The metabolic basis of arginine nutrition and pharmacotherapy

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Abstract

As an essential precursor for the synthesis of proteins and other molecules with enormous biological importance (including nitric oxide, urea, ornithine, proline, polyamines, glutamate, creatine, agmatine, and dimethylarginines), arginine displays remarkable metabolic and regulatory versatility. Evidence available to date provides a sound reason to classify arginine as an essential amino acid for young mammals (including parenterally fed human infants) and as a conditionally essential amino acid for adults under such conditions as trauma, burn injury, massive small-bowel resection, and renal failure. Arginine administration reverses endothelial dysfunction, enhances wound healing, prevents the early stages of tumorigenesis, and improves cardiovascular, reproductive, pulmonary, renal, digestive, and immune functions. Arginine or its effective precursor citrulline may hold great promise as a nutritional or pharmacotherapeutic treatment for a wide array of human diseases. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

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1. Introduction

A century after its first isolation from lupin seedlings, L-arginine (2-amino-5-guanidinovaleric acid) was identified in 1988 as the physiological precursor for nitric oxide (NO) synthesis in animal cells [1]. NO is the major endothelium-derived relaxing factor, a mediator of immune responses, a neurotransmitter, a cytotoxic free radical, and a widespread signaling molecule in the body [2]. The discovery of NO synthesis has stimulated an enormous interest in the biochemistry, nutrition, and pharmacology of arginine over the past 15 years. As such, extensive studies have been conducted to explore nutritional or therapeutic roles of arginine to treat a wide array of human diseases that are associated with a relative or absolute deficiency of arginine or with a reduced bioavailability of NO [3-5]. In addition to NO, other products of arginine catabolism, such as ornithine, proline, polyamines (putrescine, spermidine and spermine), creatine, agmatine, and glutamate (Fig. 1), may also mediate the beneficial effects of arginine [6], thus extending its physiological roles to virtually every cellular and organ function in the body. The major objective of this article is to examine the metabolic basis of arginine nutrition and pharmacotherapy.

2. Arginine synthesis and catabolism

A brief summary of arginine metabolism is necessary to appreciate the broad implications of arginine for health, disease, and clinical applications. For a general knowledge of mammalian arginine metabolism and its molecular regulation, readers are referred to the recent comprehensive reviews by Wu and Morris [6] and Morris [7]. Here, we will focus on the salient features of arginine synthesis and catabolism that are most pertinent to this article.

2.1. Endogenous synthesis of arginine

Although arginine is formed in the liver via the urea cycle, there is no net synthesis of arginine by this organ.
because an exceedingly high activity of arginase results in rapid hydrolysis of arginine. Studies over the past 25 years have established that absorptive epithelial cells of the small intestine (enterocytes) play a crucial role in whole body synthesis of citrulline, the immediate precursor of arginine [6,7]. Thus, plasma concentrations of citrulline are a useful marker for small intestinal mass, intestinal injury, and successful adaptation of the short-bowel in infants or adult humans [8-10]. Also, changes in plasma citrulline concentrations are reflective of changes in intestinal citrulline synthesis in both neonates and adults [11-13].

The mammalian pathways for citrulline and arginine synthesis are illustrated in Fig. 2. Phosphate-dependent glutaminase, proline oxidase, ornithine aminotransferase (OAT), argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and glutamate/aspartate aminotransferase are widely distributed in animal tissues, whereas carbamoylphosphate synthase-I (CPS-I), ornithine carbamoyltransferase (OCT), and N-acetylglutamate (NAG) synthase are restricted to the mammalian liver and intestinal mucosa. Note that pyrroline-5-carboxylate (PSC) synthase is located almost exclusively in intestinal mucosa for PSC synthesis from glutamine/glutamate [6,12,13]. Both enzymatic and metabolic evidence have established that PSC synthase and NAG synthase are two key regulatory enzymes in the intestinal synthesis of citrulline and arginine [6,7].

In neonates, most of the citrulline synthesized in enterocytes is converted locally to arginine because of high ASS and ASL activities, and net synthesis of arginine occurs in these cells because of the absence of arginase activity [14]. In weaned mammals, enterocytes release most of the synthesized citrulline due to a low ASS activity [14]. In both neonatal and adult mammals, citrulline released by the small intestine is not taken up by the liver, but is utilized instead for arginine synthesis in extrahepatic tissues, with the kidneys being the major site [6,7]. Because the uptake of physiological concentrations of arginine by the liver is low [15], the intestinally derived citrulline and arginine are equally effective as a source of arginine for extrahepatic tissues. However, in patients with liver transplants or trauma, a high activity of plasma arginase rapidly hydrolyzes both dietary and intestine-derived arginine, resulting in an arginine deficiency [16].

