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REVIEW

New indications and controversies in arginine therapy

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Summary
Arginine is an important, versatile and a conditionally essential amino acid. Besides serving as a building block for tissue proteins, arginine plays a critical role in ammonia detoxification, and nitric oxide and creatine production. Arginine supplementation is an essential component for the treatment of urea cycle defects but recently some reservations have been raised with regards to the doses used in the treatment regimens of these disorders. In recent years, arginine supplementation or restriction has been proposed and trialled in several disorders, including vascular diseases and asthma, mitochondrial encephalopathy lactic acidosis and stroke-like episodes (MELAS), glutaric aciduria type I and disorders of creatine metabolism, both production and transportation into the central nervous system. Herein we present new therapeutic indications and controversies surrounding arginine supplementation or deprivation.
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Introduction

L-Arginine (2-amino-5-guanidino-pentanoic acid) is an essential amino acid for birds, carnivores and young mammals, but is considered as non-essential in healthy adults.[1] Its metabolism is complex and is incompletely understood. Arginine serves as a precursor for protein synthesis as well as the synthesis of urea, nitric oxide (NO), creatine and agmatine (Fig. 1). It is a substrate for four enzymes, some of which exist as multiple isoforms: the arginases, the nitric oxide synthases (NOS), arginine:glycine amidinotransferase, and arginine decarboxylase (Fig. 1; Table 1).[2]

Differential tissue specific expression of enzymes or isoforms as well as transporters involved in arginine metabolism result in distinct metabolic outcomes. With improved understanding of the significant role NO and creatine play in the function of the central nervous-, muscular- and vascular-systems the scope of indications for treatment with arginine

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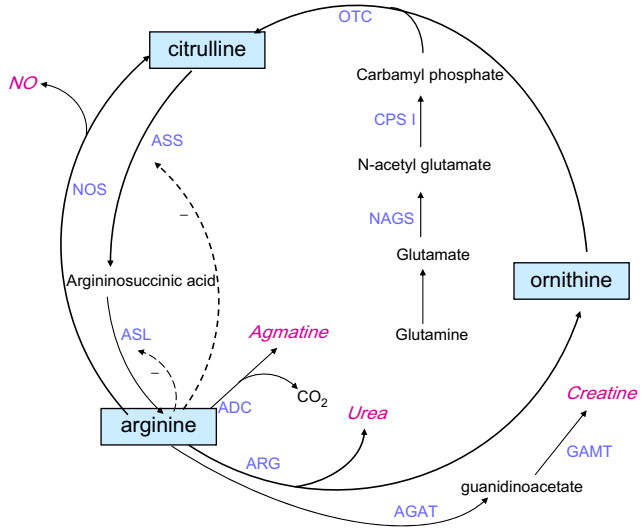

Figure 1 Arginine metabolism by four enzymes: ARG: arginase, NOS: nitric oxide synthase, ADC: arginine decarboxylase, and AGAT: arginine:glycine aminotransferase. Other enzymes in the metabolic pathways: NAGS: N-acetyl glutamate synthetase; CPS: carbamyl phosphate synthetase, OTC: ornithine transcarbamylase, ASS: argininosuccinate synthase, ASL: argininosuccinate lyase, and GAMT: guanidinoacetate methyltransferase. NO: nitric oxide — Denotes inhibition. Enzymes are denoted in blue. End metabolites are denoted in purple.

has broadened. The purpose of this review is to summarise the information regarding arginine metabolism, and to highlight new uses and controversies of arginine supplementation. These are summarised in Table 2.

Arginine homeostasis

Normal plasma arginine concentrations depend upon the age of the individual and its homeostasis is achieved primarily via its catabolism.[3] The sources of free arginine are dietary protein (with about 40% of it being metabolised in the intestine before reaching the circulation via the portal vein),[4] turnover of body proteins (approximately 85% of arginine in the circulation), and endogenous, *de novo* synthesis.[5]

