle changes in the expression and activity of AGAT may have a marked effect on vascular pathobiology. Perhaps increased levels of plasma homoarginine are associated with decreased levels of homocysteine, reflecting a change in the balance of AGAT activity between using lysine as a cosubstrate (leading to homoarginine synthesis) instead of glycine (yielding creatine and homocysteine). Might lysine be the link? We do not know, but it is important to note that all but one of the AGAT polymorphisms identified in the Choe et al study are located in intronic regions, which might possibly lead to differences in AGAT expression or tissue distribution.

Clearly, more remains to be learned about the molecular mechanisms whereby differences in plasma homoarginine

levels modulate stroke severity. It should now be clear that arginine metabolism cannot be viewed as a single pathway or even as the confluence of multiple pathways. Rather, arginine metabolism must be viewed as a biochemical system, allowing multiple points of connection and regulation within any particular cell type and differing patterns of tissue-specific enzyme expression and modulation.

One of the best-known short stories in Bradbury's *R is for Rocket* is called "A Sound of Thunder," which relates the tale of a time traveler who interferes with the thread of time by accidentally treading on a butterfly during his journey into the past. When the time traveler returns to the present after his mishap with the butterfly, he finds subtle but irrevocable changes in the world that he left behind to travel into the past. So it is for arginine metabolism: Subtle changes in levels of one metabolite may lead to profound changes in the levels or biological activities of distantly related metabolites. No matter what molecular mechanisms are ultimately identified in unraveling the association between homoarginine and disease, the Choe et al study serves as an exemplar of the need to view levels of specific metabolites as reflecting the products of interrelated and dynamic metabolic systems.

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Disclosures

None.

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