The crucial role of the small intestine in endogenous arginine synthesis is epitomized by the arginine deficiencies which result from an inherited deficiency of PSC synthase in humans [17,18], a limited supply of enteral proline in neonatal pigs [19], or massive resection of the small bowel in adult rats [12]. An analogous situation exists in strict carnivores (e.g. cats and ferrets), which synthesize very little intestinal citrulline owing to low PSC synthase and OAT activities [20]. The requirements for dietary arginine by strict carnivores are so stringent that ingestion of an arginine-free meal rapidly leads to hyperammonemia, encephalopathy, and even death [20].

2.2. Arginine catabolism

Arginine transport represents the first step of arginine utilization by cells. The most important mechanism for arginine uptake by a majority of cell types is the system y+, a high-affinity, Na+-independent transporter of basic amino acids, including arginine, lysine, histidine, and ornithine [15]. However, other transporters, such as b0,+ , B0,+ , and y+L , also transport arginine in a cell-specific manner. Once inside the cell, there are multiple pathways for arginine degradation (Fig. 2). The identification of widespread and highly regulated expression of NO synthases (NOS) and extrahepatic arginase, as well as the discovery of arginine decarboxylase and agmatinase in animal cells, are some of the most exciting developments in arginine catabolism in recent years [7].

A classic pathway for arginine degradation is initiated by arginase to synthesize urea, ornithine, proline, polyamines, glutamate, and glutamine [6]. Most cells can form these products from arginine, which is of great nutritional and physiological importance for diverse functions such as lactation, growth, development, tissue remodeling, and responses to a wide range of hormones and signaling molecules. There are two distinct isozymes of mammalian arginase (type I and type II), which are encoded by separate genes and differ in molecular and immunological properties, tissue distribution, subcellular localization, and regulation of expression [7]. Type I arginase (a cytosolic enzyme) is highly expressed in the liver as a component of the urea cycle, and to a limited extent in a few other tissues. In contrast, type II arginase (a mitochondrial enzyme) is expressed at much lower levels in extrahepatic tissues, including the kidney, brain, small intestine, endothelial...
cells, mammary gland, and macrophages [6]. The different subcellular localization of the arginase isozymes may provide a mechanism for regulating the metabolic fate of arginine. The cloning of the arginase cDNAs has now provided a powerful means to directly test this hypothesis. Recent studies have shown that arginase is limiting for polyamine synthesis in many cell types, including vascular smooth muscle cells [21,22], endothelial cells [23,24], and macrophages [25,26]. Because arginase and NOS use arginine as a common substrate, there has also been a growing interest during the past several years in the role of arginase I and II in regulating NO synthesis in mammalian cells (e.g. [21-26]).

Another well-characterized pathway for arginine utilization is creatine synthesis [6]. This pathway is initiated by arginine/glycine amidotransferase, which transfers the guanidino group from arginine to glycine to form guanidinoacetate and ornithine. This enzyme is present predominantly in the renal tubules, pancreas, and to a much lesser extent in the liver and other organs. The kidneys are the principal site of guanidinoacetate formation in vivo [27]. Guanidinoacetate is methylated by guanidinoacetate N-methyltransferase, which is located primarily in the liver, pancreas, and, to a much lesser extent, in the kidneys to form creatine. Circulating creatine is actively taken up by skeletal muscle and nerve where it is phosphorylated and

Fig. 2. Arginine metabolism in mammals. Abbreviations: ADC, arginine decarboxylase; AGA, agmatinase; AGAT, arginine:glycine amidotransferase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; AS, argininosuccinate; Asp, aspartate; BH$_4$, (6R)-5,6,7,8-tetrahydro-L-biopterin; CP, carbamoylphosphate; CPS-I, carbamoylphosphate synthetase-I (ammonia); DCAM, decarboxylated S-adenosylmethionine; Glu, glutamate; Glu, glutamine; GDH, glutamate dehydrogenase; GA, guanidinoacetate; GMAT, guanidinoacetate N-methyltransferase; CK, creatine kinase; Cr-P, creatine-phosphate; α-KG, α-ketoglutarate; MTA, methylenetetrahydrofolate; NAG, N-acetylglutamate; NAGS, N-acetylglutamate synthase; NO, nitric oxide; NOS, nitric oxide synthase; OAT, ornithine aminotransferase; OCT, ornithine carbamoyltransferase; ODC, ornithine decarboxylase; PO, proline oxidase; PSCD, pyrroline-5-carboxylate dehydrogenase; PSCR, pyrroline-5-carboxylate reductase; PSCS, pyrroline-5-carboxylate synthase; SAM, S-adenosylmethionine; SAMD, S-adenosylmethionine decarboxylase; SAHC, S-adenosylhomocysteine; SPDS, spermidine synthase.
eventually undergoes nonenzymatic and irreversible dehydration to yield creatinine, which is excreted by the kidneys. Thus, creatine homeostasis primarily involves three major organs: the kidney, liver, and skeletal muscle. Note that a large amount of dietary arginine is utilized for creatine synthesis (e.g. 2.3 g arginine per day in adult humans), and arginine plays an important role in creatine availability in vivo [6]. This view is supported by the recent finding that an arginine deficiency decreases the concentrations of arginine, guanidinoacetate and creatine in mouse tissues (brain, muscle, liver, and kidneys) [28].