Arginine is considered an essential amino acid in neonates, particularly under conditions such as stress, infection and prematurity.[1,6] *De novo* arginine synthesis in the fetus occurs in enterocytes. However, the gene expression of key enzymes of the fetal intestinal arginine-synthetic pathway, Δ1-pyrroline-5-carboxylase, argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) is low. The perinatal cortisol surge may precipitate the maturation of these enzymes and its absence in premature delivery contributes to their delayed maturation and to limited arginine synthesis in preterm infants.[7] During the suckling period, the major source for endogenous arginine biosynthesis is from citrulline in the intestine.[8] The expression of ASS and ASL peaks immediately after birth and then decreases gradually whereas at the same time arginase mRNA and activity are not detectable at all.[9] In the post-weaning phase there is a gradual transition to a pathway known as ''the intestine-renal axis'' in which citrulline is produced in the epithelial cells of the small intestine, primarily from glutamine and glutamate, and released to the circulation. The proximal tubules of the kidney then extract citrulline from the circulation and convert it to arginine.[10]

The sequence of conversion of ornithine to citrulline and to arginine does not occur only in the ''intestine-renal axis''. These reactions constitute a part of the urea cycle, the final common pathway for the excretion of waste nitrogen in mammals. The urea cycle is comprised of five enzymes: carbamyl phosphate synthetase-1 (CPS-1), ornithine transcarbamylase (OTC), ASS, ASL, and arginase. CPS-1 and OTC are mitochondrial enzymes, whereas ASS and ASL are cytosolic. Periportal hepatocytes are the only cells in which all enzymes of the urea cycle are expressed.[11]

Table 1 Arginine metabolism, its metabolic products and their physiological role

Enzyme	Metabolic product	Physiological role	Reference
Arginase	Ornithine	Ammonia detoxification through maintenance of urea cycle function	77,78
		Collagen production and airway remodelling	76,78,79
NO synthase	Nitric oxide	Endothelium-derived relaxing factor, signal transduction, mediation of immune response, neurotransmission	1,31
Arginine:glycine aminotransferase	Creatine	CNS Na^+ K^+ ATPase activity, neurotransmitter release, calcium homeostasis, maintenance of membrane potential and ion gradients	34
		CNS embryonic development	35
		CNS protection from ammonia toxicity	36,21
Arginine decarboxylase	Agmatine	Allosteric activation of NAGS	29
		Cell-signalling	15
		Neurotransmission	16
		Inhibition of cell proliferation	17
		Inhibition of NOS	18

Table 2 Arginine supplementation and restriction in disease states

Disease states	Implications of treatment	Reference
Urea cycle defects (supplementation)	Increased nitrogen disposal Increased urea flux Adequate creatine production Adequate NO production	36,47,50,51
LPI (supplementation)	Restoration of functional urea cycle	85
MELAS (supplementation)	Improved endothelial function	71,72,74
NEC (supplementation) persistent pulmonary hypertension (supplementation)	Restoration of adequate NO production and improvement of vascular stability	66,67
X-linked creatine transport defect (supplementation[a])	Substrate for CNS creatine production[a]	38
Asthma (supplementation[a])	Provision of substrate for the restoration of NO production	78,79
(Arginase inhibition)	to maintain bronchodilator tone	68
GA I: (supplementation[a])	Competitive inhibition of l-lysine across the y^+ transport system, decreased transport of l-lysine into the CNS with decreased CNS dicarboxylic acid production	58,59
Creatine deficiency due to GAMT deficiency (restriction)	Reduction of guanidinoacetic acid, an epileptogenic compound	61

LPI — Lysinuric Protein Intolerance; MELAS — myoclonic encephalopathy, lactic acidosis and stoke-like episodes; NEC — necrotising enterocolitis; GA I — glutaric aciduria type 1; and GAMT — guanidinoacetate methyltransferase.
[a] Denotes theoretical ground for supplementation or restriction but not sufficient studies yet to show clinical evidence of benefit.

