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Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine

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Abstract

Nonessential amino acids are synthesized de novo and therefore not diet dependent. In contrast, essential amino acids must be obtained through nutrition since they cannot be synthesized internally. Several nonessential amino acids may become essential under conditions of stress and catabolic states when the capacity of endogenous amino acid synthesis is exceeded. Arginine and glutamine are 2 such conditionally essential amino acids and are the focus of this review. Low arginine bioavailability plays a pivotal role in the pathogenesis of a growing number of varied diseases, including sickle cell disease, thalassemia, malaria, acute asthma, cystic fibrosis, pulmonary hypertension, cardiovascular disease, certain cancers, and trauma, among others. Catabolism of arginine by arginase enzymes is the most common cause of an acquired arginine deficiency syndrome, frequently contributing to endothelial dysfunction and/or T-cell dysfunction, depending on the clinical scenario and disease state. Glutamine, an arginine precursor, is one of the most abundant amino acids in the body and, like arginine, becomes deficient in several conditions of stress, including critical illness, trauma, infection, cancer, and gastrointestinal disorders. At-risk populations are discussed together with therapeutic options that target these specific acquired amino acid deficiencies. (*Nutr Clin Pract*. 2017;32(suppl 1):30S-47S)

Keywords

arginine; glutamine; essential amino acids; sickle cell disease; trauma; arginase; hemolysis; myeloid-derived suppressor cells

Under normal metabolic conditions, nonessential (dispensable) amino acids are synthesized de novo and therefore not diet dependent, while essential (indispensable) amino acids must be obtained through diet since they cannot be synthesized internally.

However, several nonessential amino acids become essential under conditions of stress and catabolic states when the capacity of endogenous amino acid synthesis is exceeded. These are classified as conditionally essential amino acids. Emerging evidence shows that depletion of glutamine and arginine is conditionally essential in that clinically important deficiencies occur in certain illnesses and require dietary supplementation. In healthy individuals, glutamine and arginine are traditionally classified as nonessential amino acids, playing important roles beyond protein synthesis in regulating gene expression of both transcription and translational levels.[1,2] Dietary arginine is also required for maximum neonatal growth and embryonic survival and thus is essential in infancy, while dietary glutamine is indispensable for intestinal mucosal integrity.[3–6] This review focuses on the causes and consequences of acquired arginine and glutamine deficiencies, at-risk populations, and therapeutic options that target these deficiencies.

Physiology of Conditionally Essential Amino Acids

Arginine

Arginine is a conditionally essential amino acid involved in multiple pathways in health and disease.[7,8] Arginine becomes essential under conditions of stress and catabolic states when capacity of endogenous arginine synthesis is surpassed, including hemolytic anemias,[8–12] asthma,[13–15] pregnancy, and critical illness such as sepsis, burns, and trauma.[16,17] Arginine also plays a key role in the metabolic, immune, and reparative response to trauma.[18] Serving as a substrate for protein synthesis, L-arginine is the precursor for nitric oxide (NO), polyamines, proline, glutamate, creatine, and agmatine[19,20] (Figure 1). Arginine metabolism is highly compartmentalized because its enzymes are expressed to different extents in varying tissues and cell types. Since it is involved in multiple metabolic processes, an arginine deficiency has the potential to disrupt many cellular and organ functions.[21]

Arginine is derived from dietary protein intake, body protein breakdown (approximately 80%), or endogenous de novo arginine production in the kidneys (10%–15% of the total arginine production). Arginine is absorbed in the intestine, with the

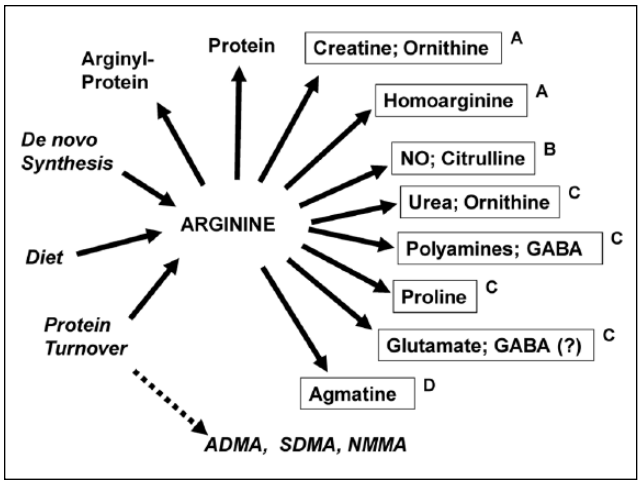

Figure 1. Sources and metabolic fates of L-arginine. Arginine is produced through de novo synthesis from citrulline primarily in the proximal tubules of the kidney, through protein turnover, or via uptake from the diet. The boxes indicate the arginine metabolites that are either immediately or ultimately generated as a consequence of the action of the following 4 enzymes that use arginine as substrate: (A) arginine/glycine amidinotransferase, (B) nitric oxide synthases (NOS), (C) arginases, and (D) arginine decarboxylase. Putrescine, spermine, and spermidine, which are the polyamines produced as downstream by-products of arginase activity. Turnover of proteins containing methylated arginine residues releases asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and N-methylarginine (NMMA), which are potent inhibitors of NOS. GABA, gamma-Aminobutyric acid; NO, nitric oxide. Reprinted with permission from Morris SM Jr. Arginine metabolism revisited [published online November 9, 2016]. *Am J Clin Nutr*. Copyright 2006 American Society for Clinical Nutrition. Figure in Morris[20] adapted with revised text from Morris SM Jr. Arginine: beyond protein. *Am J Clin Nutr*. 2006;83:508S-512S. Copyright 2006 American Society for Clinical Nutrition.

jejunum as the major absorption site, and exhibits a significant liver uptake and metabolism by arginase. Most whole-body arginine synthesis in adults is performed in a metabolic collaboration by the small intestines and kidneys in what has been termed the *intestinal-renal axis*.[22] Dietary glutamine plays an important role in this process, as 90% of circulating citrulline arises from glutamine.[23–25] Intestine-derived citrulline is released into the circulation and taken up primarily by the kidneys for arginine synthesis.[26] Therefore, damage or dysfunction of either organ system can compromise arginine bioavailability when requirements exceed production capacity: small intestinal inflammation, malabsorption, maldigestion, protein-losing enteropathy, or any condition where intestinal metabolic functions are impaired will affect availability and delivery of protein and amino acids to circulation, while renal dysfunction will affect de novo synthesis of arginine from citrulline in the kidney directly. Ultimately, global arginine bioavailability depends on the flux of other amino acids in the body, including glutamine and citrulline but also glutamate, ornithine, and lysine.[27]

Arginine is the sole substrate for NO synthesis by NO synthase (NOS) enzymes.[28] NO is a potent vasodilator essential for vascular health and homeostasis. NOS metabolizes L-arginine first to the intermediate N-hydroxy-L-arginine (NOHA) to form NO and L-citrulline. Several cofactors are necessary for normal NOS function in addition to adequate arginine bioavailability, including oxygen, nicotinamide adenine dinucleotide phosphate (NADP), tetrahydrobiopterin, and sufficient glutathione availability.[29,30]

Arginine is also metabolized by arginase, a urea cycle enzyme that catalyzes the hydrolysis of L-arginine to urea and L-ornithine, the precursor for polyamine production. Both arginase I and II isoforms are found in many cell types and are constitutively expressed in the human airways; arginase I is cytosolic and highly expressed in the liver, while arginase II is mitochondrial and extrahepatic.[31,32] Arginase I is also present in human erythrocytes, which has significant implications for hemolytic disorders, where it is aberrantly released into plasma in active form as the red blood cells rupture, mechanistically contributing to an acquired arginine deficiency syndrome.

Enzymatic properties of the arginase isozymes are very similar, with both efficiently catalyzing the conversion of arginine

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to ornithine and urea. While the affinity (K_m) of L-arginine for arginase is in the low micromolar range compared with the low millimolar range for NOS, substrate competition does occur between arginase and NOS because the speed of the reaction (V_{max}) for arginase is 1000-fold faster than NOS.[22] By competing for a common substrate, arginase reduces the bioavailability of L-arginine for NOS, therefore limiting NO production in a path toward endothelial dysfunction.

