Cellular and Physiological Effects of Arginine

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Abstract: Arginine is a semi-essential amino acid that is required during periods of maximal growth, severe stress, and injury. Arginine is a substrate for protein synthesis but also modulates cellular biochemical functions via conversion to a number of biologically active compounds. Arginine is utilized by a vast variety of metabolic pathways that produce a variety of biologically active compounds such as nitric oxide, creatine phosphate, agmatine, polyamines, ornithine, and citrulline. Arginine supply is primarily regulated by two enzyme systems: arginase (part of the urea cycle) and nitric oxide synthase. Arginine has many effects in the body that include modulation of immune function, wound healing, hormone secretion, vascular tone, insulin sensitivity, and endothelial function. Arginine mediates its effects via nitric oxide independent and dependent pathways. Nitric oxide modulates many cellular functions that include vascular tone, expression of adhesion molecules, leukocyte adhesion, and platelet aggregation. Arginine modulates the development of atherosclerotic cardiovascular disease, improves immune function in healthy and ill patients, stimulates wound healing in healthy and ill patients, and modulates carcinogenesis and tumor growth. Thus, arginine is a biologically active dietary compound with numerous physiologic and pharmacological activities.

INTRODUCTION

Arginine is a basic amino acid that carries a guanidinium group on its side chain and carries a positive charge at neutral pH [1]. It is considered to be a semi-essential amino acid; under normal conditions, the amount of arginine produced in the body is sufficient to maintain muscle and connective tissue mass. However, in times of immaturity and severe stress, endogenous synthesis of arginine is often insufficient to meet high demands. In these situations, arginine becomes an indispensable amino acid for optimal growth and maintenance of positive nitrogen balance [2].

Sources of arginine may be either exogenous (dietary) or endogenous, from whole body protein turnover, intestinal synthesis or *de novo* synthesis. The majority of plasma arginine is derived from protein metabolism and turnover. The kidney is the main site of net arginine synthesis, accounting for approximately 60% of these stores. Citrulline serves as the primary substrate, formed in the small intestine from the metabolism of dietary amino acids such as glutamine and glutamate. From the intestine, citrulline is transported to the proximal tubules where it is converted to arginine and released into the bloodstream [3].

De novo arginine synthesis accounts for only 5-15% of the endogenous supply and does not play a significant role in arginine homeostasis of the adult human. One site of arginine synthesis is the urea cycle of the liver. Tight regulation of metabolites and high levels of arginase, however, yields little if any net arginine for use by other body tissues [4]. In many other cell types, arginine may be synthesized for conversion to nitric oxide (NO) through what has been termed the citrulline/NO or arginine/citrulline cycle. The metabolic pathway for arginine is presented in Fig. (**1**).

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In infancy or times of severe stress, dietary sources of arginine play an ever important role in maintaining adequate stores. As mentioned, glutamine, glutamate and proline derived from the diet are metabolized in the small intestine to yield citrulline as a source for renal arginine production. Dietary arginine is also derived from various foods. Meat provides the largest contribution while wheat, milk, rice, corn, soy and nuts are also important sources[1]. Assuming a total daily protein intake of 100 grams/day, a typical American diet provides approximately 5.4 g/d of arginine [5]. However, only 40% of this remains available for absorption due to the relatively high arginase activity in the small intestinal mucosa. A substantial portion of dietary arginine is degraded by arginase during intestinal transit and absorption while the remainder enters the portal vein for use by the body [4].