Two novel enzymes for mammalian arginine catabolism are NOS and arginine decarboxylase [7]. NO is formed from L-arginine by one of the three NOS isoforms: nNOS (originally identified as constitutive in neuronal tissue), iNOS (originally identified as being inducible by cytokines in activated macrophages and liver), and eNOS (originally identified as constitutive in vascular endothelial cells) [29]. nNOS and eNOS are collectively termed constitutive NOS (cNOS). All three isoforms of NOS can be induced by different, appropriate stimuli through transcriptional and posttranscriptional mechanisms. Additionally, all the isoforms of NOS can be constitutively expressed in some tissues or cells, and can be found in the cytosol or particulate fractions of cells, or both [29]. A quantitatively small amount of NO is produced by nNOS or eNOS in animal cells [1], whereas much larger amounts of NO are generated by iNOS in almost all cell types stimulated by inflammatory cytokines and lipopolysaccharide [6]. Because deficiencies or excesses of NO production lead to dysfunctions of numerous and diverse organs and systems, understanding the roles of arginine in regulating NO synthesis is crucial to the health and survival of humans and animals [30].

Arginine decarboxylase (a mitochondrial enzyme) is the most recent addition to a family of mammalian arginine-metabolizing enzymes [6]. This enzyme catalyzes the synthesis of agmatine from arginine in rat brain, liver, kidney, adrenal gland, macrophages, and small intestine. There are species and perhaps developmental differences in tissue distribution of arginine decarboxylase activity. Agmatinase catalyzes the conversion of guanidino nitrogen

2.3. Metabolism of methylarginines

Due to their roles in the regulation of NO synthesis, there has been a growing interest in mammalian metabolism of methylarginines [5]. After arginine is incorporated into proteins, arginine residues are methylated by an emerging family of protein arginine N-methyltransferases (PRMT) [32]. The posttranslational modification of protein-bound arginine results in the formation of $^N\epsilon$-monomethyl-L-arginine (NMMA), $^N\epsilon^N\delta$-dimethyl-L-arginine (asymmetric dimethylarginine; ADMA), and $^N\epsilon^N\gamma$-dimethyl-L-arginine (symmetrical dimethylarginine; SDMA) (Fig. 3). All PRMT identified to date can monomethylate arginine residues in proteins. However, further dimethylation of NMMA to form ADMA and SDMA is catalyzed by type-I and -II PRMT, respectively. Most PRMT genes encode type-I PRMT, but Janus kinase binding protein-1 [32] and an estrogen receptor $\alpha$ activator [33] have recently been shown to be a type-II PRMT. All of the arginine methylation reactions involve the modification of guanidino nitrogen atoms and require S-adenosylmethionine. When proteins are degraded by proteases and peptidases, free methylarginines (NMMA, ADMA, and SDMA) are formed. A majority of free NMMA and ADMA produced in the body is metabolized by dimethylarginine dimethylaminohydrolase (DDAH), of which two isoforms have recently been identified [34]. DDAH is widespread in mammalian tissues and cells, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and endothelial cells [34], and catalyzes the hydrolysis of the C–N bond in the methylated guanidine moiety of NMMA and ADMA to form citrulline and methylamines [35]. Concentrations of free NMMA, ADMA and SDMA in plasma are low in healthy subjects (<1 μM), but can be elevated in patients with various cardiovascular and other disorders, such as diabetes, renal failure, hypercholesterolemia, atherosclerosis, schizophrenia, and multiple sclerosis [36], suggesting a role for dimethylarginines in these diseases.

3. Metabolic roles of arginine

3.1. Roles of arginine in hormone secretion, proteolysis, gene expression, and oxidative defenses

Arginine stimulates the secretion of pancreatic hormones (insulin and glucagon), anterior pituitary hormones (growth hormone and prolactin), and placental lactogen in humans and animals [37], thereby regulating the metabolism of protein, amino acids, glucose, and fatty acids, as well as conceptus development. Also, arginyl-tRNA is not only an immediate precursor for protein synthesis but participates in the posttranslational conjugation of arginine with the
3.2. Roles of arginine metabolites

Arginine also exerts its metabolic roles through the production of diverse metabolites, including NO, ornithine, polyamines, proline, glutamate, glutamine, creatine, aminotriazine, and dimethylarginines (Fig. 1). These arginine metabolites play important roles in nutrition and physiology [5-7].

