The Journal of Nutrition
Supplement: 9th Workshop on the Assessment of Adequate and Safe Intake of Dietary Amino Acids

Arginine Metabolism Revisited[1,2]

Sidney M Morris Jr.*

Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

Mammalian arginine metabolism is complex due to the expression of multiple enzymes that utilize arginine as substrate and to interactions or competition between specific enzymes involved in arginine metabolism. Moreover, cells may contain multiple intracellular arginine pools that are not equally accessible to all arginine metabolic enzymes, thus presenting additional challenges to more fully understanding arginine metabolism. At the whole-body level, arginine metabolism ultimately results in the production of a biochemically diverse range of products, including nitric oxide, urea, creatine, polyamines, proline, glutamate, agmatine, and homoarginine. Included in this group of compounds are the methylated arginines (e.g., asymmetric dimethylarginine), which are released upon degradation of proteins containing methylated arginine residues. Changes in arginine concentration also can regulate cellular metabolism and function via a variety of arginine sensors. Although much is known about arginine metabolism, elucidation of the physiologic or pathophysiologic roles for all of the pathways and their metabolites remains an active area of investigation, as exemplified by current findings highlighted in this review. *J Nutr* 2016;146(Suppl):2579S–86S.

Keywords: ADMA, arginase, argininosuccinate, homoarginine, GPCR, mTORC1, nitric oxide

Introduction

There has been considerable interest in arginine metabolism for many years, beginning with early studies on nutrition and the urea cycle, followed by studies of de novo arginine synthesis and creatine biosynthesis, and then an explosion of studies occasioned by the discovery of the biological roles of NO in the 1980s. Although many genes and proteins involved in arginine metabolism have been isolated and characterized over the past several decades, work to identify and characterize the genes and proteins involved in arginine transport and metabolism, as well as variants involved in disease, continues to the present day. The existence of multiple isoforms for some of these enzymes, as well as the dynamic regulation of their cell- and tissue-specific expression, continues to present considerable challenges for understanding arginine nutrition and metabolism at the cellular, tissue, and whole-body levels.

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 isozymes), arginases (2 isozymes), 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).

[1] Published in a supplement to *The Journal of Nutrition*. The 9th Workshop on the Assessment of Adequate and Safe Intake of Dietary Amino Acids was presented at the "9th Amino Acid Assessment Workshop" held in Paris, France, 15–16 October 2015. The conference was sponsored by the International Council on Amino Acid Science (ICAAS). The Organizing Committee for the workshop included Sidney M Morris Jr., Dennis M Bier, Luc Cynober, Motoni Kadowaki, and Rajavel Elango. The Supplement Coordinator for this supplement was D'Ann Finley, University of California, Davis. Supplement Coordinator disclosures: D'Ann Finley received travel support and compensation from ICAAS for editorial services provided for this supplement publication. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *The Journal of Nutrition*.

[2] Author disclosures: The International Council on Amino Acid Science paid SM Morris Jr.'s travel expenses to attend the 9th Workshop on the Assessment of Adequate and Safe Intake of Dietary Amino Acids and an honorarium for the preparation of this manuscript.

*To whom correspondence should be addressed. E-mail: smorris@pitt.edu.

[3] Abbreviations used: ADMA, asymmetric dimethylarginine; AGAT, arginine:glycine amidinotransferase; AGXT2, alanine-glyoxylate aminotransferase 2; ASL, argininosuccinate lyase; CAT-1, cationic amino acid transporter-1; DDAH, dimethylarginine dimethylaminohydrolase; eIF2α, eukaryotic initiation factor 2α; eNOS, endothelial NO synthase; GABA, γ-aminobutyric acid; GCN2, general control nonderepressible 2; GPCR, G-protein–coupled receptor; GPRC6A, G-protein–coupled receptor, class C, group 6, subtype A; iNOS, inducible NO synthase; mTORC1, mechanistic target of rapamycin complex 1; NMMA, N^G-monomethyl-L-arginine; nNOS, neuronal NO synthase; NOS, NO synthase; SDMA, symmetric dimethylarginine; SLC38A9, solute carrier 38A9; STM1987, *Salmonella* transmembrane 1987.

Manuscript received November 2, 2015. Initial review completed January 22, 2016. Revision accepted February 5, 2016.
First published online November 9, 2016; doi:10.3945/jn.115.226621.