In most mammalian cells, arginine requirements are met primarily by uptake of extracellular arginine via specific transport systems. The y^+ system is a high-affinity transporter of arginine, lysine and ornithine, and is considered to be the most important mechanism of arginine transport in most cells. The level of expression of system y^+ differs by cell type and may vary depending on cellular and tissue needs. Other systems involved in arginine transport include $b^{0,+}$, $B^{0,+}$ and y^{+L} [6, 7]. Genes encoding different proteins for these transport systems are found in different cell types, and their expression is regulated by specific stimuli such as stress and inflammatory cytokines [8]. The capacity for arginine transport increases to support NO synthesis, demonstrated by the fact that system y^+ expression is co-induced with inducible nitric oxide synthase (iNOS) in various cell types [9]. Conversely, other cationic amino acids and positively charged analogs may inhibit arginine uptake by system y^+. These include lysine,

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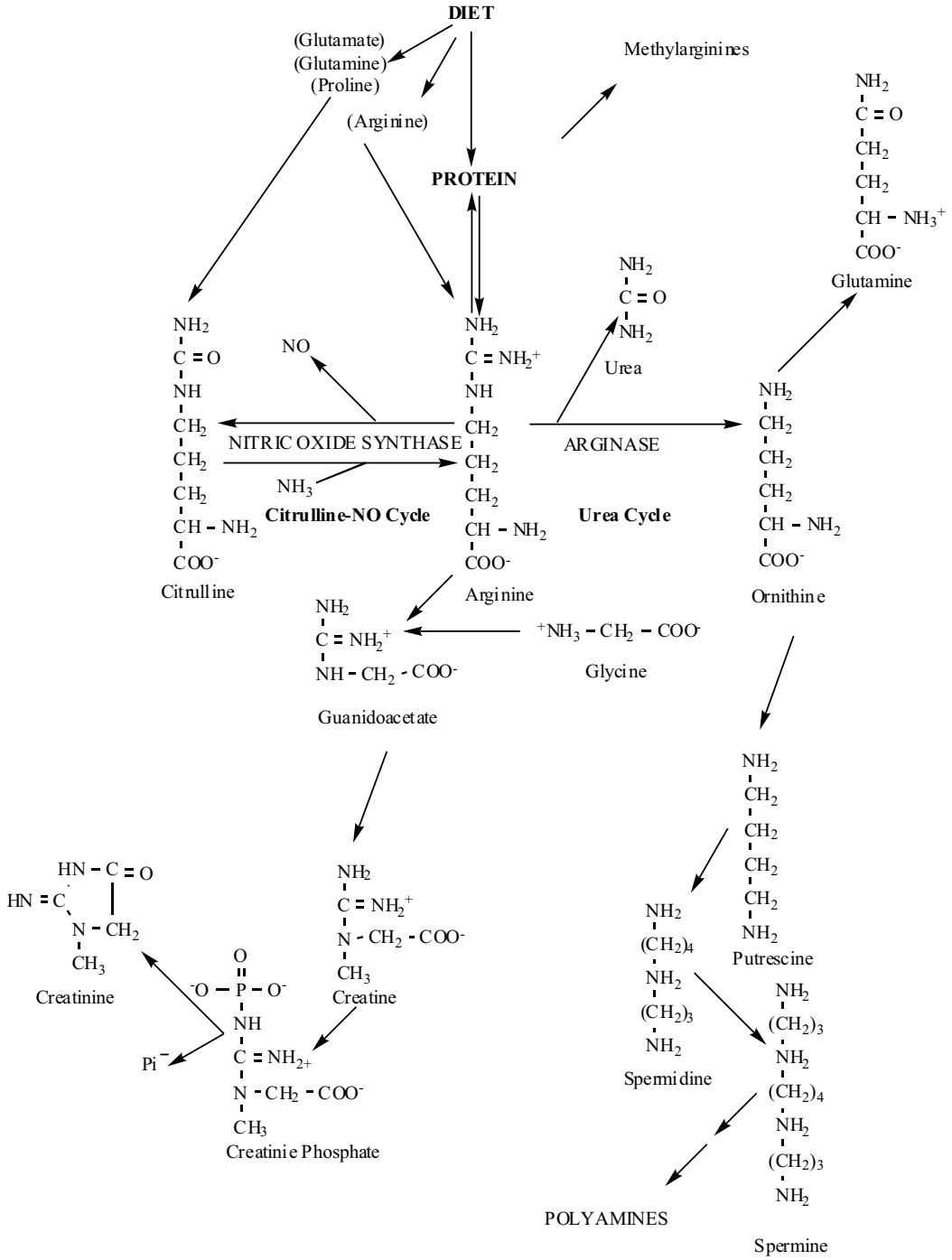

Fig. (1). Metabolism of Arginine

ornithine, canavanine and certain inhibitors of nitric oxide synthase (NOS) such as N-monomethyl-L-arginine and N-iminoethyl-L-ornithine [4]. In addition, NOS inhibitors that are taken up via system y^+ may be used to limit the availability of intracellular arginine.

