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 pKₐ 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,⁴ 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,²⁻⁵ this structural similarity has recently inspired numerous clinical studies exploring the relationship between plasma homoarginine levels and human disease states.⁶⁻¹² 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.¹⁵ 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 creatinine 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 of evidence to support an association between AGAT polymorphisms and plasma homoarginine levels. 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.¹⁵ 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 creatinine 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 of evidence to support an association between AGAT polymorphisms and plasma homoarginine levels.
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<sup>−/−</sup> knockout mice. They found that AGAT<sup>−/−</sup> 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. 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.
concentrations may be limiting for NOS in specific subcellular locales. Moreover, the presence of endogenous competitive inhibitors of NOS (discussed below) may raise the effective intracellular arginine concentration needed for full enzyme activity. In any event, the action of NOS on \( L \)-arginine yields NO plus \( L \)-citrulline, which can be recycled back to \( L \)-arginine by the sequential reactions catalyzed by argininosuccinate synthase and argininosuccinate lyase. But it appears that most of the plasma \( L \)-arginine comes either from the diet or from recycling/proteolysis of cellular proteins, with a smaller fraction synthesized from other amino acids or recycled from \( L \)-arginine (at least in most cell types). The traffic of extracellular \( L \)-arginine (and other cationic amino acids, including \( L \)-lysine) into cells occurs via specific cell surface amino acid transporters, of which CAT-1 and related transporters are the best characterized. Arginine may also be catabolized by \( L \)-AGAT, the enzyme recently implicated in homoarginine metabolism, as discussed above. However, in most cell types, arginine catabolism is catalyzed principally by a family of arginase enzymes, which yield ornithine and urea; the ornithine that is formed by arginase can by metabolized back to \( L \)-arginine in multiple enzymatic steps. Arginases are central to arginine flux in the cell because these enzymes may represent the quantitatively most important pathway for arginine catabolism, and arginase activity appears to be inhibited by homoarginine.

Let us now move on to the methylated arginine compounds, which have been definitively linked to cardiovascular disease and might represent another potential pathway whereby homoarginine may have a role. Monomethylated arginine (NMMA) and asymmetrically \( N^\omega \)-dimethylated arginine (ADMA) are potent NOS inhibitors, whereas symmetrically dimethylarginine is neither an NOS substrate or inhibitor. To form these arginine homologs, the terminal guanido nitrogen of arginine residues in proteins are methylated by a family of protein arginine methyltransferases. Methylarginines play a broad role in cellular regulation well beyond their effects on NOS. These compounds are implicated in transcriptional regulation and in the metabolism of DNA and RNA. As is the case for arginine, methylated arginine compounds are released from cellular proteins by proteolysis, possibly in the context of cellular autophagy or apoptosis; increased levels of ADMA and NMMA are clearly implicated in vascular pathology and in the inhibition of NOS activity. ADMA and NMMA can be catalyzed either by arginases or by a family of dimethyl diaminocitrulline enzymes, yielding citrulline, which can then be recycled back to arginine. It is possible that homoarginine might inhibit the actions of protein arginine methyltransferases, thereby reducing the levels of NMMA and ADMA and thus enhancing NOS activity. Arguing against this notion is the fact that plasma levels of ADMA and NMMA were unchanged in the AGATnull knockout mouse studied by Choe et al, although intracellular levels of these compounds were not measured.

Compared with the large body of clinical and biochemical data on the roles of arginine and methylated arginine compounds, much less is known about the metabolic transformations and biochemical roles of homoarginine (the Figure and Table 1). In mammals, homoarginine can come either from the diet or by synthesis from endogenous precursors; plasma homoarginine levels are \( \approx 100 \)-fold lower than those of \( L \)-arginine. Homoarginine can serve as an NOS substrate, yielding NO plus homocitrulline as coproducts. Yet the NOS K_m for homoarginine is \( \approx 20 \)-fold higher than for arginine. Moreover, homoarginine is not a very effective substrate for NOS, and it is not clear than higher homoarginine levels would necessarily lead directly to significant increases in NO synthesis. There are even some reports that homocitrulline, the enzymatic product of NOS action on homoarginine, might be associated with atherogenesis. Although homoarginine can serve as an arginase substrate with a low K_m, it is not a very good arginase substrate, and it is plausible that homoarginine could actually function as an arginase inhibitor and thereby lead to an increase in intracellular arginine. But because intracellular arginine levels are already well in excess of the NOS K_m for its
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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None.

**References**


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