Arginine is metabolised by arginase, which catalyses its conversion to L-ornithine, a key metabolite in the urea cycle. There are two distinct isoforms of arginase, which are encoded by separate genes.[12] Type I, cytosolic arginase, is expressed primarily in the liver and plays an important role in the detoxification of ammonia.[13] Type II, mitochondrial arginase, is expressed in extrahepatic tissue and is involved in a number of biosynthetic functions such as the synthesis of ornithine, proline and glutamate.[14]

Arginine decarboxylase (ADC) catalyses the conversion of arginine to agmatine (Fig. 1, Table 1), the physiological role of which has not been fully elucidated. Agmatine may be responsible for the allosteric activation of NAGS by arginine (see below). In addition, agmatine can act as a cell-signalling molecule. Agmatine-mediated augmentation of hepatic long chain fatty acid oxidation has been attributed to its glucagon-like function, leading to an increase in intracellular cAMP concentration.[15] It has been suggested that agmatine acts as a neurotransmitter,[16] as an inhibitor of cell proliferation,[17] and as an inhibitor of NOS.[18]

Different intracellular arginine pools exist in different cell types,[19] suggesting diverse effects of arginine transporters. Most of the intracellular arginine transport (~70%) occurs via the y^+ system, a group of five cationic transporters designated as CATs.[19,20] Three other transport systems exist, the y^+L, b^{o+}, and B^{o+}.[21] Arginine transport in gut epithelium and vascular endothelium are regulated by signal transduction mechanisms.[21] Mitochondrial transport is less well defined and may involve multiple transporters such as the ORNT1 transporter.[22]

Brain arginine is mostly derived from blood or from protein breakdown. In addition, citrulline, which is produced in the brain via the action of nitric oxide synthase (NOS; see below), can be recycled to arginine via the CNS expression of ASS and ASL.[23,24] Hyperammonaemia has been shown to induce gene expression of ASS and ASL in rat brain, although they do not co-locate in the same neurons, thus relying on transport mechanisms.[24–26] However, there is no *de novo* arginine synthesis from ammonia and ornithine in the brain, as CPS-1 and OTC are not expressed in brain tissue.[24] This constellation has implications on several disease states (see below).

Role of arginine in metabolic pathways

Role of arginine in ammonia detoxification

Arginine plays a critical role in ammonia detoxification and is indispensable for the normal function of the urea cycle, in which ammonia is detoxified through its metabolism into urea.[27,28] Two mechanisms have been put forth through which arginine participates in ammonia detoxification. First, it has long been accepted that arginine is an allosteric activator of N-acetyl glutamate synthetase (NAGS), which is in itself an allosteric activator of CPS-1.[11] Recently it has been suggested that arginine does not directly activate NAGS but, rather, via agmatine.[15,29] According to a second mechanism, proposed by Nissim et al, arginine entering the liver via the portal vein is metabolised by mitochondrial arginase to provide ornithine for citrulline and aspartate synthesis and for the priming of the urea cycle.[30]

Role of arginine in nitric oxide synthesis

Arginine is the physiological precursor of nitric oxide (NO), an endothelium-derived relaxing factor, a signal transduction molecule, a neurotransmitter, and a mediator of immune response.[1,31] NO production is dependent on nitric oxide synthases (NOS) (Table 1), a cytochrome P450-like enzyme family of three isoforms.[32] NOS isoforms contain a binding site for arginine, which is subsequently oxidised to NO and citrulline, a pathway commonly referred to as the L-arginine-NO pathway or the NOS reaction (Fig. 1).[33]

Role of arginine in creatine synthesis

The role of creatine in CNS metabolism has been unravelled in recent years. Creatine is involved in CNS Na^+ K^+ ATPase activity, neurotransmitter release, calcium homeostasis, and maintenance of membrane potential and ion gradients (Table 1).[34] It has been shown to play a major role in CNS embryonic development[35] including the protection of axonal growth from the toxic effect of ammonia.[21,36] Arginine serves as a precursor for creatine synthesis by providing guanidino groups.[37] It combines with glycine, a reaction catalysed by arginine:glycine aminotransferase (AGAT), to form guanidinoacetate, which is then converted to creatine by guanidinoacetate methyltransferase (GAMT) (Fig. 1). Dietary intake of creatine acts as a feedback repressor of AGAT, thus regulating the flux of arginine through this pathway.[34] Creatine is produced largely in the liver and kidney. However, AGAT and GAMT are expressed in rat brain aggregates and it appears that under normal conditions, creatine used by the brain is synthesised locally.[38]