ARGININE CATABOLISM AND METABOLIC PATHWAYS

Because it is used in metabolic processes of numerous systems, arginine may have one of several fates in the body. An important enzyme in arginine degradation is arginase, which facilitates the downstream conversion of arginine to urea, ornithine, proline, polyamines, glutamate and glutamine. There are two distinct types of mammalian arginase, Types I and II, which are encoded by separate genes. Type I arginase is a cytosolic enzyme with high levels of expression in the liver where it participates in the urea cycle by converting arginine to ornithine and urea. As a vehicle for the transport, storage, and excretion of nitrogen, arginine is essential for ammonia detoxification through the urea cycle, thus preventing metabolic derangements caused by elevations in tissue ammonia [3].

Type II arginase is a mitochondrial enzyme with low levels of expression in a variety of tissues, including the small intestine, kidney, brain, endothelium, mammary gland

and macrophages [10]. In these tissues, arginase-mediated conversion of arginine to ornithine is the initial step in polyamine synthesis and plays an important role in cellular proliferation. Ornithine is also used in proline synthesis, which is central to production of collagen and wound healing. This is illustrated by wound healing studies demonstrating that supplemental arginine increases collagen deposition and wound breaking strength [11]. Other metabolic products such as putrescine, spermine and spermidine are recognized as important components of cellular proliferation and differentiation, and depend on arginine as a synthetic precursor [12]. Finally, cells of the lactating breast use arginase-derived ornithine to produce glutamate, which is one of the most abundant amino acids in breast milk [4].

In perhaps one of its most important physiologic pathways, L-arginine is the unique substrate of nitric oxide synthase (NOS) in the production of nitric oxide. As a putative neurotransmitter and cytotoxic effector molecule, nitric oxide is also known as endothelium derived relaxing factor (EDRF), which causes vasodilatation of blood vessels [13]. With this knowledge, arginine has been proposed as a treatment for various cardiovascular diseases caused by endothelial dysfunction and limited blood flow, including congestive heart failure, coronary artery disease and impotence [14]. Other effects of arginine on the cardiovascular system are discussed below.

In another notable pathway, arginine : glycine amidinotransferase effects the transamidation between arginine and glycine to yield guanidinoacetic acid, which is then methylated to form creatine. After its release into the circulation, creatine is actively transported into skeletal muscle and nerves. It is subsequently phosphorylated to form creatine phosphate, a major source of ATP in muscle. Creatine eventually undergoes non-enzymatic and irreversible dehydration to form creatinine, which is distributed in the total body water and filtered by the kidneys. Urinary excretion of creatinine is the most widely used marker of renal function [4].

Yet another metabolic pathway for arginine involves agmatine, which is produced from arginine through the action of arginine decarboxylase. Only recently described in mammalian cells, the physiological roles of agmatine have yet to be fully elucidated. One such role may be in regulating cellular production of NO, as agmatine acts as a weak competitive inhibitor of the NOS isoenzymes [15]. Also, by inducing synthesis of antizyme, agmatine inhibits ornithine decarboxylase. Since ornithine decarboxylase is involved in the polyamine synthesis pathways, conversion of arginine to agmatine can negatively affect cell proliferation. In the central nervous system, agmatine binds to both α_2-adrenergic and imidazoline receptors, which also suggests a role in cell signaling [4, 16].

Clearly, arginine is involved in a number of important biochemical processes. However, arginine metabolism also has effects at the systemic level. Metabolic processes involving arginine influence the cardiovascular, endocrine and immune systems as described below. These processes are potential therapeutic targets for a number of clinical disease states including atherosclerosis, diabetes, sepsis and regulation of wound healing.