Arginase is also released as a result of inflammatory signaling and upon cell damage and cell death.[8,21,33] The liver contains the largest amount of arginase I in the body, highly concentrated in the cytosol of hepatic cells as part of the urea cycle. Arginase can therefore be considered a "liver function test"[34]; liver trauma or conditions inducing hepatic inflammation associated with elevated plasma levels of transaminases or other liver enzymes will also will give rise to hepatic-arginase release in its active form[35] into circulation with consequences on arginine bioavailability.

Finally, adequate arginine bioavailability plays an important role in normal immunologic function and host defense. Arginase I is constitutively expressed in granulocyte subsets (collagenase granules) and is released locally and/or systemically upon immune activation. In addition, immune activation during certain illnesses, through the release of mediators (eg, prostaglandin E1 and E2; interleukins 4, 10, and 13; catecholamines; and certain growth factors), induces the accumulation of a heterogeneous group of mostly immature myeloid cells that express arginase I. In immune organs, immature myeloid cells expressing arginase effectively deplete arginine from the surrounding environment. Arginine metabolism is involved in macrophage class transition from M1 (inflammatory; macrophages expressing NOS) to M2 (resolution of inflammation and healing macrophage; macrophages expressing arginase).[36] Arginine is an essential amino acid for normal proliferation and maturation of human T cells,[33,37–39] while arginine depletion will induce T-cell dysfunction and increase susceptibility to infection. This latter phenomenon has important implications for trauma and critical illness.

Arginine deficiency syndromes. There are at least 2 broad categories of arginine deficiency syndromes involving either T-cell dysfunction or endothelial dysfunction, depending on the disease state under which arginine deficiency occurs.[21] Whether hepatic, immune activation or from hemolyzed red blood cells (RBCs) during hemolysis or transfusion,[40–42] the clinical consequences of excess extracellular arginase will be similar[9,11] regardless of the cellular origin.

T-cell dysfunction. T-cell dysfunction has been linked to acute nutrition deficiencies in prior studies of trauma patients.[43,44] T cells are exquisitely sensitive to nutrition status, particularly to levels of key amino acids.[45] These include arginine, which is essential for naive T-cell activation, as well as glutamine and branched-chain amino acids, which are required for anabolic processes (eg, induced expression of relevant cytokines and receptors) needed for activated T cells to undergo polarization and properly implement their function.[33,38,39,46] T-cell proliferation is significantly blunted, production of interferon-γ and interleukin 2 is inhibited, and T-lymphocyte–mediated cytotoxicity and memory responses are nearly completely abolished when arginine is depleted.[47–49] The provision of arginine to culture media has been shown to restore T-lymphocyte function.[50] Plasma arginine levels drop acutely after trauma within minutes to hours,[16,51] while arginase activity is increased.[17,33,52–55] Plasma arginine levels may remain low for up to a week or longer in severe injury.[17,48] Accumulating evidence demonstrates that decreased arginine availability by myeloid-derived suppressor cells (MDSCs) is a cause of T-cell dysfunction after physical injury, coinciding with an induction of MDSC-expressing arginase I.[33,37,39] However, as previously mentioned, arginase is also released from other cell types with implications in trauma, including hepatocytes, as arginase concentrations correlate strongly to liver function enzymes.[8,9,12,21,56,57] Ultimately, decreased arginine bioavailability will inhibit T-cell function and potentially increase susceptibility to infection after injury.[16,18,33,39,49,53] Packed RBC transfusions often required in cases of more severe trauma will therefore compound the effect of immune-mediated arginase on the arginine metabolism and T-cell function[40–42] in patients injured by trauma with additional release of erythrocyte arginase, particularly when older-age blood is used.[58,59]

Hemolysis, arginine deficiency, and endothelial dysfunction. When released into plasma, the contents of RBCs disrupt vascular health and contribute to endothelial dysfunction.[12,60] Hemolysis itself will drive arginine consumption (Figure 2). Over the past 15 years, a paradigm has matured, linking hemolysis to vasculopathy in sickle cell disease (SCD),[11,61,65,66] with implications to other hemolytic conditions, including malaria,[67,68] thalassemia,[9,69] and even iatrogenic hemolytic processes like the transfusion of aged stored blood.[70,71] Rapid consumption and decreased production of NO is a fundamental aspect of this model. Under normal conditions, hemoglobin is safely packaged within the erythrocyte plasma membrane; however, during hemolysis, it is decompartmentalized and released into plasma where it rapidly reacts with and destroys NO.[72] This results in abnormally high NO consumption, the formation of reactive oxygen species, and a state of NO resistance.[60]

Plasma from patients with SCD contains cell-free hemoglobin, which stoichiometrically consumes micromolar quantities of NO and abolishes forearm blood flow responses to NO donor infusions.[72] NO interacts with hemoglobin in a reaction that is rate limited by diffusion to the heme group within hemoglobin[73]:

$$HbFe^{II}O_2 + NO \rightarrow HbFe^{III} + NO_3^-.$$

This dioxygenation reaction occurs when oxygenated hemoglobin reacts with an NO to form methemoglobin (FE^{III}) and nitrate and effectively destroys NO activity. Extracellular

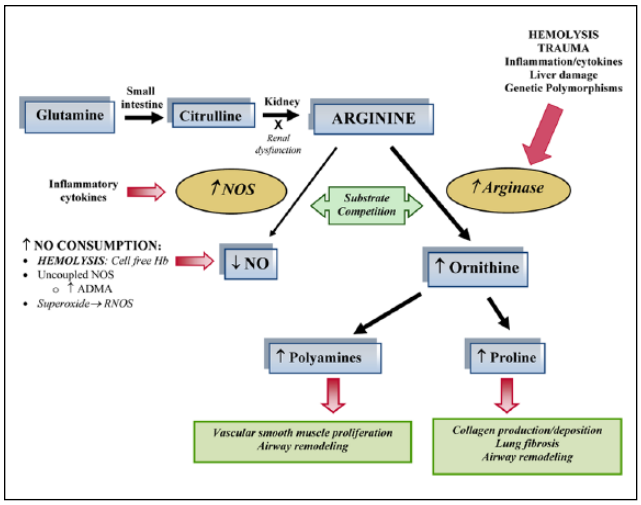

Figure 2. Altered arginine metabolism in hemolysis. Dietary glutamine serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway. Arginine is synthesized endogenously from citrulline primarily via the intestinal-renal axis. Arginase and nitric oxide synthase (NOS) compete for arginine, their common substrate. In sickle cell disease and thalassemia, bioavailability of arginine and nitric oxide (NO) are decreased by several mechanisms linked to hemolysis and oxidative stress. Endothelial dysfunction resulting from NO depletion and increased levels of the downstream products of ornithine metabolism (polyamines and proline) likely contribute to the pathogenesis of lung injury, fibrosis, and pulmonary hypertension. This disease paradigm has implications for all hemolytic processes. ADMA, asymmetric dimethylarginine; RNOS, reactive nitrogen oxide species. Reprinted with permission from Morris CR. Mechanisms of vasculopathy in sickle cell disease and thalassemia. *Hematol Am Soc Hematol Educ Program*. 2008:177-185. Copyright 2008 American Society of Hematology.

hemoglobin also consumes NO approximately 1000-fold more rapidly than cytoplasmic hemoglobin, producing a state of decreased NO bioavailability.[72] As such, NO scavenging has been a major contributor to pathological consequences of many blood substitutes that involve hemoglobin-based oxygen carriers.[74] In vivo, the ability of hemoglobin to react with NO produced by endothelium is limited by compartmentalization of hemoglobin within the erythrocyte. Another major contribution of reduced NO scavenging by RBCs is thought to be due to the cell-free zone, where blood flow leads to a pressure gradient that pushes RBCs to the center of vessels and away from the endothelium where NO is made. Free radicals like superoxide elevated in SCD will further consume NO.[75] Ultimately, the process of hemolysis disrupts NO homeostasis and is a key feature to endothelial dysfunction in SCD.[60]