3.2.1. Nitric oxide

NO is the major endothelium-derived relaxing factor, a mediator of the immune response, a neurotransmitter, a cytotoxic free radical, and a widespread signaling molecule [2]. Thus, NO participates in virtually every cellular and organ function in the body. In blood vessels, NO released by endothelial cells activates guanylyl cyclase in smooth muscle cells, thereby elevating cellular cGMP concentrations and causing smooth muscle relaxation [2]. As such, physiological concentrations of NO produced by eNOS are essential for regulating vascular tone and hemodynamics. In addition, NO stimulates angiogenesis (the formation of new blood vessels from pre-existing vessels), which plays an important role in physiological events (e.g. wound healing, vascular remodeling, ovulation, and placental growth) and in pathological conditions (e.g. tumor growth, myocardial infarction, and diabetic retinopathy) [41]. NO inhibits leukocyte adhesion, platelet aggregation, superoxide generation, the expression of vascular cell adhesion molecules and monocyte chemotactic peptides, smooth muscle cell proliferation, and the release of endothelin-1 (a vasoconstrictor) [4]. Thus, NO is a novel vasodilator, antiatherogenic, antiproliferative, and antithrombotic factor.

It is important to recognize that NO is an oxidant and inhibitor of enzymes that contain an iron–sulfur center, and that NO reacts with H$_2$O$_2$ to form peroxynitrite (ONOO$^-$), another potent oxidant [40]. NO and peroxynitrite readily oxidize biomolecules (e.g. proteins, amino acids, lipids and DNA), which leads to cell injury and death. This cytotoxic effect of NO is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system, but is deleterious to mammalian cells and mediates the pathogenesis of many diseases, including the autoimmune destruction of pancreatic $\beta$-cells in type-I diabetes mellitus, arthritis, glomerulonephritis, and neurological disorders [42]. In addition, when excess NO is produced by all NOS isoforms under septic and inflammatory conditions, life-threatening hypotension occurs in animals and humans [2].

3.2.2. Ornithine and polyamines

Besides its role in hepatic ureagenesis, ornithine is an immediate precursor for the synthesis of putrescine, which is converted to spermidine and spermine (Fig. 2). Interestingly, a recent study reported a novel role for ornithine in mitochondrial integrity and function, as it inhibited cytochrome c release and apoptosis in MDA-MB-468 cells induced by $N^\omega$-hydroxy-L-arginine, an intermediate of arginine in the NOS reaction [43]. As antioxidants, polyamines protect cells from oxidative damage [44]. As polycationic compounds, polyamines participate in many cellular processes through binding with RNA, DNA, nucleotide triphosphates, proteins, and other negatively charged molecules.
Polyamines regulate gene expression, signal transduction, ion channel function, DNA and protein synthesis, apoptosis, and N-methyl-D-aspartate (NMDA) receptor activity [44]. Thus, polyamines are essential for cell proliferation, differentiation, and function. When cells are stimulated with growth factors, one of the first crucial events is the induction of polyamine synthesis, which precedes increases in DNA replication and protein synthesis. An increase in arginase I or II expression promotes the proliferation of endothelial cells and vascular smooth muscle cells, but depletion of cellular polyamines by inhibition of arginase or ornithine decarboxylase (ODC) arrests cell growth [21,22,24].

Note that an increase in cellular ornithine concentration owing to an OAT deficiency results in gyrate atrophy of the choroid and retina in adult humans and mice [45]. Thus, an excess of ornithine can lead to cell damage, but the underlying mechanism remains unknown. Decreasing circulating or tissue concentrations of ornithine will likely prevent gyrate atrophy. Indeed, a long-term (12 month) restriction of dietary arginine intake has recently been reported to substantially reduce plasma ornithine concentration and completely prevent retinal degeneration in OAT-deficient mice [46]. The metabolic roles of ornithine are likely cell- and tissue-specific.

3.2.3. P5C and proline

P5C produced from arginine-derived ornithine enters the cytosol for conversion to proline by the NADPH-dependent P5C reductase (Fig. 2), thereby regulating the cellular ratio of [NADPH]/[NADP+]. An increase in the conversion of P5C into proline stimulates glucose metabolism via the pentose cycle to increase the provision of both ribose-5-phosphate and NADPH in some cell types, such as tumor cells [47]. This results in enhanced synthesis of purines and cell proliferation. In addition, because NADPH is required for the synthesis of fatty acids, superoxide, and NO, and for the conversion of glutathione disulfide (oxidized glutathione) to glutathione (a major antioxidant) [40], the formation of P5C and proline from arginine plays a role in regulating cellular redox status. Furthermore, proline is a precursor for protein synthesis. Particularly, proline plus hydroxyproline (a posttranslational derivative of proline) account for one third of total amino acid residues in collagen protein [37]. As such, proline availability is crucial for collagen synthesis, extracellular matrix generation, wound healing, and tissue remodeling.