EFFECTS OF ARGININE ON THE CARDIOVASCULAR SYSTEM

One of the most important functions of arginine is in the cardiovascular system. As the lone substrate for the NOS enzymes, L-arginine is converted to NO and citrulline [17]. The primary function of NO is vasodilatation, in both the normal physiologic state as well as in pathologic states such as sepsis.

NO is generated by the nitric oxide synthases (NOS), of which there are three known isoforms. These enzymes contain regions of high sequence homology, yet there are features that reflect specific functions characteristic of each isoform. Neuronal NOS (nNOS) was the first of the isoforms to be identified and cloned. In addition to its presence in neuronal tissues, a role for nNOS in regulating vascular tone has also been identified [18]. eNOS, found in the vascular endothelium, provides constitutive levels of NO in order to maintain vascular tone. Endothelium-derived NO activates guanylyl cyclase to generate cyclic GMP, which causes smooth muscle relaxation.

eNOS and nNOS activity depend on calcium and calmodulin, as increases in intracellular calcium effect modest increases in NO production. Additional cofactors in this pathway include nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (BH_4), flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) [14]. Elevated extracellular arginine levels have also been shown to drive endothelial NO production.

While eNOS and nNOS are responsible for basal levels of NO production, iNOS, the inducible form of nitric oxide synthase, increases NO production in response to inflammatory stimuli. In infection, the presence of bacterial endotoxin activates a systemic inflammatory response, generating various inflammatory cytokines. The endotoxin and cytokines have potent effects of activating iNOS in the production of NO. In this manner, significantly increased levels of NO may lead to pathologic vasodilatation and hypotension. As a result, NOS inhibitors have been investigated for possible therapeutic roles in the treatment of sepsis [19]. Other effects of arginine on the immune system are discussed below.

Other NO-Dependent Effects

There are several other NO-dependent effects of arginine on the cardiovascular system. In general, these effects reduce the degree of endothelial dysfunction associated with cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes, smoking and obesity. Basal release of NO is essential for control of blood pressure; in both animals and humans, arginine or NO deficiency results in hypertension. Mice with deficient eNOS have elevated blood pressure as well as demonstrated insulin resistance. In normal animals, long-term inhibition of NOS induces severe hypertension. However, in models of hypertensive animals, administration of L-arginine has been shown to attenuate increases in both systolic and diastolic blood pressures [20].

In humans, intravenous arginine infusion reduces blood pressure and renal vascular resistance in essential hypertensive patients who have normal or insufficient renal

function [21]. Endothelial dysfunction resulting from either pulmonary or salt-induced hypertension can be prevented by administration of either oral or intravenous arginine [22]. Finally, studies of infants with pulmonary hypertension have demonstrated improved systemic oxygenation, reduced pulmonary vascular resistance and increased cardiac output when treated with arginine infusion [23].

Cigarette smoke induces endothelial dysfunction through the actions of oxygen-derived radicals and prooxidants, especially in the aorta and coronary vasculature. Studies have demonstrated that NO synthesis is impaired in the presence of cigarette smoke. This is the basis for using supplemental arginine in an attempt to reverse these changes. Indeed, in both human and animal models, administration of oral arginine prevents smoking-induced endothelial dysfunction. Even in long-term smokers, coronary vasomotor function can be normalized with intravenous arginine [22].

Hypercholesterolemia, a known risk factor for atherosclerotic disease, is also associated with endothelial dysfunction. An early event in the development of atherosclerosis, impaired endothelium-dependent vasodilatation has been demonstrated in hypercholesterolemic patients [24]. This may occur either as a result of decreased NO synthesis or activity, or by accelerated inactivation of NO after release by the endothelium [25]. However, the derangement of endothelium-dependent vasodilatation due to hypercholesterolemia can be improved and even reversed with administration of L-arginine [24, 26].