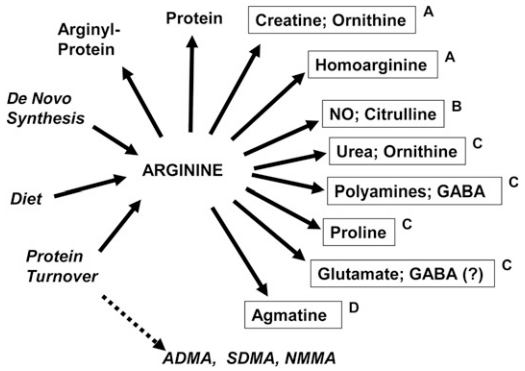

FIGURE 1 Outline of the metabolic origins and fates of arginine in mammals. The boxes indicate the arginine metabolites that are either immediately or ultimately generated as a consequence of the action of the following enzymes that use arginine as substrate: A, arginine: glycine amidinotransferase; B, NO synthases; C, arginases; D, arginine decarboxylase. Putrescine, spermine, and spermidine are the polyamines designated here. ADMA, asymmetric dimethylarginine; GABA, γ-aminobutyric acid; NMMA, N^G-monomethyl-L-arginine; SDMA, symmetric dimethylarginine. Adapted from reference 1 with permission.

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 (**Figure 2**), 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 (7–9) (via pathways initiated by either diamine oxidase or monoamine oxidase) 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) (Figure 2).

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 methyl transferases (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 N^G-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)]. Although

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

Metabolite	Produced by
Arginine	Argininosuccinate lyase
Ornithine	Arginase, ornithine aminotransferase, arginine:glycine amidinotransferase
Citrulline	Ornithine transcarbamylase, NOSs (NOS-1, NOS-2, NOS-3), dimethylarginine dimethylaminohydrolase
Urea	Arginases I and II, agmatinase
NO	NOSs (NOS-1, NOS-2, NOS-3), heme-associated globins (e.g., deoxyhemoglobin, myoglobin),[2] various molybdenum metalloenzymes (e.g., sulfite oxidase, mARC proteins)[2]
Creatine	Guanidinoacetate methyltransferase
Homoarginine	Arginine:glycine amidinotransferase
Guanidinoacetate	Arginine:glycine amidinotransferase
Agmatine	Arginine decarboxylase
Proline	Pyrroline-5-carboxylate reductase
Glutamate	Pyrroline-5-carboxylate dehydrogenase, glutaminase,[2] amino acid transaminases[2]
Putrescine	Ornithine decarboxylase, agmatinase
GABA	Glutamate decarboxylase, aldehyde dehydrogenase, ``GABA deacetylating enzyme´´

[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.