The simultaneous release of erythrocyte-arginase metabolizes arginine during hemolysis,[12] further diminishing NO bioavailability. Asymmetric dimethylarginine (ADMA) is an arginine analogue and competitive NOS inhibitor[76] that is also released from the erythrocyte during hemolysis,[77,78] further compromising arginine bioavailability. Formation of superoxide from enzymatic oxidases such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase,[75] and uncoupled endothelial NOS[79] will also react with and scavenge NO, further amplifying a state of NO resistance. Consequently, smooth muscle guanylyl cyclase is not activated and vasodilation is inhibited. NO destruction by hemoglobin can also cause further impairment in vascular endothelial function via activation of adhesion molecules and potent vasoconstrictors such as endothelin 1.[60] This phenomenon, foreshadowed by evidence of NO scavenging and oxidant stress with early generation blood substitutes,[80] has now been implicated as a mechanism of NO depletion in the RBC storage lesion[70] and other hemolytic conditions such as thalassemia, malaria, and paroxysmal nocturnal hemoglobinuria.[67,81–83]

SCD. SCD, an inherited hemolytic anemia, is a quintessential arginine deficiency syndrome.[21,84] Normal arginine metabolism is impaired through various mechanisms (Figure 3) that contribute to endothelial dysfunction, vaso-occlusion, pulmonary complications, risk of leg ulcers, and early mortality.[8,11] The arginine dysregulation in SCD occurs as a result of increased destruction, decreased production, and intercellular transport anomalies[8,11] and will manifest similarly in other hemolytic conditions. Excess arginase activity is the hallmark of the arginine-deficient state of SCD (Figure 4).[12]

Adults with SCD are arginine deficient at steady state,[85–87] while children have plasma levels that are similar to pediatric normal controls.[87] The arginine deficiency develops with age and is likely the consequence of longstanding hemolysis over time. Although the altered arginine metabolome differs in children compared with adults, plasma arginine concentration decreases significantly in all ages during acute disease-specific complications and is associated with low NO metabolite levels.[87–89] Of interest, low plasma arginine levels predicted clinical need for admission in children with SCD and pain presenting for emergency care, while plasma NO metabolite levels did not,[87] suggesting a role for arginine bioavailability during pain events that goes beyond NO production.

Uptake of arginine into the cells occurs via the cationic amino acid transporter (CAT) protein, which is also responsible for ornithine and lysine uptake. Plasma arginine levels in adults with SCD are approximately 40–50 μM at baseline,[87,88] low compared with normal controls (80–100 μM) and well below the affinity constant (K_m) for the CAT protein (100–150 μM). Accordingly, even mild fluctuations in extracellular arginine concentration may significantly affect cellular arginine uptake and bioavailability. Also, the fact that both ornithine and lysine share the same CAT protein with arginine makes them obligate competitive inhibitors.[8] Intracellular arginine transport can be further compromised due to elevated ADMA, a competitive inhibitor of arginine transport known to be increased in SCD.[77]

Global arginine bioavailability ratio: a biomarker of arginine deficiency? SCD-related arginine deficiency is most remarkably associated with elevated arginase activity and a low arginine/

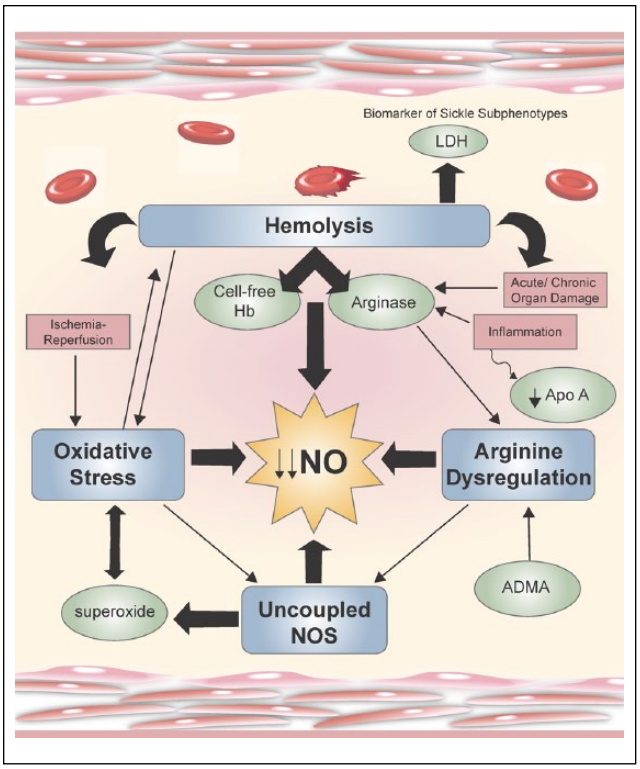

Figure 3. Mechanisms of endothelial dysfunction. Hemolysis, arginine dysregulation, oxidative stress, and uncoupled nitric oxide synthase (NOS) are key mechanisms that contribute to the complex vascular pathophysiology of sickle cell disease and other hemolytic conditions. These events limit nitric oxide (NO) bioavailability through several paths that ultimately provoke increased consumption and decreased production of the potent vasodilator, NO. Although often discussed independently, there is significant overlap closely linking these pathways of endothelial dysfunction that prohibit determining cause and effect. The contribution of inflammation coupled with antioxidant, glutathione, and apolipoprotein A-1 (Apo A) depletion; ischemia-reperfusion injury; and acute as well as chronic end-organ damage obscures mechanistic boundaries further. During hemolysis, cell-free hemoglobin and arginase are simultaneously released from the erythrocyte and profoundly contribute to low NO bioavailability. Lactate dehydrogenase (LDH) is also released from the erythrocyte and represents a convenient biomarker of hemolysis. ADMA, asymmetric dimethylarginine; Hb, hemoglobin. Reprinted with permission from Morris CR. Mechanisms of vasculopathy in sickle cell disease and thalassemia. *Hematol Am Soc Hematol Educ Program*. 2008:177-185. Copyright 2008 American Society of Hematology.

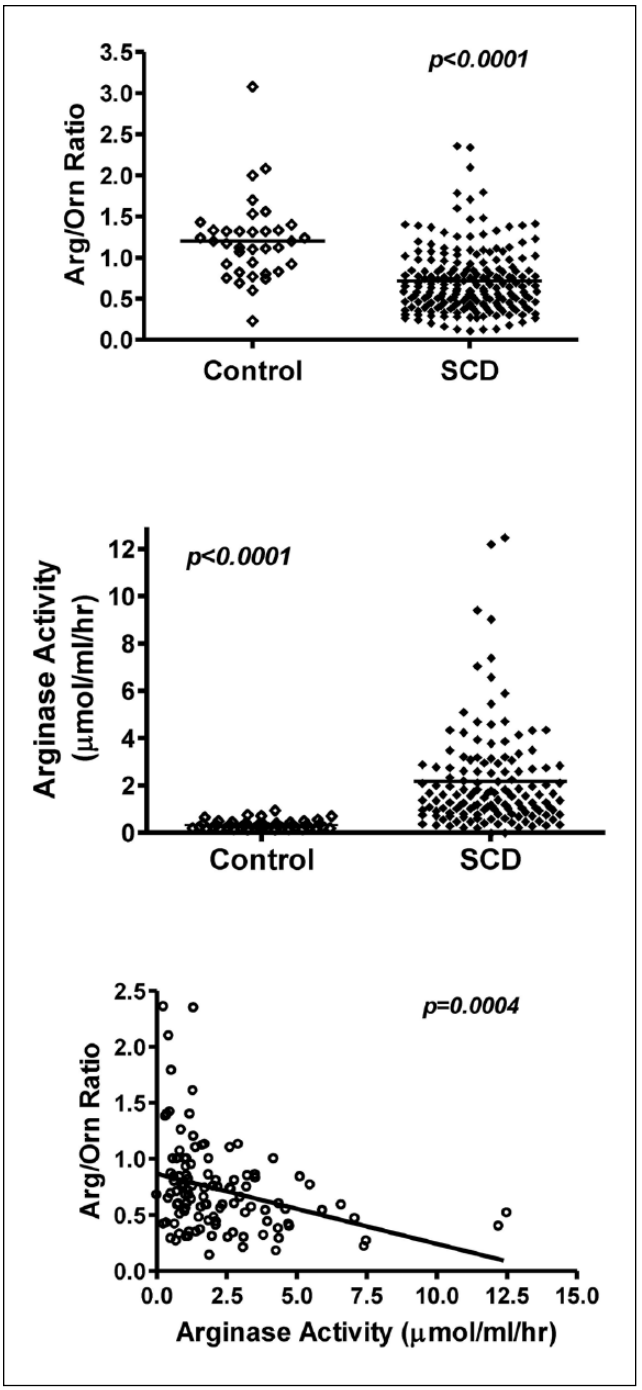

Figure 4. Association of arginine/ornithine (Arg/Orn) ratio with plasma arginase activity in patients with sickle cell disease (SCD). (A) Arg/Orn ratio in controls vs patients with SCD. (B) Plasma arginase activity in controls vs patients with SCD. (C) Correlation of plasma arginase activity to Arg/Orn ratio. Reprinted with permission from Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA*. 2005;294(1):81-90. Copyright 2005 American Medical Association.