One of the first steps in the formation of atherosclerotic plaque involves recruitment and adhesion of inflammatory cells to the vascular endothelium. By downregulating the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) by endothelial cells, NO decreases leukocyte adhesion to the endothelium [27, 28]. NO also inhibits platelet adhesion and aggregation in both direct and indirect fashion. cGMP, an intermediate product of the NO/EDRF pathway, works synergistically with prostacyclin from the vasculature to increase platelet concentrations of cAMP; increased cAMP concentrations in the platelet consequently inhibit aggregation. NO also directly inhibits platelet adhesion at the endothelium [22].

In patients with diabetes, serum arginine concentrations are lower than those of normal subjects. When administered to patients with diabetes, oral arginine lowers blood pressure and improves overall hemodynamic function. Similarly, in animal studies, giving either intravenous or oral arginine lowers mean blood pressures and reduces platelet aggregation. Patients with insulin resistance may also benefit from arginine supplementation, as insulin sensitivity and insulin-mediated vasodilatation are both improved following intravenous arginine infusion [22].

In addition to inhibiting endothelial cell apoptosis, NO stimulates endothelial cell proliferation and angiogenesis, thereby playing an important role in wound healing and the microcirculation. Also, by inhibiting the release of endothelin-1, a vasoconstrictor, NO enhances its vasodilator effect [29]. Other vascular effects include limiting superoxide generation by NADPH oxidase as well as limiting the expression of monocyte chemotactic peptides. NO may also inhibit endothelial cell apoptosis while limiting proliferation of vascular smooth muscle cells. Finally, iNOS-derived NO produced by vascular smooth muscle cells inhibits oxidation of LDL, a critical and early event in atherogenesis [25]. Thus, in addition to its effects on vasorelaxation and angiogenesis, NO may act as a unique antiatherogenic, antiproliferative and antithrombotic factor [30].

Decreased eNOS activity appears to be the central mechanism for endothelial dysfunction in states as diverse as atherosclerosis, hypertension, hyperlipidemia, aging and trauma. The intracellular concentrations of arginine (>100 μmol) are far above the Km of eNOS (2-10 μmol), yet exogenous arginine can restore NO-mediated responses in animals and humans. Termed the "arginine paradox," this phenomenon is not fully understood, but may involve an inbalance between expression and function of arginase and NOS [31]. Furthermore, it provides for possible intervention either locally, by modulating the arginine catabolism, or systemically by providing supplemental arginine.

NO-Independent Effects

Arginine also exerts several hemodynamic effects independent of its association with NO. In atherogenesis, arginine may act as an antioxidant to inhibit superoxide release by endothelial cells, thereby reducing lipid peroxidation induced by copper moieties. Since arginine is a basic amino acid at neutral pH, it may contribute to regulation of blood and intracellular pH through depolarization of cell membranes. High arginine concentrations also decrease blood viscosity by regulating the binding of macromolecules to erythrocytes [32]. Apart from NO-dependent vasodilatory effects, arginine can modulate vascular reactivity by directly binding to ATP-sensitive potassium channels [33].

As a stimulator of insulin, glucagon and prolactin secretion, arginine plays a role in the metabolism of glucose, protein and lipids, all of which are factors closely linked to atherogenesis. Arginine is an inhibitor of angiotensin-converting enzyme (ACE), thereby enhancing the vasodilatory effects of NO. Arginine also stimulates fibrinogenolysis by enhancing plasmin generation and fibrin degradation. In addition to NO-dependent effects on platelet aggregation, arginine inhibits the formation of thromboxane B_2 and the platelet-fibrin complex. Finally, arginine may directly inhibit leukocyte adhesion to the non-endothelial matrix independent of NO production [22].