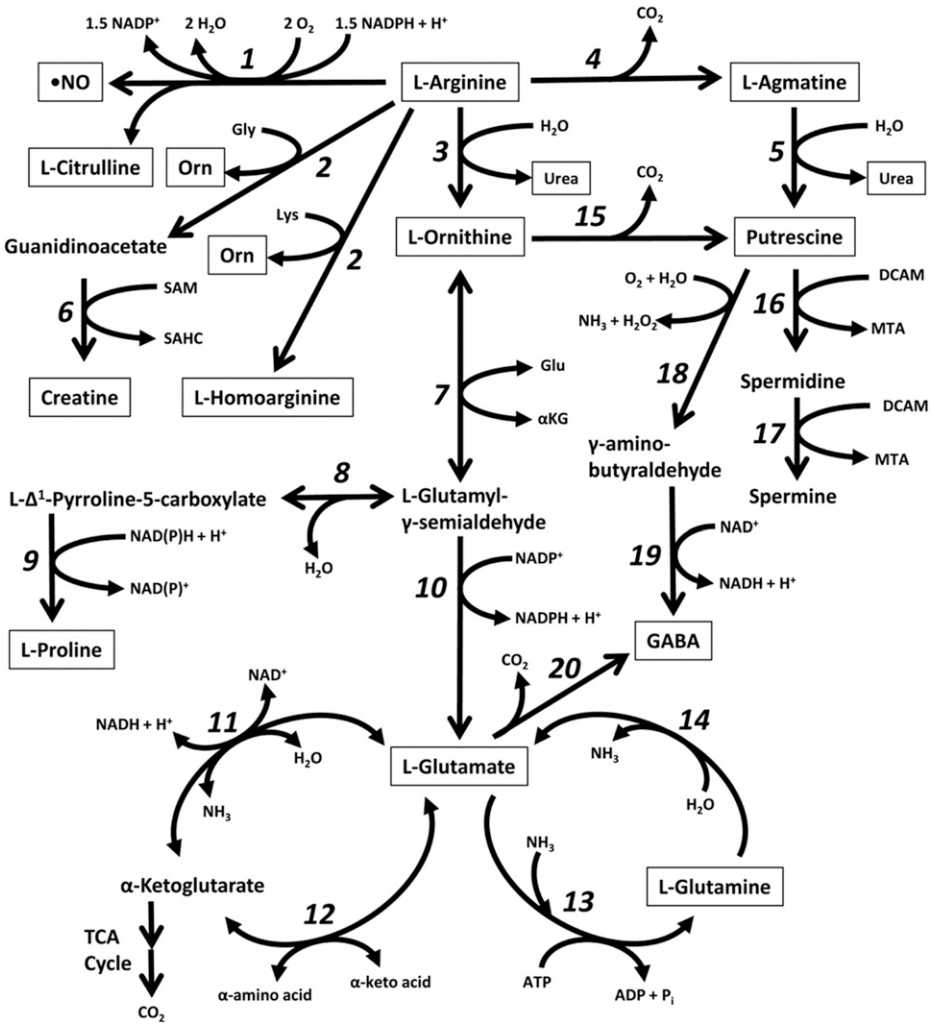

FIGURE 2 Pathways of arginine catabolism. Enzymes that catalyze the indicated reactions are as follows: 1, NO synthases; 2, arginine:glycine amidinotransferase; 3, arginase; 4, arginine decarboxylase; 5, agmatinase (agmatine ureohydrolase); 6, guanidinoacetate N-methyltransferase; 7, ornithine aminotransferase; 9, pyrroline-5-carboxylate reductase; 10, pyrroline-5-carboxylate dehydrogenase; 11, glutamate dehydrogenase; 12, alanine aminotransferase, aspartate aminotransferase, or branched-chain amino acid aminotransferase; 13, glutamine synthetase; 14, glutaminase; 15, ornithine decarboxylase; 16, spermidine synthase; 17, spermine synthase; 18, diamine oxidase; 19, aldehyde dehydrogenase; and 20, glutamate decarboxylase. Complete oxidation of arginine-derived α-ketoglutarate occurs via the citric acid cycle. Step 8 is a spontaneous, nonenzymatic reaction. DCAM, decarboxylated *S*-adenosylmethionine; Glu, L-glutamate; MTA, methylthioadenosine; SAHC, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; αKG, α-ketoglutarate. Adapted from reference 6 with permission.

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-(N^G,N^G-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 N_α-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. In addition to causing morbidity and mortality due to hyperammonemia, deficiencies in any of the first 4 enzymes of the urea cycle will impair capacity for endogenous arginine synthesis via the intestinal-renal axis; thus, arginine becomes an essential dietary amino acid for these individuals. 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 groups have reported 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., ^{15}N-labeled compared with ^{13}C-labeled or ^{2}H-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 permeablized 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 so 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 isozymes 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 NOS; 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 atherogenesis. 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 also can 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 nonderepressible 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 mRNA 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 ArtI 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

The sole author had responsibility for all parts of the manuscript.