ornithine ratio[84,90] that correlates to markers of hemolysis.[8,84,91] The arginine/ornithine ratio also correlates to mortality in SCD[84,92] and may represent an easily attainable blood biomarker of arginase activity and disease severity. Given that de novo synthesis of arginine occurs from citrulline in the kidneys, including citrulline in the ratio (arginine/[ornithine + citrulline]), termed the *global arginine bioavailability ratio* (GABR), escalates the value of this analysis to identify increased risk of death by taking into account the impact of renal dysfunction on arginine bioavailability[12,93] (Figure 5). The GABR is a more accurate reflection of arginine bioavailability than plasma arginine alone,

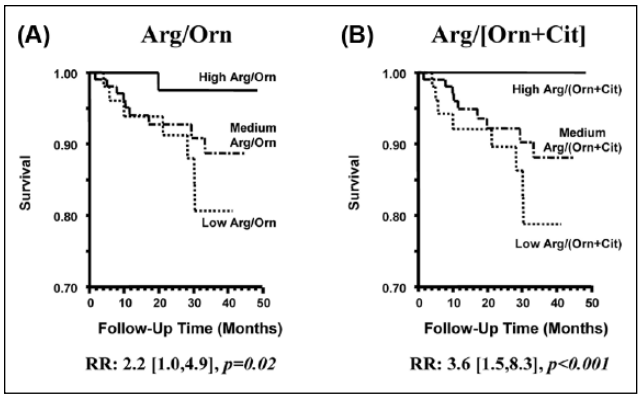

Figure 5. Association of global arginine bioavailability ratios (GABRs) with mortality in sickle cell disease: Kaplan-Meier survival plots. (A) Survival for 3 categories of the arginine/ornithine (Arg/Orn) ratio: high = upper quartile, >0.8690; medium = 25th–75th percentiles, >0.4385 and ≤0.8690; low = lower quartile, ≤0.4385. (B) Survival for 3 categories of the arginine/(ornithine + citrulline) (Arg/[Orn + Cit]) ratio: high = upper quartile, >0.6254; medium = 25th–75th percentiles, >0.3245 and ≤0.6254; low = lower quartile, ≤0.3245. Reprinted with permission from Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA*. 2005;294(1):81-90. Copyright 2005 American Medical Association.

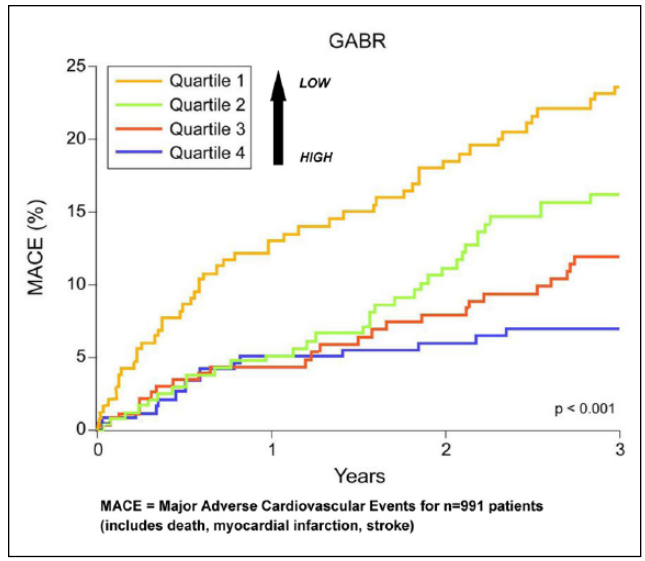

Figure 6. Kaplan-Meier survival analysis for patients with 3-year incidence of major adverse cardiac events (MACE) according to global arginine bioavailability ratio (GABR) by arginine quartiles. Low GABR is associated with increased risk of nonfatal myocardial infarction, nonfatal stroke, or death within 3 years of follow-up. Reprinted with permission from Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J Am Coll Cardiol*. 2009;53(22):2061-2067. Copyright 2009 Elsevier.

considering the complexity of intracellular arginine pools and compartmentalization of arginine. It is interesting to note that independent of SCD, low global arginine bioavailability is associated with major adverse cardiovascular events, including mortality in patients screened for cardiovascular disease[94] (Figure 6) and mortality risk in malaria,[67] and is associated with pulmonary hypertension risk.[95–97] More recently, a low GABR has also been implicated in additional conditions of endothelial dysfunction, including cardiopulmonary dysfunction in patients with thalassemia,[9] heart failure,[98] advanced left ventricular diastolic dysfunction, increased severity of right ventricular systolic dysfunction, and poorer long-term adverse clinical outcomes in nonhemolytic patients with chronic systolic heart failure[98] and diabetes.[99] Low global arginine bioavailability may be exacerbated further by the presence of elevated ADMA, an established biomarker of cardiovascular disease. Circulating ADMA levels are high in several conditions of endothelial dysfunction, including SCD[77,100,101] and thalassemia,[102] and are also linked to increased mortality.[77] Although hemolysis is clearly a path to creating an arginine deficiency syndrome, arginine deficiency is also induced through other mechanisms that contribute to endothelial dysfunction and cardiovascular disease.

Low arginine bioavailability itself will further compromise NO bioavailability. Under conditions of low arginine or cofactor tetrahydrobiopterin availability,[103] NOS is uncoupled, producing reactive oxygen species in lieu of NO,[104] which will further consume NO. Upregulation of NOS would therefore enhance oxidative stress when arginine, tetrahydrobiopterin, or other NOS cofactors like glutathione are deficient[29,30,105] and NOS becomes uncoupled. This process is supported by studies in transgenic sickle cell mice that demonstrate that NOS activity is paradoxically increased[106] and uncoupled[79] in a disease state involving a marked decrease in NO bioavailability. SCD therefore represents a model of global disruption of the arginine-NO pathway through multiple mechanisms. Ultimately, patients who develop an amino acid deficiency are the most likely subgroup to benefit from amino acid replacement therapy. Future clinical trials should include screening patients with SCD for plasma amino acids to determine whether the presence and severity of arginine deficiency correlate with clinical response to nutrition interventions.