Animal studies have demonstrated many beneficial effects of arginine in the development of atherosclerotic disease. Arginine supplementation reduces the size of atherosclerotic lesions and intimal thickening in hypercholesterolemic animals as well as in those fed high-cholesterol diets [34-36]. In response to endothelial injury such as that which results from balloon angioplasty, arginine reduces intimal thickening and increases endothelium-dependent vasorelaxation after injury [37, 38]. Several other animal studies have demonstrated that L-arginine supplementation decreases leukocyte adhesion, platelet aggregation and macrophage accumulation in atherosclerotic lesions [39, 40]. In animals fed hypercholesterolemic diets, arginine has also been shown to decrease serum cholesterol,

LDL, VLDL and triglyceride levels. This may be accomplished by arginine-associated inhibition of phospholipid synthesis in the liver [41]. Also, higher serum concentrations of arginine yield a lower insulin-to-glucagon ratio. This is a major determinant of balance between lipogenesis and lipolysis, as a reduction in this ratio leads to decreased activity of the hepatic lipogenic enzymes.

NO plays an important role in regulating cerebral vascular tone and circulation, and NO deficiency contributes to large cerebral infarct size. Previous animal studies have shown that arginine administration improves tissue preservation during reperfusion and increases regional blood flow in focal cerebral ischemia [22]. Arginine also protects cardiac, hepatic, intestinal and lung microcirculation from ischemia and reperfusion injury. These findings may have important implications for cardiac arrest, organ transplantation and other surgical procedures requiring periods of ischemia and reperfusion.

Human studies have also demonstrated positive effects of arginine on the cardiovascular system. However, there is a higher degree of variability in these results, which may be due to greater diversity of the study groups as well as limited study size. Coronary blood flow, leukocyte adhesion, adhesion molecule expression, platelet aggregation and adhesion, limb ischemia, exercise tolerance and myocardial infarction have all been evaluated [17, 26]. Many of these studies have failed to show significant benefit in healthy subjects who did not have underlying endothelial dysfunction. In contrast, studies of subjects at risk for or with existing vascular disease have demonstrated favorable effects from exogenous arginine administration [25, 26].

When given to patients with coronary artery disease, supplemental arginine improves cardiovascular function, increases exercise capacity and reduces myocardial ischemia. Intravenous infusion of arginine in patients with severe congestive heart failure results in improved renal hemodynamics and enhanced cardiac performance. Clearly, through both NO-associated and NO-independent effects, arginine plays an important role in modulation of cardiovascular function. This has wide potential clinical application for the treatment of coronary and peripheral vascular diseases, ischemia/reperfusion injury and heart failure. Therapeutic modalities, including dietary supplements, are currently in development [22].

EFFECTS OF ARGININE ON THE IMMUNE SYSTEM

Supplemental arginine therapy produces beneficial effects on the immune system, particularly on thymus-dependent and T cell-dependent immune reactions. Early animal experiments demonstrated thymotropic effects of arginine manifested as increased thymic weight and thymic lymphocyte content as well as enhanced reactivity of thymic lymphocytes [42]. Arginine supplementation also increases thymic lymphocyte blastogenesis and diminishes posttraumatic thymic involution and T cell suppression [43]. Also, in response to Concanavalin-A (Con A) or phytohemagglutinin (PHA), cultured human peripheral blood lymphocytes undergo mitogenesis; this response is diminished if the cells are cultured in arginine-free medium.

Dietary supplementation with ornithine induces similar thymotrophic effects [44]. However, treatment with citrulline, spermine, spermidine, agmatine and putrescine do not affect thymic size or cellularity, suggesting that the thymotrophic effects of arginine and ornithine are not mediated directly via polyamine synthesis. It appears that an intact hypothalamic-pituitary axis is necessary for some of the benefits of arginine supplementation; effects on post-wounding weight loss, wound breaking strength and changes in thymic weight are absent in hypophysectomized mice [11].

Studies in athymic mice have shown that arginine therapy increases extrathymic T cells both in number and mitogenic function. Delayed-type hypersensitivity responses are enhanced as well, suggesting a mechanism for T cell maturation and function that is independent of the thymus [45]. It also appears that arginine differentially affects T cell subpopulations. In response to arginine supplementation, *in vitro* proliferation of CD-8 + cytotoxic T cells is enhanced in a dose-dependent manner; a modest increase in IL-2 production is demonstrated as well. In the same experiment, however, proliferation of CD-4+ helper T cells is not affected [46].