References

1. Morris SM Jr. Arginine: beyond protein. Am J Clin Nutr 2006;83 (Suppl):508S–12S.
2. Boudko DY. Bioanalytical profile of the L-arginine/nitric oxide pathway and its evaluation by capillary electrophoresis. J Chromatogr B Analyt Technol Biomed Life Sci 2007;851:186–210.
3. van Eijk HM, Luiking YC, Deutz NE. Methods using stable isotopes to measure nitric oxide (NO) synthesis in the L-arginine/NO pathway in health and disease. J Chromatogr B Analyt Technol Biomed Life Sci 2007;851:172–85.
4. Wu G, Meininger CJ. Analysis of citrulline, arginine, and methylarginines using high-performance liquid chromatography. Methods Enzymol 2008;440:177–89.
5. Martens-Lobenhoffer J, Bode-Boger SM. Mass spectrometric quantification of L-arginine and its pathway related substances in biofluids: the road to maturity. J Chromatogr B Analyt Technol Biomed Life Sci 2014;964:89–102.
6. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J 1998;336:1–17.
7. Seiler N, Al-Therib MJ. Putrescine catabolism in mammalian brain. Biochem J 1974;144:29–35.
8. Yamasaki EN, Barbosa VD, De Mello FG, Hokoc JN. GABAergic system in the developing mammalian retina: dual sources of GABA at early stages of postnatal development. Int J Dev Neurosci 1999;17:201–13.
9. Kim JI, Ganesan S, Luo SX, Wu YW, Park E, Huang EJ, Chen L, Ding JB. Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons. Science 2015;350:102–6.
10. Mistry SK, Burwell TJ, Chambers RM, Rudolph-Owen L, Spaltmann F, Cook WJ, Morris SM Jr. Cloning of human agmatinase: an alternate path for polyamine synthesis induced in liver by hepatitis B virus. Am J Physiol Gastrointest Liver Physiol 2002;282:G375–81.
11. Jahan S, Davie JR. Protein arginine methyltransferases (PRMTs): role in chromatin organization. Adv Biol Regul 2015;57:173–84.
12. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. Mol Cell 2009;33:1–13.
13. Wei H, Mundade R, Lange KC, Lu T. Protein arginine methylation of non-histone proteins and its role in diseases. Cell Cycle 2014;13:32–41.
14. Hu RG, Brower CS, Wang H, Davydov IV, Sheng J, Zhou J, Kwon YT, Varshavsky A. Arginyltransferase, its specificity, putative substrates, bidirectional promoter, and splicing-derived isoforms. J Biol Chem 2006;281:32559–73.
15. Kashina AS. Protein arginylation: over 50 years of discovery. Methods Mol Biol 2015;1337:1–11.
16. Wang J, Han X, Wong CC, Cheng H, Aslanian A, Xu T, Leavis P, Roder H, Hedstrom L, Yates JR III, et al. Arginyltransferase ATE1 catalyzes midchain arginylation of proteins at side chain carboxylates in vivo. Chem Biol 2014;21:331–7.
17. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. BioEssays 2003;25:1106–18.
18. Sell DR, Monnier VM. Conversion of arginine into ornithine by advanced glycation in senescent human collagen and lens crystallins. J Biol Chem 2004;279:54173–84.
19. Morris SM Jr. Arginine metabolism: boundaries of our knowledge. J Nutr 2007;137(Suppl):1602S–9S.
20. Wu Z, Hou Y, Hu S, Bazer FW, Meininger CJ, McNeal CJ, Wu G. Catabolism and safety of supplemental L-arginine in animals. Amino Acids 2016;48:1541–52.
21. McNeal CJ, Meininger CJ, Reddy D, Wilborn CD, Wu G. Safety and effectiveness of arginine in adults. J Nutr 2016;146(Suppl):2587S–93S.
22. Rosenthal MD, Carrott PW, Patel J, Kiraly L, Martindale RG. Parenteral or enteral arginine supplementation safety and efficacy. J Nutr 2016;146(Suppl):2594S–600S.