Glutamine

Glutamine is one of the most abundant amino acids in the human body, comprising approximately half of the free amino acids in the blood and muscle.[107] Classified as a nonessential amino acid, glutamine can be produced in the body by conversion from the amino acid, glutamic acid, primarily by the skeletal muscle and liver. Glutamine has important physiologic functions, mainly serving as a precursor in the synthesis of other amino acids and glucose for energy. In addition to its role as an arginine precursor, glutamine is also the precursor of nicotinamide adenine dinucleotide (NAD) and glutathione, therefore possessing an important antioxidant role as well. Glutamine

is an important oxidative fuel for rapidly proliferating cells such as those in the gastrointestinal tract, immune system, reticulocytes, and fibroblasts. Glutamine can also function as a signaling molecule, particularly under catabolic conditions, and enhances stress-induced heat shock protein expression, which improves cell survival against a variety of stressful stimuli.[108] Glutamine is naturally found in dietary protein and, like arginine, is a conditionally essential amino acid under conditions of catabolic stress and critical illness as well as in preterm infants.[109–111] Demands for glutamine in critical illness are met in part by skeletal muscle proteolysis and release of large amounts of glutamine to maintain normal concentrations in the plasma, resulting in depletion of glutamine stores. A low plasma glutamine level in critically ill patients in the intensive care unit (ICU) at the time of admission was found to be an independent predictor of mortality.[112]

Endogenous glutamine production can be impaired in situations of abnormal muscle protein metabolism, including severe burns and trauma.[5,110,113] Reduced concentrations of plasma glutamine have also been reported in children with hemolytic conditions, including SCD,[105] malaria,[114] and β-thalassemia,[115] which may be related to the growth impairment commonly observed in these children. Moreover, low erythrocyte glutamine levels may contribute to alterations in the erythrocyte redox environment, thereby increasing RBC vulnerability to hemolysis.[105] Of interest, we found that erythrocyte glutamine levels correlated inversely with pulmonary hypertension severity in patients with SCD, a complication linked to hemolytic rate.[105]

Glutamine also contributes to normal intestinal barrier function[6,116] and can become deficient in some intestinal diseases, including Crohn's disease, diarrheal illness, and short gut syndrome.[116] As mentioned earlier, glutamine is converted to citrulline in enterocytes as part of the "intestinal-renal axis" access. In humans, citrulline is an amino acid involved in intermediary metabolism, is not incorporated in proteins, and is not found in high concentration in the diet. The end products of glutamine metabolism are the main source of circulating citrulline, produced by small bowel enterocytes. For this reason, citrulline, like glutamine, also functions as an arginine precursor.[27]

Patient Populations Who May Benefit From Conditionally Essential Amino Acid Supplementation

A drop in concentration of any amino acid does not necessarily mean that a clinically significant deficiency exists. A nutrition deficiency is only present if the biological processes that depend on the nutrient are compromised and if this compromise leads to abnormal physiologic responses that are causative of poor clinical outcomes. Based on the principles of causality, the poor outcomes linked to the deficiency should ultimately be reversed by therapeutic replacement of that amino acid.[117] Accumulating data support the notion of an arginine deficiency syndrome that may benefit from supplementation in trauma[47,117] and in SCD[8] that meets these criteria of causality. For example, pulmonary hypertension[90] and leg ulcers[118–121] are SCD-related clinical phenotypes of arginine deficiency that are reversed with arginine supplementation. A number of additional candidate conditions, however, may benefit from targeted therapy with conditionally essential amino acids, although more research is warranted.

Critical illness is associated with an adaptive metabolic stress response that is characterized by muscle catabolism. A reduced supply of amino acids from the diet or increased demand for amino acids from catabolic disease will contribute to increased protein degradation from muscle, which is the largest reservoir of protein, to ensure bodily functions. A protein-enriched diet has been shown to increase protein synthesis and thereby improve the protein balance in the setting of increased protein catabolism during illness.[122,123] Wound burden also affects protein requirements, and adult studies demonstrate that commonly estimated protein requirements for wound patients are too low. Better tools and procedures for protein assessment and maintenance need to be implemented to achieve best clinical outcomes.[124]

Trauma carries a significant financial burden, with greater than 15 million injuries per year in the United States alone. Approximately 10% of all trauma patients develop wound infections, with infection risk increasing to 30% in those who remained in the ICU for over 48 hours. Infections are the leading cause of late organ failure and potentially contribute to 10% of all trauma-related deaths.[117] Infections dramatically increase morbidity of trauma patients and significantly increase length of stay, the need for institutional rehabilitation, and cost.[47,117] Like trauma, infections after elective surgery are also a major healthcare problem.[125,126] Strategies aimed at infection prevention after trauma should result in a significant decrease in morbidity, mortality, and cost.[117] Adults and children with critical illness, burns, T-cell dysfunction from chronic inflammatory diseases, trauma, and elective surgery represent patient populations who may benefit from conditionally essential amino acid supplementation.

The process of hemolysis creates an arginine deficiency syndrome.[8] Of hemolytic conditions, the role of arginine deficiency has been most extensively studied in SCD,[8] but those with malaria,[127–129] thalassemia,[9] or cardiopulmonary bypass and patients at risk for complications from blood transfusion, particularly of older blood products,[58] may also benefit from arginine supplementation.

Patients with intestinal compromise, malabsorption, certain chronic diseases like celiac disease and cystic fibrosis, or specific complications of pregnancy like preeclampsia may also benefit from nutritionally targeted approaches. An increased incidence of pulmonary hypertension in patients with SCD and thalassemia associated with increasing severity of arginine deficiency, particularly as indicated by a reduced GABR, may represent another population with an arginine deficiency syndrome.[9,12] The severity of pulmonary hypertension in SCD

and thalassemia is strongly associated with biomarkers of hemolytic rate, similar to the severity of arginine deficiency itself, and likely represents the common mechanistic denominator.[9,12] Low erythrocyte glutamine concentration is also associated with pulmonary hypertension in SCD.[105] Although pulmonary hypertension is a rare disease that occurs more frequently in all hemolytic conditions,[61,66] nonhemolytic pulmonary hypertension is also associated with a low GABR and excess arginase activity[95,130,131] and evidence of response to arginine replacement under certain conditions.[90,132–135] It is interesting to note that many pulmonary diseases are associated with increased arginase activity and an arginine deficiency, including cystic fibrosis, acute asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and bronchopulmonary dysplasia.[130] Further study is needed in these and in patients with endothelial dysfunction and cardiovascular disease to identify any potential benefits of targeted amino acid therapies. However, 1 study demonstrating increased mortality in the arginine arm of a randomized placebo-controlled trial of low-dose oral arginine (3 g, 3 times a day) argues against its use following acute myocardial infarction.[136] We speculate, however, that the low doses used in the study may have been insufficient to overcome the impact of arginase and ADMA on global arginine bioavailability, especially since previous studies have shown that low-dose arginine is unlikely to affect NO synthesis.[137]

Sepsis is a unique condition of impaired arginine bioavailability, at least in some patients,[138–141] that warrants caution given the association of excessive NO production as a potential causative factor in the pathophysiology of septic shock, and studies showed increased mortality by blocking NOS[142] or by using an arginine-fortified immunonutrition.[143] The arginine deficiency of sepsis is thought to be the result of decreased arginine uptake and an impaired arginine de novo synthesis from citrulline, in combination with an enhanced arginine catabolism by the upregulation of arginase and the inflammatory NOS (iNOS) in the immune response, similar to other arginine deficiency syndromes. More recent studies of arginine monotherapy in septic patients resulted in increased arginine concentrations and also increased NO production without adverse events.[144,145] Therefore, good-quality studies examining arginine monotherapy are essential to define the clinical usage of arginine in critically ill, septic patients.

Methods of Providing Conditionally Essential Amino Acids

Supplementation with amino acid monotherapy targeting the deficiency is a logical therapeutic option to restore decreased conditionally essential amino acids and can be provided through both enteral and parenteral routes. Immunonutrition is another option, which uses formulas with combinations of nutrients, including macronutrients, vitamins, minerals, and trace elements thought to affect inflammation and resistance to disease, typically fortified with arginine and/or glutamine. Mixed results have been reported across various disease states, which prohibit the establishment of a clear consensus.[33,126,146–148] However, data supporting benefits in subgroups of patients require further consideration, particularly when the specific amino acid deficiency has established clinical consequences. The American Society for Parenteral and Enteral Nutrition (ASPEN) recommends that patients who undergo major neck or abdominal cancer surgery, have trauma or burns, or are critically ill and on mechanical ventilation should receive enteral formulations that are supplemented with arginine, nucleic acid, ω-3 fatty acids, and antioxidants.[149]