3.2.4. Guanidinoacetate and creatine

Guanidinoacetate is neurotoxic, and thus its conversion to creatine is of physiological importance. Creatine plays a major role in energy metabolism in skeletal muscle and neuronal cells. Recent studies have shown that creatine has antioxidant, antitumor, antiviral, and antidiabetic effects, and prevents hypoxic, ischemic, neurodegenerative, or muscle damage [27]. The methylation of guanidinoacetate to form creatine consumes more methyl groups than all other methylation reactions combined [48]. Thus, creatine synthesis from arginine plays an important role in regulating the availability of the methyl group donor for other methylation reactions, such as the synthesis of methionine from homocysteine, an independent risk factor for cardiovascular disease [30]. Indeed, dietary supplementation of creatine decreases, while dietary supplementation of guanidinoacetate increases, plasma homocysteine concentration in rats [48]. Dietary creatine supplementation, which decreases arginine/glycine amidinotransferase expression, has a potential to increase the availability of arginine for NO generation. Thus, creatine may be beneficial for cardiovascular function by increasing endothelial NO synthesis and reducing homocysteine production. The importance of creatine biosynthesis is graphically demonstrated by the finding that a recently identified deficiency of guanidinoacetate N-methyltransferase in humans causes a severe creatine deficiency and developmental abnormalities in muscle and brain during early infancy [49]. Also, arginine deficiency results in abnormal neuromotor behavior and impaired muscle development in mice [28,50].

3.2.5. Agmatine

Agmatine is a ligand for α2-adrenergic and imidazoline receptors in rat brain [51], thus serving as a signaling molecule. Agmatine has also been shown to stimulate glomerular filtration rate, inhibit the activities of all NOS isoforms, monoamine oxidase, and ODC, and reduce cell proliferation [6]. Because circulating concentrations of agmatine are relatively low compared with its concentrations used for in vitro and in vivo studies, the physiological significance of agmatine in humans and animals remains to be determined. As mentioned above, agmatine may be a novel substrate for polyamine synthesis in animal tissues, but experimental data are lacking.

3.2.6. Glutamate and glutamine

Glutamate participates in multiple biochemical reactions [52]. Glutamate is a key component of the glutamate/aspartate shuttle for the transfer of cytosolic NAD(P)H to mitochondria for oxidation in many cell types. Glutamate is a substrate for the synthesis of proteins, glutamate, γ-aminobutyric acid (GABA; another cell-signaling molecule), glutathione, and NAG (an allosteric activator of CPS-I), and is the principal excitatory neurotransmitter in the brain. As a blocker of the nonallosteric feedback inhibition of γ-glutamylcysteine synthetase by glutathione [53], glutamate upregulates glutathione synthesis in animal cells. As an inhibitor of kidney-type glutaminase, glutamate regulates glutamine hydrolysis by extrahe-
N.-acetylgalactosamine, and UDP-glutamate), aminosugars (glucosamine-6-phosphate, UDP-N-acetylgalactosamine, and UDP-N-acetylglucosamine, a precursor for the formation of all macromolecules containing amino sugars), nucleotides, NAD(P)⁺, glucose (both in liver and kidneys), urea (via glutaminase and glutamate dehydrogenase), GABA (via glutamate), and glutathione (via glutamate) [55,56]. Glutamine is a major fuel for rapidly dividing cells, including enterocytes, tumor cells, lymphocytes, and erythrocytes. Through renal ammoniagenesis by glutaminase, glutamine plays a crucial role in regulating acid–base balance. Interestingly, glutamine is required for iNOS expression in activated macrophages [30], and is an inhibitor of arginine synthesis from extraacellular citrulline [6] and endothelial NO production [56]. Through glutamine synthetase, glutamine serves as a scavenger of ammonia, a small molecule that is highly toxic to animal tissues, particularly the brain, probably due to the depletion of α-ketoglutarate in the Krebs cycle and an inhibition of NO-dependent blood flow. Glutamine may increase protein synthesis, inhibit protein degradation, and stimulate glycogen synthesis in skeletal muscle [55,57]. Importantly, glutamine participates in the intercellular glutamate–glutamine cycle between astrocytes and presynaptic neurons in the brain, the intercellular glutamate–glutamine cycle between periportal and perivenous hepatocytes in liver, and the interorgan transport of nitrogen and carbon primarily among skeletal muscle, liver, kidneys, small intestine, and lymphoid organs [55].