23. Peranzoni E, Marigo I, Dolcetti L, Ugel S, Sonda N, Taschin E, Mantelli B, Bronte V, Zanovello P. Role of arginine metabolism in immunity and immunopathology. Immunobiology 2007;212:795–812.
24. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, Carey Satterfield M, Smith SB, Spencer TE, Yin Y. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 2009;37:153–68.
25. Morris SM Jr. Recent advances in arginine metabolism: roles and regulation of the arginases. Br J Pharmacol 2009;157:922–30.
26. Munder M. Arginase: an emerging key player in the mammalian immune system. Br J Pharmacol 2009;158:638–51.
27. Haines RJ, Pendleton LC, Eichler DC. Argininosuccinate synthase: at the center of arginine metabolism. Int J Biochem Mol Biol 2011;2:8–23.
28. Luiking YC, Ten Have GA, Wolfe RR, Deutz NE. Arginine de novo and nitric oxide production in disease states. Am J Physiol Endocrinol Metab 2012;303:E1177–89.
29. Bahri S, Zerrouk N, Aussel C, Moinard C, Crenn P, Curis E, Chaumeil JC, Cynober L, Sfar S. Citrulline: from metabolism to therapeutic use. Nutrition 2013;29:479–84.
30. Popolo A, Adesso S, Pinto A, Autore G, Marzocco S. L-Arginine and its metabolites in kidney and cardiovascular disease. Amino Acids 2014;46:2271–86.
31. Breuillard C, Cynober L, Moinard C. Citrulline and nitrogen homeostasis: an overview. Amino Acids 2015;47:685–91.
32. Feun LG, Kuo MT, Savaraj N. Arginine deprivation in cancer therapy. Curr Opin Clin Nutr Metab Care 2015;18:78–82.
33. Monné M, Miniero DV, Daddabbo L, Palmieri L, Porcelli V, Palmieri F. Mitochondrial transporters for ornithine and related amino acids: a review. Amino Acids 2015;47:1763–77.
34. Qiu F, Huang J, Sui M. Targeting arginine metabolism pathway to treat arginine-dependent cancers. Cancer Lett 2015;364:1–7.
35. Kayacelebi AA, Langen J, Weigt-Usinger K, Chobanyan-Jurgens K, Mariotti F, Schneider JY, Rothmann S, Frolich JC, Atzler D, Choe CU, et al. Biosynthesis of homoarginine (hArg) and asymmetric dimethylarginine (ADMA) from acutely and chronically administered free L-arginine in humans. Amino Acids 2015;47:1893–908.
36. Leiper J, Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. Cardiovasc Res 1999;43:542–8.
37. Blackwell S. The biochemistry, measurement and current clinical significance of asymmetric dimethylarginine. Ann Clin Biochem 2010;47:17–28.
38. Schwedhelm E, Boger RH. The role of asymmetric and symmetric dimethylarginines in renal disease. Nat Rev Nephrol 2011;7:275–85.
39. Raptis V, Kapoulas S, Grekas D. Role of asymmetrical dimethylarginine in the progression of renal disease. Nephrology (Carlton) 2013;18:11–21.
40. Brinkmann SJ, de Boer MC, Buijs N, van Leeuwen PA. Asymmetric dimethylarginine and critical illness. Curr Opin Clin Nutr Metab Care 2014;17:90–7.
41. Lüneburg N, Xanthakis V, Schwedhelm E, Sullivan LM, Maas R, Anderssohn M, Riederer U, Glazer NL, Vasan RS, Boger RH. Reference intervals for plasma L-arginine and the L-arginine:asymmetric dimethylarginine ratio in the Framingham Offspring Cohort. J Nutr 2011;141:2186–90.
42. van der Zwan LP, Scheffer PG, Dekker JM, Stehouwer CD, Heine RJ, Teerlink T. Systemic inflammation is linked to low arginine and high ADMA plasma levels resulting in an unfavourable NOS substrate-to-inhibitor ratio: the Hoorn Study. Clin Sci (Lond) 2011;121:71–8.
43. Cardounel AJ, Cui H, Samouilov A, Johnson W, Kearns P, Tsai AL, Berka V, Zweier JL. Evidence for the pathophysiological role of endogenous methylarginines in regulation of endothelial NO production and vascular function. J Biol Chem 2007;282:879–87.