The beneficial effects of arginine on the immune system are not limited to T cells. In fact, arginine appears to be a requirement for differentiation of pro-B cells to pre-B cells in bone marrow. In addition, low levels of circulating arginine are associated with decreased size and number of intestinal Peyer's patches as well as serum IgG levels [47]. Arginine also enhances the cytotoxic activities of both natural killer (NK) cells and lymphokine-activated killer (LAK) cells [44].

Arginine has a number of important effects on the macrophage. First, phagocytic activity is enhanced by the presence of arginine. In addition, macrophage cytotoxic activity depends solely on a metabolic pathway that utilizes arginine to produce NO via iNOS. The macrophage is the body's predominant source of iNOS, which provides NO in response to systemic inflammatory stimuli. TNF-α, a macrophage-derived peptide with both antitumor action and modulation of immune and inflammatory reactions, is also secreted in greater amounts in response to arginine supplementation [48].

Other immunomodulatory effects of arginine have been demonstrated in several experimental models. The effects of depressed delayed-type hypersensitivity response associated with extreme young or old age are diminished with arginine supplementation [49]. In a model of bacterial peritonitis, arginine supplementation increased overall survival in rats undergoing cecal ligation and puncture [50]. Also, guinea pigs subjected to 30% total body surface burns had a greater survival rate with dietary arginine supplementation [51]. In experimental renal transplantation, arginine exerts a net anti-inflammatory effect, improving function in grafts exhibiting moderate interstitial rejection [52].

There are two pathways that appear to be important for arginine and its role in the immune system. As the lone substrate for NO synthesis, arginine may be used by iNOS to produce local or systemic vasodilatation. The other pathway involves arginase, which converts arginine to

ornithine and urea, with ornithine widely recognized as a principle precursor for both proline and polyamine synthesis. It appears that the pattern of cytokine release by the inflammatory system dictates which pathway is followed [53]. When the cellular immune response is activated, secretion of cytokines such as IL-1, IL-2, γ-IFN and TNF-α predominates. This evokes a systemic inflammatory response in the body and iNOS expression is subsequently upregulated. While excessive NO production is associated with sepsis and hemodynamic instability, iNOS expression is necessary in order for the body to clear infecting organisms. This activation is also important for normal T cell function and development. Conversely, when the cellular immune response is downregulated, expression of IL-4, IL-10 and IL-13 predominates. This leads to increased arginase expression and subsequent availability of ornithine for polyamine and proline synthesis. Arginase function is central to regulating overall arginine availability, and competes with iNOS for arginine stores.

With these effects in mind, arginine therapy has been used to improve outcomes in critically ill patients. In one study of ICU patients, dietary supplementation with "immune-enhancing formulas" was associated with diminished overall mortality, incidence of bacteremia and nosocomial infections when compared with controls [54]. In critically ill children, arginine is an essential amino acid, as demonstrated by impaired arginine homeostasis and overall negative arginine balance [55]. This is important in the treatment of premature infants, as arginine therapy has been associated with decreased incidence of necrotizing enterocolitis (NEC), a potentially devastating complication of prematurity [56].

Meta-analyses of immunonutrition in critically ill patients confirm that there are benefits to dietary supplementation in this patient population [57-59]. These include fewer infectious complications and infection rates as well as decreased number of ventilator-dependent days and overall length of stay. However, the association with lower overall mortality is lost in the meta-analyses. Some have even argued that arginine therapy may be harmful in grossly septic patients due to overproduction of iNOS-induced NO [60]. While there are some clear benefits to dietary supplementation in some patient populations, optimal therapy should be directed toward those in which the potentially harmful effects can be minimized.