For arginine-deficiency syndromes, improved arginine bioavailability through arginine supplementation, either orally or parenterally, may restore important physiologic processes, including organ perfusion, immune function, protein synthesis, and wound healing. However, metabolism of arginine by excess arginase may limit its potential to maximally affect NO synthesis.[8,11] Since glutamine and citrulline can serve as a precursor for the de novo production of arginine through the citrulline-arginine pathway,[22,24,150] they represent novel therapies for T-cell dysfunction in trauma and hemolysis-associated arginine-NO dysregulation that may bypass at least a portion of arginase metabolism. Oral citrulline supplementation has been shown to increase plasma arginine levels, while glutamine depletion, the main precursor of citrulline, depletes plasma citrulline,[151] thereby potentially modifying arginine bioavailability as well. L-citrulline supplementation dose-dependently increases plasma L-arginine levels in healthy human volunteers more effectively than equivalent doses of arginine itself.[152] In fact, data suggest that oral supplementation of citrulline may be more efficient than oral arginine supplementation during inflammatory conditions.[153] Citrulline also increases plasma arginine levels in SCD.[154,155] Interestingly, in a pharmacokinetics study, we found that 10 g of oral glutamine significantly increased both glutamine and arginine bioavailability in patients with SCD at risk for pulmonary hypertension within 4 hours,[150] while glutamine-enriched enteral nutrition (EN) improved arginine bioavailability in patients with multiple trauma.[156] Combination amino acid therapy also warrants consideration. In an animal model, coadministration of arginine and citrulline resulted in a more rapid increase in plasma arginine levels and marked enhancement of NO bioavailability than supplementation of the single amino acids alone.[157] In a murine model, glutamine supplementation improved bioavailability of glutamine and arginine in the fetal plasma and placental fluids.[158] Finally, improving arginine bioavailability through inhibition of arginase is an active area of interest. Very recently, an oral arginase inhibitor has been developed for human use with anticipated phase 1 clinical trials for solid tumors recruiting soon. The potential benefits of arginase inhibitors for arginine deficiency syndromes remain to be determined, but a promising recent study demonstrated that arginase inhibition improved NO bioavailability and attenuated systemic and pulmonary vascular endothelial dysfunction in transgenic mice with SCD.[159]

Therefore, arginase represents a potential therapeutic target in the treatment of cardiovascular dysfunction in hemolytic anemias.

Arginine Supplementation

On average, an individual is likely to consume 2–7 g/d arginine, with plasma arginine levels influenced by dietary intake of arginine.[160–163] L-arginine has been shown to be well absorbed orally in many animal and human trials, reaching peak levels in humans after approximately 2 hours after administration.[4,164,165] Arginine is largely metabolized by the liver and, after filtration by the kidney, is actively reabsorbed in the distal tubules. Utilization of plasma arginine depends on the status of the host (eg, stress, activity, nutrition, inflammatory state), and bioavailability will likely be limited in conditions of elevated arginase activity.[90] Dietary amino acids are usually metabolized within 4–6 hours, suggesting that multiple doses are needed, with bioavailability of arginine estimated to approach 50% after oral administration. A variety of dosing strategies has been used in human trials, with current trend using 6–30 g/d L-arginine in 3 divided doses,[90,137,165–167] with low toxicity.

Arginine therapy in SCD. Promising data on arginine monotherapy have been published from phase 2 randomized controlled trials for treatment of chronic refractory leg ulcers and vaso-occlusive pain in patients with SCD.[10,119,120] In transgenic mouse models of SCD, arginine supplementation inhibits the RBC Gardos channels,[168] reduces RBC density, improves perfusion, and reduces inflammation,[169] lung injury, microvascular vaso-occlusion, and mortality.[78,170,171] Arginine supplementation also has increased erythrocyte glutathione levels in both mouse[170] and human trials.[172] Mechanistically, low arginine bioavailability is associated with acute pain, pulmonary hypertension, leg ulcers, and early mortality.[8,10,84,90] Rapid healing of leg ulcers was reported with oral[90] and intravenous (IV) arginine-butyrate in patients with SCD and thalassemia.[118] A randomized controlled phase 2 trial of IV arginine-butyrate for patients with SCD and chronic recalcitrant leg ulcers confirmed the initial anecdotal observations.[120] Short-term oral arginine therapy improved pulmonary hypertension in SCD[90] and acutely increased both plasma and exhaled NO when administered to ethnically matched normal controls and patients hospitalized for pain.[88,173] Anecdotal cases of immediate improvement in SCD-associated priapism in the emergency department setting have also been reported.[8,174] When arginine is given to patients with SCD at steady state, a paradoxical decrease in NO metabolites occurs that is not overcome by higher doses,[88] clearly indicating that arginine is metabolized differently in SCD compared with control subjects. However, when arginine is given during an acute pain event, a robust dose-dependent increase in NO metabolites is observed.[88] This suggests that arginine is also metabolized differently in SCD at steady state compared with times of acute illness, including pain and acute chest syndrome.[87,88,173] Similar observations were made with respect to arginine pharmacokinetics in moderate compared with severe malaria, suggesting that a greater consumption of arginine may occur when the disease state is more severe.[127–129,175] Since elevated plasma arginase concentration and activity has been reported in SCD,[84,90,173] higher doses of arginine may therefore be necessary to reach maximum benefits for patients with SCD and other hemolytic disorders. In patients with moderately severe malaria, a bolus treatment of arginine in doses of 3, 6, or 12 g over 30 minutes significantly improved endothelial function.[68,175] Dosing simulations suggest, however, that continuous arginine infusions over 6, 8, or 12 hours maintain plasma arginine concentration above the K_m for CAT-1 for the duration of the infusion compared with bolus dosing. Bolus dosing provided concentrations above the K_m for 50% of the patients at 2 hours and only 25% at 3 hours.[175] It remains to be determined if continuous arginine infusions are superior to 30-minute bolus dosing of L-arginine in SCD, but this question is currently being studied in ongoing pharmacokinetics studies (clinicaltrials.gov identifier: NCT02447874).

In a single-center randomized, double-blinded, placebo-controlled trial of parenteral arginine therapy in children with SCD and pain requiring hospitalization,[10] a significant reduction in total parenteral opioid use by 54%, lower pain scores at discharge, and a clinically relevant trend in decreased length of hospital stay by 17 hours were reported in the arginine arm compared with placebo. A second phase 2 trial is currently under way (clinicaltrials.gov identifier: NCT02536170), and a multicenter phase 3 trial in children with SCD and pain is in development.

Arginine therapy in trauma. Arginine supplementation enhances wound healing following trauma and hemorrhagic shock.[7,176–178] Supplemental arginine in enteral feeding is readily absorbed and mainly metabolized into ornithine, presumably by arginase,[179] which may augment wound healing. Immunonutrition support with arginine can enhance inflammatory and immunologic responses in animal models and in humans.[180] Studies using commercial formulas with high arginine content in critically ill patients were associated with a significant reduction in infectious complications and a trend toward a lower mortality rate compared with other immune-enhancing diets, while other studies show potential harm in patients with septic shock.[181] However, methodological weaknesses are present in most published studies to date. The benefits of EN using arginine-fortified formulas are most significant in the surgical patients.[180] There is a paucity of data on nutrition support for the critically ill child, which remains a controversial topic.[182,183] One randomized controlled trial of an arginine/glutamine-fortified formula in 40 ventilated pediatric patients with traumatic brain injury showed no difference in outcomes compared with standard formula, but with a sample size of 20 children per arm, the study was insufficiently

powered to answer questions on mortality and other clinical outcomes. In this study, nitrogen balance by day 5 became positive in 69.2% vs 30.8% of children who received immunonutrition vs regular formula, respectively ($P < .05$).[184] This observation is important in light of a recent multicenter, prospective, cohort study of 1245 critically ill pediatric patients that demonstrated decreased 60-day mortality in those with adequate enteral protein intake.[123] However, the issue around nutrition in critically ill children represents a critical gap in our knowledge surrounding optimal treatment of these children.