44. Davids M, Richir MC, Visser M, Ellger B, van den Berghe G, van Leeuwen PA, Teerlink T. Role of dimethylarginine dimethylaminohydrolase activity in regulation of tissue and plasma concentrations of asymmetric dimethylarginine in an animal model of prolonged critical illness. Metabolism 2012;61:482–90.
45. Davids M, Teerlink T. Plasma concentrations of arginine and asymmetric dimethylarginine do not reflect their intracellular concentrations in peripheral blood mononuclear cells. Metabolism 2013;62:1455–61.
46. Ogawa T, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. J Biol Chem 1989;264:10205–9.
47. Rodionov RN, Jarzebska N, Weiss N, Lentz SR. AGXT2: a promiscuous aminotransferase. Trends Pharmacol Sci 2014;35:575–82.
48. Pope AJ, Karrupiah K, Kearns PN, Xia Y, Cardounel AJ. Role of dimethylarginine dimethylaminohydrolases in the regulation of endothelial nitric oxide production. J Biol Chem 2009;284:35338–47.
49. Hu X, Atzler D, Xu X, Zhang P, Guo H, Lu Z, Fassett J, Schwedhelm E, Boger RH, Bache RJ, et al. Dimethylarginine dimethylaminohydrolase-1 is the critical enzyme for degrading the cardiovascular risk factor asymmetrical dimethylarginine. Arterioscler Thromb Vasc Biol 2011;31:1540–6.
50. Martens-Lobenhoffer J, Bode-Boger SM. Amino acid N-acetylation: metabolic elimination of symmetric dimethylarginine as symmetric N (alpha)-acetyldimethylarginine, determined in human plasma and urine by LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2015;975:59–64.
51. Marini JC, Agarwal U, Didelija IC. Dietary arginine requirements for growth are dependent on the rate of citrulline production in mice. J Nutr 2015;145:1227–31.
52. Marini JC, Didelija IC, Fiorotto ML. Extrarenal citrulline disposal in mice with impaired renal function. Am J Physiol Renal Physiol 2014;307:F660–5.
53. Ligthart-Melis GC, Vermeulen MA, van Leeuwen PA, Deutz NE. Glutamine: precursor or nitrogen donor for citrulline synthesis? Am J Physiol Endocrinol Metab 2010;299:E683; author reply: E4.
54. Marini JC, Didelija IC, Castillo L, Lee B. Glutamine: precursor or nitrogen donor for citrulline synthesis? Am J Physiol Endocrinol Metab 2010;299:E69–79.
55. Marini JC, Didelija IC, Lee B. Reply to Ligthart-Melis et al [letter]. Am J Physiol Endocrinol Metab 2010;299:E684.
56. Ligthart-Melis GC, Marini JC, Engelen MP, Deutz NE. Glutamine supplementation, citrulline production, and de novo arginine synthesis: is there a relation? Am J Clin Nutr 2015;101:890–2.
57. Buijs N, Brinkmann SJ, Oosterink JE, Luttikhold J, Schierbeek H, Wisselink W, Beishuizen A, van Goudoever JB, Houdijk AP, van Leeuwen PA, et al. Reply to GC Ligthart-Melis et al [letter]. Am J Clin Nutr 2015;101:892–3.
58. Tomlinson C, Rafii M, Ball RO, Pencharz P. Arginine synthesis from enteral glutamine in healthy adults in the fed state. Am J Physiol Endocrinol Metab 2011;301:E267–73.
59. Tomlinson C, Rafii M, Ball RO, Pencharz PB. Arginine can be synthesized from enteral proline in healthy adult humans. J Nutr 2011;141:1432–6.
60. Ligthart-Melis GC, van de Poll MC, Boelens PG, Dejong CH, Deutz NE, van Leeuwen PA. Glutamine is an important precursor for de novo synthesis of arginine in humans. Am J Clin Nutr 2008;87:1282–9.
61. Marini JC. Arginine and ornithine are the main precursors for citrulline synthesis in mice. J Nutr 2012;142:572–80.
62. Marini JC. Interrelationships between glutamine and citrulline metabolism. Curr Opin Clin Nutr Metab Care 2016;19:62–6.
63. Cheung CW, Cohen NS, Raijman L. Channeling of urea cycle intermediates in situ in permeabilized hepatocytes. J Biol Chem 1989;264:4038–44.
64. Pastor CM, Morris SM Jr., Billiar TR. Sources of arginine for induced nitric oxide synthesis in the isolated perfused liver. Am J Physiol 1995;269:G861–6.
65. Simon A, Plies L, Habermeier A, Martine U, Reining M, Closs EI. Role of neutral amino acid transport and protein breakdown for substrate supply of nitric oxide synthase in human endothelial cells. Circ Res 2003;93:813–20.
66. Goodwin BL, Solomonson LP, Eichler DC. Argininosuccinate synthase expression is required to maintain nitric oxide production and cell viability in aortic endothelial cells. J Biol Chem 2004;279:18353–60.