Role of arginine in regulating its homeostasis

Arginine plays an essential role in maintaining its own homeostasis by selectively regulating several key enzymes involved in its metabolism. High concentration of arginine leads to inhibition of ASS and ASL activities (Fig. 1).[39] The exact mechanisms of ASS and ASL regulation is not clear but regulation of ASS seems to involve post-transcriptional regulatory mechanism(s).[40] Under conditions of its deprivation, arginine has been shown to decrease translation of inducible NOS (iNOS) mRNA [41] and to decrease the stability of the iNOS protein.[42] Under these conditions, arginine has been shown to increase transcription and translation of the *CAT-1* gene and CAT-1 mRNA, respectively.[43] Taken together, these mechanisms preserve arginine availability under conditions of arginine deprivation and increase its utilisation under conditions of abundance.[43] However, these mechanisms vary from one cell type to another and further research is needed to completely unravel the mechanisms by which arginine regulates its own homeostasis.

Arginine supplementation in urea cycle defects

In patients with urea cycle defects (UCD), except in arginase deficiency, arginine becomes an essential amino acid.[44] Moreover, due to its diverse effects, arginine deficiency is specifically linked to particular complications of some of the UCD. For example, Summar et al. postulated that polymorphisms in the *CPS-1* gene lead to altered carbamyl phosphate synthetase function and decreased arginine availability as substrate for NO production.[45] Under certain environmental situations these polymorphisms may play a role in the pathogenesis of disease states such as persistent pulmonary hypertension of the newborn, post-cardiac surgery pulmonary hypertension and bone marrow transplant complications.[46,47] Impaired intracellular arginine flux has been postulated in the pathogenesis of the complications of ASL deficiency, namely cognitive deficiency, and progressive hepatic disease,[48] both of which are disproportionate to the frequency and severity of hyperammonaemia in patients with this disorder.

In addition to specific UCD enzyme deficiencies, disruption of specific transport mechanisms may lead to arginine deficiency and hyperammonaemia. For example, a defect in the $y+L$ transport system is responsible for Lysinuric Protein Intolerance (LPI), an inborn error of lysine, cystine, ornithine and arginine transport across renal and intestinal baso-lateral membrane.[49] In LPI, arginine deficiency leads to intellectual deficit, which results from a disruption of the urea cycle but is disproportionate to the level of hyperammonaemia. It is conceivable that additional, yet unknown, inborn errors of arginine transport could lead to perturbation of the urea cycle.

It follows that arginine supplementation plays a major role in the treatment of UCD. Arginine supplementation is aimed at restoring ammonia detoxification, NO production and creatine production, as well as enhancing protein synthesis. In ASS deficiency, one mole of nitrogen can be removed for every mole of arginine metabolised through the urea cycle. In ASL deficiency, two moles of nitrogen can be removed for every mole of arginine metabolised through the urea cycle.[47] Arginine supplementation leads to higher levels of ornithine and citrulline (Fig. 1), which are excreted in the urine and represent a significant proportion of waste nitrogen excretion. In CPS-1 and OTC deficiencies, arginine supplementation may be replaced by citrulline, which carries the advantage of using an additional atom of nitrogen in the synthesis of arginine.[50] *In vivo* measurement of urea flux has been shown to increase, after the administration of arginine in patients with urea cycle defects.[51]

Inhibition of creatine production has been shown both *in vitro* in rat brain cell aggregates exposed to ammonia [21,38] and *in vivo* in patients with various UCDs.[52] Substitution with arginine or citrulline normalised the levels of metabolites. These findings suggest that arginine supplementation may be critical in restoring physiological creatine synthesis in patients with a UCD. Finally, the clinical observation of improved growth (height) in patients with UCD treated with arginine can be explained by the role of arginine in growth hormone and, possibly, insulin secretion.[53]