3.3. Nitrogen balance and metabolic needs

Arginine is a building block for tissue protein synthesis. It has recently been recognized that arginine is the most abundant carrier of protein nitrogen in animals [59]. Arginine must be provided from the diet to support nitrogen balance and the growth of strict carnivores (e.g., cats and ferrets) and birds [6,20]. Dietary arginine is also necessary for optimal growth of young omnivores including rats, pigs, and dogs [20,45,60]. In contrast, a dietary deficiency of arginine does not affect nitrogen balance in healthy adults, including humans, rats, pigs, and dogs [20,45,60]. Thus, on the basis of nitrogen balance and growth, arginine was originally classified as an essential (indispensable) amino acid for young mammals, and as a nonessential (dispensable) amino acid for healthy adults, including humans, rats, and pigs [61]. This definition, however, has significant conceptual and practical limitations, because nitrogen balance studies do not take into consideration the diverse, crucial roles of arginine metabolites (e.g., NO polyamines and creatine) in nutrition and physiology, and are not sufficiently sensitive to fully evaluate dietary requirements of arginine for metabolic needs by the body [6]. Indeed, adult mammals fed an arginine-deficient diet do exhibit metabolic, neurological, reproductive, and developmental disorders. For example, in mature dogs fed an arginine-free diet, there is both metabolic and clinical evidence of a severe arginine inadequacy, including hyperammonemia, increased urinary excretion of orotic acid, decreased food intake, sialorrhea, emesis, muscle tremors, and coma [20]. In addition, feeding an arginine-deficient diet to adult men for 9 days decreases sperm counts by ~90% and increases the percentage of nonmotile sperm by ~10-fold [62]. Furthermore, a maternal deficiency of arginine in adult female rats results in increased fetal resorption, retarded intrauterine growth, decreased number of live fetuses, and increased perinatal mortality [63,64]. These striking observations argue that functional needs other than nitrogen balance and growth should be an important criterion for the classification of arginine as an essential or nonessential amino acid for mammals, including humans. It is now recognized that arginine is a conditional essential amino acid for adult mammals (including humans
and rats), under such conditions as trauma, burn injury, massive small-bowel resection, and renal failure [12,37,65].

There is considerable debate about whether arginine is an essential amino acid for human infants [45]. On the basis of a nitrogen balance study involving only two term infants (1.5–3-week-old) and one preterm infant (3.5-month-old), Snyderman et al. [66] concluded that arginine was not an essential amino acid for human neonates. Unfortunately, this study involved only a limited number of enterally fed infants for a relatively short period (14–35 days). Additionally, neither metabolic indicators of an arginine deficiency (e.g. plasma concentrations of arginine and ammonia, and urinary excretion of orotic acid and ammonia) nor physiological parameters (e.g. cardiovascular, pulmonary, intestinal, muscular, immunological, and neurological functions) were measured by Snyderman et al. [66]. Feeding an arginine-deficient diet to infants likely results in a low plasma concentration of arginine in infants, which may affect all of the above metabolic and physiological parameters, as recently shown for arginine-deficient mice [28,50]. Remarkably, hypoargininemia and hyperammonemia frequently occur in preterm infants and term neonates maintained on total parenteral solution [19,67-69] likely due to inadequate synthesis of citrulline and arginine by the small intestine [3], and these neonates exhibit increased severity of respiratory distress syndrome, decreased systemic oxygenation, and increased incidence of necrotizing enterocolitis [68-70]. Importantly, recent studies have shown that increasing provision of exogenous arginine prevents hyperammonemia and necrotizing enterocolitis in preterm infants [71], persistent pulmonary hypertension in term newborn infants [72], as well as hyperammonemia and death in parenterally fed neonates [19]. These recent findings demonstrate that arginine is an essential amino acid for human infants maintained on total parenteral nutrition. It remains to be defined whether arginine is an essential amino acid for enterally fed human infants, as previously demonstrated for other young mammals (including rats and pigs).

An arginine deficiency results in hyperammonemia, increased plasma glutamine concentration, and a rapid increase in urinary excretion of orotate in growing mammals (e.g. rats, dogs, cats, mice, hamsters, rabbits, and guinea pigs) and some adult animals (e.g. dogs and cats) [20,45,61,63,74]. Arginine is an allosteric activator of NAG synthase, the enzyme catalyzing the conversion of NAG from carbamoylphosphate to glutamine by glutamine synthetase [6]. NAG is an essential cofactor for CPS-I, the enzyme catalyzing the formation of carbamoyl phosphate from ammonia and bicarbonate. In addition, arginine is an immediate precursor of ornithine, which is required for the conversion of carbamoylphosphate into citrulline by OCT. In the liver, when the fluxes through CPS-I and OCT are reduced due to a low availability of arginine, ammonia and carbamoylphosphate exit mitochondria into the cytosol, where ammonia is incorporated into glutamine by glutamine synthetase for carbamoylphosphate synthesis (Fig. 4). An increase in the cytosolic availability of carbamoylphosphate enhances the formation of orotate [73]. This metabolite is released by the liver into the circulation when the rate of its production is greater than the rate of its conversion to orotidine monophosphate, and then excreted by the kidneys (Fig. 4). Thus, urinary excretion of orotate provides a noninvasive, simple, and sensitive indicator of an arginine deficiency for young mammals and mature carnivores [20,45,61,74]. However, there is little information about the effect of dietary arginine intake on orotate production in humans. Interestingly, in adult patients with sickle cell anemia, a marked decrease in plasma arginine concentration (~53%) is associated with a three-fold increase in urinary excretion of orotate, as compared with age-matched healthy subjects [75]. We predict that urinary excretion of orotic acid is a useful indicator of arginine nutritional status in human infants. Clinical studies are required to support this proposition.