67. Topal G, Brunet A, Walch L, Boucher JL, David-Dufilho M. Mitochondrial arginase II modulates nitric-oxide synthesis through nonfreely exchangeable L-arginine pools in human endothelial cells. J Pharmacol Exp Ther 2006;318:1368–74. Erratum in: J Pharmacol Exp Ther 2006;319(1):506.
68. Karbach S, Simon A, Slenzka A, Jaenecke I, Habermeier A, Martine U, Forstermann U, Closs EI. Relative contribution of different l-arginine sources to the substrate supply of endothelial nitric oxide synthase. J Mol Cell Cardiol 2011;51:855–61.
69. Shen LJ, Beloussow K, Shen WC. Accessibility of endothelial and inducible nitric oxide synthase to the intracellular citrulline-arginine regeneration pathway. Biochem Pharmacol 2005;69:97–104.
70. Closs EI, Simon A, Vekony N, Rotmann A. Plasma membrane transporters for arginine. J Nutr 2004;134(Suppl):2752S–9S; discussion: 65S–67S.
71. Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, Kanai Y. CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch 2004;447:532–42.
72. Closs EI, Boissel JP, Habermeier A, Rotmann A. Structure and function of cationic amino acid transporters (CATs). J Membr Biol 2006;213:67–77.
73. Simon A, Karbach S, Habermeier A, Closs EI. Decoding the substrate supply to human neuronal nitric oxide synthase. PLoS One 2013;8:e67707.
74. Qualls JE, Subramanian C, Rafi W, Smith AM, Balouzian L, DeFreitas AA, Shirey KA, Reutterer B, Kernbauer E, Stockinger S, et al. Sustained generation of nitric oxide and control of mycobacterial infection requires argininosuccinate synthase 1. Cell Host Microbe 2012;12:313–23.
75. Rapovy SM, Zhao J, Bricker RL, Schmidt SM, Setchell KD, Qualls JE. Differential requirements for L-citrulline and L-arginine during antimycobacterial macrophage activity. J Immunol 2015;195:3293–300.
76. Fakler CR, Kaftan HA, Nelin LD. Two cases suggesting a role for the L-arginine nitric oxide pathway in neonatal blood pressure regulation. Acta Paediatr 1995;84:460–2.
77. Brunetti-Pierri N, Erez A, Shchelochkov O, Craigen W, Lee B. Systemic hypertension in two patients with ASL deficiency: a result of nitric oxide deficiency? Mol Genet Metab 2009;98:195–7.
78. Erez A, Nagamani SC, Shchelochkov OA, Premkumar MH, Campeau PM, Chen Y, Garg HK, Li L, Mian A, Bertin TK, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med 2011;17:1619–26.
79. Su Y. Regulation of endothelial nitric oxide synthase activity by protein-protein interaction. Curr Pharm Des 2014;20:3514–20.
80. Xiong Y, Yu Y, Montani JP, Yang Z, Ming XF. Arginase-II induces vascular smooth muscle cell senescence and apoptosis through p66Shc and p53 independently of its L-arginine ureahydrolase activity: implications for atherosclerotic plaque vulnerability. J Am Heart Assoc 2013;2:e000096.
81. Xiong Y, Yepuri G, Forbiteh M, Yu Y, Montani JP, Yang Z, Ming XF. ARG2 impairs endothelial autophagy through regulation of MTOR and PRKAA/AMPK signaling in advanced atherosclerosis. Autophagy 2014;10:2223–38.
82. Davids M, Ndika JD, Salomons GS, Blom HJ, Teerlink T. Promiscuous activity of arginine:glycine amidinotransferase is responsible for the synthesis of the novel cardiovascular risk factor homoarginine. FEBS Lett 2012;586:3653–7.
83. Choe CU, Atzler D, Wild PS, Carter AM, Boger RH, Ojeda F, Simova O, Stockebrand M, Lackner K, Nabuurs C, et al. Homoarginine levels are regulated by L-arginine:glycine amidinotransferase and affect stroke outcome: results from human and murine studies. Circulation 2013;128:1451–61.
84. März W, Meinitzer A, Drechsler C, Pilz S, Krane V, Kleber ME, Fischer J, Winkelmann BR, Bohm BO, Ritz E, et al. Homoarginine, cardiovascular risk, and mortality. Circulation 2010;122:967–75.
85. Atzler D, Mieth M, Maas R, Boger RH, Schwedhelm E. Stable isotope dilution assay for liquid chromatography-tandem mass spectrometric determination of L-homoarginine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:2294–8.
86. Jaźwińska-Kozuba A, Martens-Lobenhoffer J, Kruszelnicka O, Rycaj J, Chyrchel B, Surdacki A, Bode-Boger SM. Opposite associations of plasma homoarginine and ornithine with arginine in healthy children and adolescents. Int J Mol Sci 2013;14:21819–32.