The dose of arginine supplementation (usually as arginine hydrochloride) used in the treatment of UCD is dependent on the age of the patient and the precise enzyme deficiency. Published dosages range between 100 and 700 mg/kg body weight per day.[44,54–57] However, concern has been raised recently about the safety of high arginine doses in general and in ASL deficiency specifically. In theory, supplementing with arginine will not only increase ornithine levels, but also those of citrulline and argininosuccinate. Given that argininosuccinate is a tricarboxylic acid, it has been suggested that trapping of this metabolite in the brain could be the pathogenetic mechanism responsible for the poor cognitive outcome of patients with ASL deficiency, akin to the putative pathogenetic role of dicarboxylic acids in causing central nervous system pathology in glutaric aciduria type 1.[58,59] Moreover, high cerebral levels of guanidinoacetate have been identified in patients with ASS and ASL deficiencies receiving high dose arginine supplementation ($\geq$700 mg/kg body weight per day),[60] which may be toxic.[61] A counter argument could be that CNS arginine deficiency may lead to deficient NO and creatine production, leading to the clinical phenotype. Indeed, some patients with ASL deficiency have required high doses of arginine in order to replenish the urea cycle during times of metabolic decompensation.[62]

A natural model of the possible toxic effects of high arginine concentrations is Hyperargininaemia, a rare UCD caused by arginase deficiency, leading to impaired hydrolysis of arginine to urea and ornithine.[63-65] The clinical phenotype is strikingly different to other UCD, involving variable degrees of cognitive deficits, epilepsy, and a progressive spastic diplegia.[63] The possibility that chronic high tissue concentrations of arginine may play a role in the pathogenesis of the clinical phenotype is supported by the observation that treating affected patients from birth can prevent the clinical phenotype from evolving.[64]

Arginine supplementation in disorders of the vascular system

Arginine exerts an effect on the vascular system both via NO-dependent and NO-independent mechanisms. For example, impaired NO production due to arginine deficiency has been implicated in perturbed intestinal vascular stability predisposing to the formation of necrotising enterocolitis (NEC). Indeed, provision of exogenous arginine to preterm infants has potential to prevent NEC,[66] and persistent pulmonary hypertension.[67] Newly developed arginase inhibitors enhance relaxation of smooth muscle in sphincter muscle[68] and in penile tissue.[69] However, a recent report indicates that in adults, long-term administration of L-arginine does not increase nitric oxide synthesis or improve vascular reactivity.[70] The vascular consequences of long-term administration of arginine to infants have not been reported.

Arginine supplementation in MELAS

The cause of stroke-like episodes in patients with the mitochondrial disorder MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) remains controversial. Administration of L-arginine to patients with MELAS during acute stroke-like episodes led to improved microcirculation and reduced tissue injury from ischaemia[71] and improved clinical and MRS findings.[72] In a subsequent, larger study, a decrease in clinical severity and frequency of stroke-like episodes has been demonstrated in 24 MELAS patients treated prophylactically with arginine.[73] The exact mechanism of the protective effect of arginine is not known. One hypothesis considers that these episodes are related to segmental impairment of vasodilatation in intracerebral arteries. Koga et al. have demonstrated endothelial dysfunction in MELAS patients via flow-mediated vasodilation, a situation that is improved by the administration of arginine.[74] This work suggests that patients with MELAS may benefit from arginine supplementation, in both the acute stroke-like phase and the interictal period. It is plausible that intracellular reactive oxygen species, which are abundant in mitochondrial cytopathy, inactivate NO and/or interact with the unpaired electron in NO to produce peroxynitrite, a toxic reactive nitrogen species. On its part, peroxynitrite has been shown to increase the arginine transport system y^+ in glial cells, replenishing neuronal arginine concentration.[75] Supplementation with arginine could lead to enhanced NO production and the resultant vasodilatation may reduce ischaemic damage, energy failure and lactate accumulation,[72] but further research is needed to unveil the mechanism by which arginine modulates the clinical phenotype of MELAS.