4. Bioavailability and safety of arginine administration

Dietary arginine intake by the average American adult has been estimated to be 5.4 g/day [45]. In both humans and
animals, owing to a relatively high arginase activity in the small intestinal mucosa, ~40% of dietary arginine is degraded during absorption and the remainder enters the portal vein [54,76]. Because the amino acid transport system y+ is virtually absent from hepatocytes, > 85% of the arginine delivered to the liver is not taken up by this organ [6]. On the basis of the digestibility of protein-bound arginine being 90% in adults and neonates [54], only ~50% of the dietary arginine enters the systemic circulation. Thus, intravenous arginine administration, which bypasses gastrointestinal metabolism, is a more effective means to provide exogenous arginine to extra-gastrointestinal tissues, compared with oral arginine administration. Nevertheless, both acute and chronic (e.g. 12 week) oral administration of arginine (15–21 g/day in three divided doses) increase plasma arginine concentrations in healthy and hypercholesterolemic humans [5,77,78].

Arginine is a stable nutrient in an aqueous solution, and is not destroyed by sterilization conditions (e.g. high temperature and high pressure). Arginine is not toxic, and its administration is generally safe for humans and animals. For example, intravenous arginine infusion (up to 0.5 g arginine-HCl per kg body wt for infants, or 30 g arginine-HCl for adults over 30–60 min) or oral arginine administration (9 g arginine-HCl per day for adults) has no adverse effects on humans [72,78,79]. However, higher oral doses of arginine-HCl are occasionally associated with nausea, gastrointestinal discomfort, and diarrhea [5], which may result from a rapid and excess production of NO by the gastrointestinal tract and from impaired intestinal absorption of other dietary basic amino acids (e.g., lysine and histidine). A solution to this potential problem may be the alternative use of L-citrulline, an effective precursor for arginine synthesis [30]. As a neutral amino acid, L-citrulline does not compete with basic amino acids for transport by cells, its conversion to arginine consumes 1 mol of ammonia in the form of aspartate, and its administration does not require equimolar HCl [4]. Thus, enteral or parenteral L-citrulline may be particularly useful for patients with elevated ammonia concentrations, impaired arginine transport, or enhanced intestinal arginine catabolism.

5. Clinical applications of arginine

The knowledge of arginine metabolism has provided a biochemical basis for its use to prevent and treat a wide array of human diseases and to develop pharmacotherapeutic strategy. A defect in NO synthesis from arginine results in abnormalities in nervous, muscular, circulatory, respiratory, digestive, urinary, reproductive, endocrine, and immune systems [3-5]. As such, increasing arginine provision is likely beneficial for patients with these disorders. In contrast, excessive production of NO is destructive to cells and may mediate the pathogenesis of autoimmune diseases, allograft rejection, and septic shock [42]. Likewise, physiological concentrations of polyamines are essential to proliferation and differentiation of mammalian cells, but excessive production of these metabolites due to overexpression of ODC promotes tumor growth [3,6]. Thus, it would not be advisable to administer arginine to patients with severe infections, active inflammatory or autoimmune disorders, active malignancy (e.g. late stages of tumorigenesis), or pathological angiogenesis.

Compelling evidence shows that enteral or parenteral administration of arginine reverses endothelial dysfunction associated with major cardiovascular risk factors (hypercholesterolemia, smoking, hypertension, diabetes, obesity/insulin resistance, and aging) and ameliorates many common cardiovascular disorders (coronary and peripheral arterial disease, ischemia/reperfusion injury, heart failure, erectile dysfunction, pre-eclampsia, and sickle cell anemia) [3-5,80]. In addition, dietary arginine supplementation decreases plasma glucose concentration in diabetic rats [81] likely due to NO-mediated increases in blood flow, glucose uptake by skeletal muscle, and insulin sensitivity in tissues. Arginine administration is also beneficial for improving reproductive, pulmonary, renal, gastrointestinal, liver, and immune functions, and for facilitating wound healing [3,37]. These effects of arginine are summarized in Table 1. Most of the beneficial effects of exogenous provision of arginine have been ascribed to increased plasma and intracellular concentrations of arginine for the synthesis of NO, proline, polyamines, and protein (Table 1). In addition, glutamate, GABA, creatine and perhaps agmatine may also mediate the effects of arginine on the muscular and nervous systems. A better understanding of arginine metabolism via NOS, arginase, and other pathways will help in the design of effective therapeutic interventions for various diseases.