87. Kayacelebi AA, Beckmann B, Gutzki FM, Jordan J, Tsikas D. GC-MS and GC-MS/MS measurement of the cardiovascular risk factor homoarginine in biological samples. Amino Acids 2014; 46:2205–17.
88. May M, Kayacelebi AA, Batkai S, Jordan J, Tsikas D, Engeli S. Plasma and tissue homoarginine concentrations in healthy and obese humans. Amino Acids 2015;47:1847–52.
89. Pilz S, Meinitzer A, Tomaschitz A, Drechsler C, Ritz E, Krane V, Wanner C, Boehm BO, Marz W. Low homoarginine concentration is a novel risk factor for heart disease. Heart 2011;97:1222–7.
90. Atzler D, Gore MO, Ayers CR, Choe CU, Boger RH, de Lemos JA, McGuire DK, Schwedhelm E. Homoarginine and cardiovascular outcome in the population-based Dallas Heart Study. Arterioscler Thromb Vasc Biol 2014;34:2501–7.
91. Pilz S, Teerlink T, Scheffer PG, Meinitzer A, Rutters F, Tomaschitz A, Drechsler C, Kienreich K, Nijpels G, Stehouwer CD, et al. Homoarginine and mortality in an older population: the Hoorn study. Eur J Clin Invest 2014;44:200–8.
92. Atzler D, Schwedhelm E, Choe CU. L-homoarginine and cardiovascular disease. Curr Opin Clin Nutr Metab Care 2015;18: 83–8.
93. Pilz S, Meinitzer A, Gaksch M, Grubler M, Verheyen N, Drechsler C, Hartaigh BO, Lang F, Alesutan I, Voelkl J, et al. Homoarginine in the renal and cardiovascular systems. Amino Acids 2015;47:1703–13.
94. Tsikas D, Wu G. Homoarginine, arginine, and relatives: analysis, metabolism, transport, physiology, and pathology. Amino Acids 2015;47:1697–702.
95. Hecker M, Walsh DT, Vane JR. On the substrate specificity of nitric oxide synthase. FEBS Lett 1991;294:221–4.
96. Moali C, Boucher JL, Sari MA, Stuehr DJ, Mansuy D. Substrate specificity of NO synthases: detailed comparison of L-arginine, homo-L-arginine, their N omega-hydroxy derivatives, and N omega-hydroxynor-L-arginine. Biochemistry 1998;37:10453–60.
97. Reczkowski RS, Ash DE. Rat liver arginase: kinetic mechanism, alternate substrates, and inhibitors. Arch Biochem Biophys 1994;312:31–7.
98. Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. Nature 2015;517:302–10.
99. Gallinetti J, Harputlugil E, Mitchell JR. Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. Biochem J 2013;449:1–10.
100. Lee J, Ryu H, Ferrante RJ, Morris SM Jr., Ratan RR. Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. Proc Natl Acad Sci USA 2003;100: 4843–8.
101. Fernandez J, Yaman I, Sarnow P, Snider MD, Hatzoglou M. Regulation of internal ribosomal entry site-mediated translation by phosphorylation of the translation initiation factor eIF2alpha. J Biol Chem 2002;277:19198–205.
102. Hatzoglou M, Fernandez J, Yaman I, Closs E. Regulation of cationic amino acid transport: the story of the CAT-1 transporter. Annu Rev Nutr 2004;24:377–99.
103. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr 2006;136(Suppl):227S–31S.
104. Ban H, Shigemitsu K, Yamatsuji T, Haisa M, Nakajo T, Takaoka M, Nobuhisa T, Gunduz M, Tanaka N, Naomoto Y. Arginine and leucine regulate p70 S6 kinase and 4E-BP1 in intestinal epithelial cells. Int J Mol Med 2004;13:537–43.
105. Nakajo T, Yamatsuji T, Ban H, Shigemitsu K, Haisa M, Motoki T, Noma K, Nobuhisa T, Matsuoka J, Gunduz M, et al. Glutamine is a key regulator for amino acid-controlled cell growth through the mTOR signaling pathway in rat intestinal epithelial cells. Biochem Biophys Res Commun 2005;326:174–80.
106. Bauchart-Thevret C, Cui L, Wu G, Burrin DG. Arginine-induced stimulation of protein synthesis and survival in IPEC-J2 cells is mediated by mTOR but not nitric oxide. Am J Physiol Endocrinol Metab 2010;299:E899–909.
107. Wauson EM, Guerra ML, Dyachok J, McGlynn K, Giles J, Ross EM, Cobb MH. Differential regulation of ERK1/2 and mTORC1 through T1R1/T1R3 in MIN6 cells. Mol Endocrinol 2015;29:1114–22.
108. Wauson EM, Zaganjor E, Lee AY, Guerra ML, Ghosh AB, Bookout AL, Chambers CP, Jivan A, McGlynn K, Hutchison MR, et al. The G protein-coupled taste receptor T1R1/T1R3 regulates mTORC1 and autophagy. Mol Cell 2012;47:851–62.
109. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. An amino-acid taste receptor. Nature 2002;416:199–202.
110. Toda Y, Nakagita T, Hayakawa T, Okada S, Narukawa M, Imai H, Ishimaru Y, Misaka T. Two distinct determinants of ligand specificity in T1R1/T1R3 (the umami taste receptor). J Biol Chem 2013;288: 36863–77.
111. Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD, Chantranupong L, Comb W, et al. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. Science 2015;347:188–94.
112. Rebsamen M, Pochini L, Stasyk T, de Araujo ME, Galluccio M, Kandasamy RK, Snijder B, Fauster A, Rudashevskaya EL, Bruckner M, et al. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. Nature 2015;519:477–81.
113. Kuang D, Yao Y, Lam J, Tsushima RG, Hampson DR. Cloning and characterization of a family C orphan G-protein coupled receptor. J Neurochem 2005;93:383–91.
114. Wellendorph P, Hansen KB, Balsgaard A, Greenwood JR, Egebjerg J, Brauner-Osborne H. Deorphanization of GPRC6A: a promiscuous L-alpha-amino acid receptor with preference for basic amino acids. Mol Pharmacol 2005;67:589–97.
115. Conigrave AD, Hampson DR. Broad-spectrum L-amino acid sensing by class 3 G-protein-coupled receptors. Trends Endocrinol Metab 2006;17:398–407.
116. Pi M, Quarles LD. Multiligand specificity and wide tissue expression of GPRC6A reveals new endocrine networks. Endocrinology 2012;153:2062–9.
117. Wauson EM, Lorente-Rodriguez A, Cobb MH. Minireview: nutrient sensing by G protein-coupled receptors. Mol Endocrinol 2013; 27:1188–97.
118. Clemmensen C, Smajilovic S, Wellendorph P, Brauner-Osborne H. The GPCR, class C, group 6, subtype A (GPRC6A) receptor: from cloning to physiological function. Br J Pharmacol 2014;171:1129–41.
119. Mills E, Petersen E, Kulasekara BR, Miller SI. A direct screen for c-di-GMP modulators reveals a Salmonella Typhimurium periplasmic L-arginine-sensing pathway. Sci Signal 2015;8:ra57.
120. Römling U. Small molecules with big effects: cyclic di-GMP-mediated stimulation of cellulose production by the amino acid L-arginine. Sci Signal 2015;8:fs12.
121. Chantranupong L, Scaria SM, Saxton RA, Gygi MP, Shen K, Wyant GA, Wang T, Harper JW, Gygi SP, Sabatini DM. The CASTOR Proteins Are Arginine Sensors for the mTORC1 Pathway. Cell 2016;165:153–64.