Immunonutrition with arginine and ω-3 fatty acids. Arginine, when supplemented with polyunsaturated ω-3 fatty acids (PUFAs) and nucleotides, has consistently demonstrated a clinical benefit in patients undergoing surgery.[55,185–187] Docosahexaenoic acid (DHA) is a PUFA highly enriched in the brain and is recognized as an essential nutrient for proper development of brain function. Optimizing the nutrition DHA status in neural tissue may allow significantly improved resilience of the central nervous system to injury, optimizing recovery. Research demonstrates substantial anti-inflammatory and neuroprotective effects of DHA and eicosapentaenoic acid (EPA),[188–190] with evidence supporting improved mitochondrial function.[191] EPA improves muscle protein quality, specifically by decreasing mitochondrial protein carbamylation, a posttranslational modification that is driven by inflammation.[192] A better understanding of the observed benefits of EPA and DHA has recently been supported by discovery and elucidation of the specialized pro-resolving molecules (SPMs). These compounds, which are endogenously produced from EPA and DHA substrates, have conclusively and consistently shown to enhance resolution of inflammation, improve bacterial killing by macrophages, and promote tissue regeneration.[193] Nitration of PUFAs yields nitro derivatives (NO_2-FA). These pluripotent signaling molecules are generated in vivo as an adaptive response to oxidative inflammatory conditions and manifest predominantly anti-inflammatory signaling reactions.[194,195] The nitration of PUFAs by arginine represents an additional plausible mechanism of immunonutrition benefits. Most commercially available enteral formulas now contain PUFAs.

Glutamine Supplementation

Glutamine, administered both orally and parenterally, has been extensively studied[23,24,109,196,197] in many clinical scenarios, including trauma, the ICU setting, burns, low birth-weight infants, and in conditions that involve gut inflammation.[5,110,198–202] Many experts believe that glutamine supplementation is essential when parenteral nutrition (PN) is being provided.[203,204] Glutamine-enriched diets show good overall tolerance, improvement of immunologic aspects in multiple trauma patients, cost reduction in critically ill patients, and improvement of mucositis in postchemotherapy patients. The doses given and the duration of therapy vary widely depending on the pathologic condition, but intake of 20–30 g/d is generally well tolerated.[202] Over the past 10 years, clinical trials of glutamine supplementation in critical illness, surgical stress, multiple trauma, and cancer have shown benefit with regard to mortality, length of stay, and infectious morbidity,[156,205] while studies demonstrating lack of benefit have also been reported.[147] However, early studies were relatively small and from single centers. More recent multicenter, randomized controlled trials, including the REducing Deaths due to OXidative Stress (REDOXS) study and MetaPlus trial in critically ill adults, demonstrating evidence of harm with respect to increased mortality, have significantly dampened enthusiasm and give equipoise to the large body of supporting evidence,[206–209] leading to the 2016 ASPEN guidelines recommendation against use of glutamine in critically ill adults.[149] The REDOXS study provided both IV and enteral supplementation,[207] while other trials used one or the other exclusively. It appears that dose and route of administration clearly influence the benefit observed from glutamine administration.[5] Further research is needed to address the controversies.

Niihara and colleagues[210,211] and Zerez et al[212] have found glutamine therapy to be beneficial in SCD, using a dose of 30 g/d without adverse events. These studies demonstrated improvement in NAD redox potential in all patients with SCD investigated. Since low erythrocyte glutamine bioavailability is associated with severity of pulmonary hypertension risk in patients with SCD,[105] glutamine therapy may decrease oxidative stress and hemolysis as well. In addition, there were consistent reports of improved general clinical condition in such areas as energy level and chronic pain levels,[210–212] as well as decreased incidence of SCD pain episodes and hospitalization in phase 2 and phase 3 trials.[213] Although glutamine has been granted orphan drug status for SCD, it has not yet been approved by the Food and Drug Administration. Like arginine, glutamine is also available without prescription as a dietary supplement and may function as an arginine prodrug similar to citrulline.

Conclusion

Amino acid therapy for critical illness, trauma, and hemolysis-associated arginine deficiency represents a promising field for further exploration and clinical application. Alterations in both arginine and glutamine bioavailability have been established, associated with impaired cellular physiology related to endothelial dysfunction and T-cell dysfunction. Both arginine and glutamine bioavailability play a significant role in the metabolic, immune, and reparative response to trauma.[18] The most appropriate enteral formula for trauma patients and the critically ill has not yet been determined, but the benefits of arginine-fortified formulas are most significant in the surgical patients.[180] In SCD, arginine deficiency is associated with a clinical phenotype of acute pain, pulmonary hypertension, leg ulcers, and early mortality[8,10,84,90]; the impact of arginine

supplementation on complications of SCD more likely represents the treatment of a nutrition deficiency than a pharmacological effect. Low erythrocyte glutamine levels are also associated with pulmonary hypertension risk.[105] Promising data from phase 2 and phase 3 trials of arginine and glutamine supplementation in SCD[10,120,211,213,214] support the future use of these nutrition interventions.

The clinical phenotype of conditionally essential amino acid deficiency syndromes needs to be more accurately defined, mechanisms of action require further research, and biomarkers of deficiency are essential to identify subpopulations of patients who are most likely to benefit from targeted nutrition interventions.

Key Points

- L-arginine and L-glutamine are conditionally essential amino acids that become essential under conditions of stress and catabolic states when the capacity of endogenous amino acid synthesis is exceeded, including critical illness, trauma, hemolysis, and gastrointestinal disorders.
- Sickle cell disease and trauma represent arginine deficiency syndromes that may benefit from arginine replacement therapy.
- There are at least 2 broad categories of arginine deficiency syndromes involving either T-cell dysfunction or endothelial dysfunction, depending on the disease content under which arginine deficiency occurs.
- The global arginine bioavailability ratio may represent a novel biomarker of arginine deficiency and warrants further study.
- Arginine and glutamine-fortified immunonutrition is an attractive way to treat acquired amino acid deficiencies, but further study is needed to best identify subpopulations who will benefit while minimizing potential adverse events.

Statement of Authorship

C. R. Morris contributed to the conception/design of the review article, and drafted the manuscript; and J. Hamilton-Reeves, R. G. Martindale, M. Sarav, and J. B. Ochoa Gautier contributed to the interpretation of the data presented and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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Discussion

Robert G. Martindale: Isn't it true that only about 3% of the arginine goes into the nitric oxide pathway and most of it is by arginase? Can we modulate the amino acid turnover enough to run that 3% a little harder?

Claudia R. Morris: Yes, that is true. The complexity of arginine metabolism is important. Once you actually start converting arginine to nitric oxide by the enzyme nitric oxide synthase (NOS), you have an intermediate product made—N-ω-hydroxy-L-arginine (NOHA), which is a physiologic arginase inhibitor that will help push arginine metabolism toward nitric oxide production. When arginine levels are low, NOS will uncouple and produce superoxide in lieu of nitric oxide. Superoxide is a reactive oxidant species that will scavenge nitric oxide so you have both decreased production and increased consumption of nitric oxide. If you can sufficiently replenish arginine to recouple NOS, then you've got a functional enzyme with respect to nitric oxide production. Elevated levels of methylated arginine compounds like asymmetric dimethylarginine (ADMA) can also impact NOS function by acting as a competitive arginine analogue, thereby compromising cardiovascular health. This effect can be attenuated by arginine supplementation. So there are a number of mechanisms that are working together which can be influenced by supplementation with arginine, which in turn are going to affect the overall nitric oxide bioavailability. Vaso-occlusive pain is a major complication of sickle cell disease. Although sickle cell disease is an arginine-deficient syndrome at baseline, arginine levels drop acutely when patients present with a pain episode. We found that the plasma arginine concentration was predictive of need for admission in children when they showed up in the emergency department with vaso-occlusive pain. However, levels of nitric oxide metabolites were not. There's more to the whole arginine story beyond nitric oxide. Arginine is actually the substrate for 4 sets of enzymes, although NOS gets the most attention. When one considers the other aspects of arginine metabolism, including the formation of creatine and its potential impact on methylation, ornithine production by arginase, and other pathways where arginine acts as a substrate, it is easy to see that we're overly focused on nitric oxide.

Robert G. Martindale: We see low arginine levels in 2 conditions—in certain tumors like renal cell carcinoma, head and neck cancers, and about a third of the melanomas, and we also see low arginine in pregnancy. When is it pathologic and when is it not pathologic? Should we be replacing arginine in pregnancy? Should we allow low arginine to occur in tumors?