6. Concluding remarks and perspectives

An important concept that has emerged from this review is that arginine displays remarkable metabolic and regulatory versatility in mammals. Thus, the beneficial or destructive roles of arginine critically depend on the relative activities of arginine-catabolizing enzymes, and precise regulation of these enzymes has important implications for health and disease. Much of the data regarding the role of arginases in regulating NO, polyamine, proline and glutamate synthesis have been generated from in vitro studies [6,7,82], and need to be verified in vivo. This can be accomplished using the recently developed arginase-I and -II knock-out mice models [83,84]. In addition, the recent cloning of cDNA for mouse NAG synthase [85], a key
Roles of arginine in mammals and possible mediators

<table>
<thead>
<tr>
<th>Roles of arginine</th>
<th>Effect</th>
<th>Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hormone secretion</strong></td>
<td></td>
<td></td>
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<tr>
<td>Insulin and glucagon</td>
<td>↓</td>
<td>NO and PA?</td>
</tr>
<tr>
<td>Growth hormone and prolactin</td>
<td>↓</td>
<td>NO and Ornithine?</td>
</tr>
<tr>
<td>Placental lactogen</td>
<td>↓</td>
<td>NO and Ornithine?</td>
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<tr>
<td><strong>Reproduction</strong></td>
<td></td>
<td></td>
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<tr>
<td>Spermatogenesis and male fertility</td>
<td>↓</td>
<td>NO, PA and PS</td>
</tr>
<tr>
<td>Ovulation and ovarian steroidogenesis</td>
<td>↓</td>
<td>NO and PA</td>
</tr>
<tr>
<td>Embryo implantation and fetal growth</td>
<td>↓</td>
<td>NO, PA and PS</td>
</tr>
<tr>
<td>Placental angiogenesis and growth</td>
<td>↓</td>
<td>NO, PA and PS</td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Preeclampsia in human pregnancy</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Uterine contractility and preterm labor</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Endothelial dysfunction in patients with CVRF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Smoking, hypertension and diabetes</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Obesity/insulin resistance and aging</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Cardiovascular disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary and peripheral arterial diseases</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Ischemia/reperfusion injury</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Heart failure and stroke</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Immune function</strong></td>
<td></td>
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<tr>
<td>T-cell proliferation and B-cell maturation</td>
<td>↓</td>
<td>NO, PA and PS</td>
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<tr>
<td>Antibody production by B-cells</td>
<td>↓</td>
<td>NO, PA and PS</td>
</tr>
<tr>
<td>Killing of pathogens</td>
<td>↓</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Digestive disorders</strong></td>
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<td></td>
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<tr>
<td>Gastrointestinal and liver injury</td>
<td>↑</td>
<td>NO and PA</td>
</tr>
<tr>
<td>Necrotyzing enterocolitis</td>
<td>↑</td>
<td>NO and PA</td>
</tr>
<tr>
<td><strong>Nitrogen disposal</strong></td>
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<tr>
<td>Nitrogen balance</td>
<td>↓</td>
<td>PS</td>
</tr>
<tr>
<td>Hyperammononemia</td>
<td>↑</td>
<td>NAG and Ornithine</td>
</tr>
<tr>
<td>Orotic aciduria</td>
<td>↑</td>
<td>NAG and Ornithine</td>
</tr>
<tr>
<td><strong>Tumor growth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumorigenesis at early stages</td>
<td>↑</td>
<td>NO</td>
</tr>
<tr>
<td>Tumorigenesis at late stages</td>
<td>↓</td>
<td>NO, Proline, Ornithine and PS</td>
</tr>
<tr>
<td><strong>Cell signaling</strong></td>
<td></td>
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<tr>
<td></td>
<td>↓</td>
<td>NO, Glutamate, GABA, agmatine and DA</td>
</tr>
<tr>
<td><strong>Wound healing and angiogenesis</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>↓</td>
<td>NO, Proline and PS</td>
</tr>
<tr>
<td><strong>Skeletal muscle and brain function</strong></td>
<td></td>
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<tr>
<td></td>
<td>↓</td>
<td>Creatine, NO, Glutamate and PS</td>
</tr>
<tr>
<td><strong>Renal disease with systemic hypertenion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>NO</td>
</tr>
</tbody>
</table>

* Adapted from Wu and Meininger [4], Wu et al. [3], Barbul [37], and Cooke et al. [5]. CVRF, cardiovascular risk factors. DA, dimethylarginines. GABA, γ-aminobutyric acid. NAG, N-acetylglutamate. NO, nitric oxide; PA, polyamines; PS, protein synthesis. (?), unknown. ↓, Increase/improve. ↑, Prevent.

enzyme for hepatic ureagenesis [6] and perhaps also for intestinal synthesis of citrulline and arginine, will provide a powerful tool to study the molecular regulation of NAG synthase expression in mammals. Furthermore, because arginine and citrulline were recently found to be unusually abundant in porcine allantoic fluid (e.g. 4–6 mM arginine on day 40 of gestation) [86,87] and in ovine allantoic fluid (e.g. 9.7 mM citrulline on day 60 of gestation) [88], the roles of arginine or citrulline in conceptus development need to be defined. We predict that arginine or citrulline (via oral or intravenous provision) will provide an effective nutritional or pharmacotherapeutic treatment for a wide array of disorders in cardiovascular, reproductive, pulmonary, renal, digestive, and immune systems. This “arginine/citrulline solution” may hold great promise for improved health and well-being in humans and animals. We are also open to the exciting possibility that new roles for arginine in regulating the synthesis of NO and other biologically important metabolites remain to be discovered.

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References

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