Arginine supplementation in asthma

Using microarray analysis, Zimmerman et al. have demonstrated high arginase I and arginase II, as well as CAT-2 expression profiles in lung tissue from murine models of asthma and in bronchoalveolar lavage cells from human patients with asthma.[76,77] In addition, enhanced arginase activity was found in the plasma of patients with asthma.[78] These findings suggest that arginine availability for NO production is decreased in these patients, at least in some tissues, leading to perturbation of the basal bronchodilator tone and potentially to the production of reactive nitrogen species and further cellular damage.[78,79] In a recent study on guinea pig tracheal preparations, NO deficiency, presumably the result of competition between NOS and arginase, was shown to underlie the impaired airway smooth muscle relaxation, which was restored using a specific arginase inhibitor.[80] It should be noted, however, that the decreased arginine bioavailability in these patients may in fact result from increased demand for NO production to maintain bronchodilator tone.[78] Taken together, these findings form a rationale for arginine supplementation in asthma. However, another consequence of increased arginine catabolism via arginase is increased production of ornithine, a precursor of proline and polyamine. These alterations can lead to collagen synthesis and cell proliferation and, hence, to airway wall thickening and remodelling.[76] Further research is needed to resolve the potential risk of providing additional substrate for arginase activity and hence for airway wall remodelling.

Arginine restriction/supplementation in cerebral creatine deficiencies

Three disorders of creatine deficiency have been described: GAMT and AGAT deficiencies, which are enzyme deficiencies, and the X-linked creatine transporter deficiency (X-L CTD). The notion that guanidinoacetate, which accumulates in GAMT deficiency (Fig. 1), may be toxic to the brain led to the recommendation of dietary arginine restriction in this disorder.[61] The clinical and neuroradiological response to creatine supplementation in X-L CTD has been disappointing.[81] However, supplemented arginine may potentially serve as a substrate for cerebral creatine production in the treatment of the X-L CTD, as it carries the advantage of gaining access to the central nervous system across the blood brain barrier via the cationic amino acid system y^+ transport system, which is not defective in X-L CTD. Further studies are required in order to substantiate the benefit of arginine treatment in X-L CTD.[38]

Arginine supplementation in glutaric aciduria type I?

The pathogenesis of glutaric aciduria type I (GAI), a rare cerebral organic acid disorder due to glutaryl-CoA dehydrogenase deficiency, is not clear but restricted passage of toxic dicarboxylic acids across the blood brain barrier

has recently been implicated in the neurological complications of the disorder.[58,59] Dietary lysine restriction has been practiced as a therapeutic option for GAI in many centres, with a view of decreasing the availability of this amino acid as a precursor for the synthesis of toxic dicarboxylic acids.[58] However, treatment of GAI patients with low-lysine low-tryptophan formulae has led to nutritional deficiency in these amino acids. Given that lysine transport across the blood–brain barrier is facilitated by the cationic amino acid system y^+ transport system, which is a competitive process, it has been suggested that arginine supplementation may competitively inhibit the delivery of lysine into the CNS, avoiding the potential complications of the lysine and tryptophan restricted diet.[59] To date, there are no reports on the effect of such supplementation in this disorder yet.

Arginine overdose

Excess arginine can lead to overproduction of NO with consequent vasodilatation and hypotension.[82] Care must be taken when administering intravenous arginine, as fatal cases of arginine hydrochloride overdose have been reported in instances of drug calculation errors, marked by severe hyponatraemia (possibly due to a surge in NO production leading to natriuresis[83]) with subsequent fatal central pontine and extrapontine myelinolysis.[84]

Conclusions and future perspectives

The role of arginine in the pathophysiology and therapy of urea cycle defects has long been of interest to those involved in the study and management of inborn errors of metabolism. In recent years, the role of this versatile amino acid in the pathophysiology of many other diseases of various systems is emerging, and arginine supplementation is advocated to counteract the complications of these diseases. However, several questions regarding arginine metabolism require further research in order to develop potential therapies using this amino acid. For example, the metabolism of arginine and its transport mechanisms in the CNS are not completely understood. How is it regulated and to what extent is it dependent on local enzymes and transporters or on transport across the blood brain–barrier? In addition, the precise role, if any, of several of arginine metabolites (e.g., agmatine) have not been fully elucidated. What is the role of these metabolites in the CNS? What are the safety ranges of arginine therapy and how does arginine supplementation affect the transport and metabolism of other amino acids? Answers to these questions and a better understanding of the diversity of arginine metabolism will lead to the development of medications aimed at improving endothelial function, vascular and bronchial smooth muscle function as well as CNS creatine and NO production.

Conflict of interest statement

The authors have no financial or other relations that could lead to conflict of interest.

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