Claudia R. Morris: I wish I had the answer to that question. Part of arginine depletion is physiologic, contributing to NOS uncoupling that can be a protective immunologic response against infection or tumors. However, low arginine bioavailability can also cause some immune dysregulation and T-cell dysfunction as seen in trauma patients. One example is in pregnancy, where some dampening of the mother's immune response is actually critical for the health of the baby. But there are some early studies to suggest that severe arginine deficiency in pregnancy is actually a bad thing as well. So it may be a physiologic component of pregnancy that if at the extreme may benefit from arginine replacement. It's an area that needs more work. I'm not quite sure what to say about tumors. Sid Morris and I have had conversations about this in the sense of that being a theoretic concern for long-term arginine therapy. By giving arginine, are we going to trigger growth of some tumors? There's nothing in the literature to suggest that that is true. I think that for some tumors, arginine depletion is part of the host's own protective defense mechanism, particularly if there is an impact on tumor-arginase interactions that can impede tumor cell growth. But there's also the other potential consequence if your immune system is suppressed by a low arginine state, that tumor growth could be exacerbated. Just like in malaria, where the parasite is protected if your immune system

is depressed through arginase-generated arginine depletion, the ability to fight the infection or the tumor is compromised. There are groups that are looking at arginase inhibition as a therapy for cancer. So, outcome depends on what kind of tumor and the specific environment that you're dealing with.

Jill Hamilton-Reeves: Just to follow up on that point. Clinical context and timing are really important in interpreting what you're seeing in amino acid profiles. I really like the idea of looking at ratios. It seems like a very logical way to interpret what's going on in a complex system, with lots of moving parts. An analogy would be the ω-3 fatty acid index, which has been really helpful when you're trying to modulate inflammation. The global arginine bioavailability ratio is really provocative. I work in not sickle cell disease but in trauma. We've been looking at plasma amino acid profiles in trauma. One of the pieces that I've struggled with is that when we looked at the global arginine bioavailability ratio, it really wouldn't have been interpreted as favorably, because ornithine is so high. But when you want wound repair, I don't really see that as a bad thing. It is important to be mindful about the clinical context. As a nutritionist and dietitian, how do we translate this into monitoring people? There is the dogma that if you give a single or just a handful of amino acids at a time, then you can cause more disturbances overall in amino acid metabolism. So, practically, how can clinicians monitor and adjust amino acids?

Claudia R. Morris: As I mentioned, I do have some concerns about arginine fueling a fire with all this excess arginase in sickle cell disease that is released into plasma from the erythrocyte during hemolysis. As an emergency medicine physician, we see many patients with sickle cell disease and acute pain, and we have published data from a phase 2 randomized controlled trial that show benefits with respect to pain when we supplement with arginine acutely for a short time during their hospital stay. As far as longer term therapy, we need more clinical studies, but I think we have to worry about that balance. My research focus has been on arginine and glutamine therapy in sickle cell disease, but I've actually looked at the entire amino acid panel in this situation. Many of the essential and nonessential amino acids are actually low in patients with sickle cell disease in addition to arginine. All of those amino acid perturbations are going to have a physiologic effect. We were focusing initially on arginine and its metabolites, but then I looked at other amino acids that were abnormal. One of my collaborators from Oakland, Jung Suh, PhD, pointed out that one related cluster involved glutamine, which can function as an arginine precursor, and the amino acid substrates for glutathione, a potent antioxidant. It turns out that glutathione levels are low in the erythrocytes of patients with sickle cell disease, which makes them more vulnerable to hemolysis. By trying to look at the bigger picture regarding amino acids, that's how I also ended up studying glutamine and glutathione in our patients with sickle cell disease. The third cluster of abnormal amino acid patterns included tryptophan, tyrosine, and phenylalanine, important amino acid precursors for neurotransmitters. This was an interesting observation because many patients with sickle cell disease experience depression and may have other neurocognitive issues. This has not yet been well studied. There could be problems with just focusing on one area of amino acid depletion without looking at it more comprehensively.

Menaka Sarav: The kidneys play an important role in the citrulline to arginine conversion. Kidneys are also important in glutamine uptake and aminogenesis. When you're treating your patients with sickle cell, do you pay attention to chronic kidney disease (CKD)? Do you treat patients with CKD as a separate patient population?

Claudia R. Morris: Patients with sickle cell disease often have abnormal renal function at baseline, and it tends to worsen with age. In my arginine and glutamine trials, I excluded patients who had severe renal dysfunction, as an abundance of cation could lead to adverse events. What we've noticed in our patients with pulmonary hypertension is that more than half of them actually have renal dysfunction. You have all these complicated compensatory mechanisms to maintain your arginine bioavailability in health, which is why arginine is considered a conditionally essential amino acid. The body has the ability to synthesize arginine in the kidneys through the intestinal-renal axis. Sickle cell disease is a condition where you start losing those compensatory mechanisms one by one, leading to lower than normal plasma arginine levels. Once the kidney function goes, that's when we see arginine bioavailability drop significantly as mortality risk increases. When we're designing studies, there is concern that patients with renal dysfunction may not do well with these amino acid supplements, particularly arginine in the form of arginine hydrochloride, which theoretically may potentiate acidosis in patients with poor kidney function.

Menaka Sarav: CKD patients are not eating well, and then there's hemodialysis, which affects the amino acid composition.

Claudia R. Morris: We have a young nephrology fellow who has found interest in the arginine panacea. We're specifically looking at some of those questions and how it links to cardiovascular disease and CKD.

L. John Hoffer: Two questions I asked myself are whether arginine is a conditionally essential amino acid and whether glutamine is a conditionally essential amino acid? Also, who would benefit from administration of these particular amino acids? There's reasonably good data suggesting that in certain situations, large doses of arginine separate from the protein dose, can fuel certain metabolic pathways for specific targeted functions. These doses of arginine would be out of line with what would be taken in a normal diet. So we're really talking about pharmacology in a certain sense. In the

practice of clinical medicine, how does that fit into a diet? Arginine could be called conditionally essential, but I probably wouldn't use that language because it's more like a drug. When it comes to glutamine, I don't understand why anyone would think that it is a conditionally essential amino acid. For example, what's the glutamine turnover of a normal person measured with glutamine tracers? Let's say it is approximately 50 grams per day, maybe 60 grams per day. What's the glutamine turnover of a patient on PN that has no glutamine in it? Again it is about 50 or 60 grams per day. Why is that? Because glutamine is a nonessential amino acid. It's part of the nonessential pool of amino acids. That means every amino acid sends its nitrogen to bind with the infinite source of carbon to produce glutamine. We have enormous amounts of glutamine being produced in our bodies every day. So when I ask myself to derive the final suggestion for the purpose of our conference, how would we improve glutamine administration to our patients, I suggest it is by giving them more protein. Because protein has to be catabolized. If we're in neutral balance, we're catabolizing protein as fast as we're consuming it. And we're converting all of that protein to glutamine, at least potentially, if the body wants to do that. For me glutamine should be a nonissue. The glutamine trials that have been carried out used doses of glutamine that are a fraction of the endogenous production rate. If you want to give more glutamine or arginine to patients, you don't have to provide a specialized product; just give them more protein. Because in fact, the amounts of arginine present in most standard parenteral amino acid solution are quite generous.

Claudia R. Morris: Usually, I would agree with a more balanced, higher protein diet approach. However, sickle cell disease and trauma, for instance, are unique clinical scenarios where a true arginine deficiency syndrome may exist due to the consequences of pathologically excessive arginase. In situations of arginine deficiency, parenteral or enteral arginine monotherapy or the utilization of oral glutamine as an arginine prodrug may have clinical benefit.

Frederick A. Moore: I want to comment on this story about MDSCs and trauma. The MDSC story is applicable to many different diseases. It's part of what's called emergency myelopoiesis. It happens in cancer and it happens in sepsis. We just published a paper showing that MDSCs have increased expression or expansion, up to 28 days after severe sepsis, and that these MDSCs suppress T-cell proliferation. The persistent elevation in MDSCs is associated with nosocomial infections, prolonged ICU stays, and disposition to nonhome destinations. This MDSC story involves a lot of patients and, in particular, patients in sepsis. If you believe this MDSC story, then arginine actually is a very important thing to give septic patients. I agree that you're not going to give arginine to somebody who's in septic shock when iNOS is expressed. But the problem is when patients survive sepsis, which many people do, they are then set on to a trajectory of very poor outcomes that's related to a persistent inflammatory state that is driven by MDSCs. Arginine could be very beneficial in those patients.