ARGININE AND WOUND HEALING

Several studies have demonstrated beneficial effects of arginine in wound healing. The importance of arginine in wound healing was first noted in experiments in which animals were fed arginine-deficient diets. Animals subject to the experimental diet exhibited significant post-operative weight loss and had a mortality rate of almost 50% when compared to control animals fed a diet sufficient in arginine. Wound breaking strength and collagen deposition were also decreased with respect to controls [3]. Other studies have demonstrated beneficial effects of supplemental arginine. Rat studies in which animals were fed parenteral solutions containing higher levels of arginine demonstrated that supplemented animals not only exhibited enhanced immune function, but that their wounds had increased amounts of collagen and higher wound breaking strength [61].

Several factors contribute to enhanced wound healing with arginine supplementation. NO is produced in wounds at levels higher than in non-wounded tissues as demonstrated by the presence of nitrite and nitrate, both of which are stable NO-derived end products. NO is generated from all three NOS isoforms in a variety of cells associated with wound repair. These include platelets (nNOS, iNOS), polymorphonuclear cells (iNOS), endothelial cells (eNOS), keratinocytes (eNOS, iNOS) as well as macrophages (iNOS) and fibroblasts (eNOS, iNOS) [62, 63]. Macrophages appear in wounds shortly after injury, respond to local cytokines such as IL-1, TNF-α and γ-IFN, and promote the inflammatory cascades necessary for healing [64]. NO levels in wounds peak approximately 24 hours following injury. This is followed by sustained production with a slow steady decline for up to 10 days [65].

The importance of NO in wound healing is illustrated by the fact that iNOS knockout mice, despite adequate supplementation with arginine, exhibit impaired wound healing [66]. Furthermore, use of NO or NOS inhibitors such as N^G-nitro-L-arginine methyl ester (NMMA) and S-methylisothiouronium, respectively, yields experimental wounds that exhibit both lower levels of NO synthesis as well as impaired wound healing [64, 67]. Another key cellular element of wound healing, the wound fibroblast, is also influenced by local iNOS and NO levels. Dermal fibroblasts derived from iNOS knockout mice demonstrate a lower proliferative rate as well as decreased collagen synthesis and wound contraction [68].

NO can also modulate cytokine expression in wounds. IL-8 is a chemoattractant molecule whose gene promoter region is activated by NO. In negative feedback fashion, IL-8 can suppress iNOS expression in polymorphonuclear cells. NO also activates TGF-β1, another important chemoattractant; in turn, TGF-β1 suppresses NO production. In addition, expression of monocyte chemoattractant protein-1 (MCP-1) is downregulated in response to NO *in vitro*, as is IL-6. Conversely, IL-1 production is increased in the presence of NO. Taken together, it has been postulated that NO may play a role in regulating the inflammatory phase of wound repair following injury [69].

Arginase also plays a key role in the wound healing process. In the first five days after wounding, iNOS is the predominant enzyme in the local wound environment. As iNOS activity decreases, that of arginase increases. The increase in arginase activity corresponds to a high rate of collagen synthesis and demand for hydroxyproline in the wound. Arginine is also consumed in the arginase-dependent formation of urea and ornithine. Ornithine is subsequently converted to hydroxyproline and proline, fundamental components of collagen and wound healing. Ornithine is also used for polyamine synthesis in cellular proliferation [66]. One possible mechanism behind these effects is related to the secretagogue effect of arginine on growth hormone and IGF-1. This is illustrated in hypophysectomized animals, in which there is no positive effect of arginine on wound healing [3].

Using a micromodel, which allows for the study of human fibroblastic responses and wound collagen

deposition, we carried out an initial study of 36 young healthy human volunteers (ages 25-35 years) randomized into three groups (a) 30 g arginine HCl daily supplements (24.8 g free arginine); (b) 30 g arginine aspartate (17 g free arginine); and (c) placebo. Arginine supplementation at both doses significantly increased the amount of hydroxyproline deposition at the wound site [70]. A second study evaluated 30 elderly volunteers (age > 70 years) who were given daily supplements of 30 g of arginine aspartate (17 g free arginine) or placebo. Arginine supplementation significantly enhanced wound collagen accumulation without any effect on wound DNA or total protein content. Arginine supplementation had no effect on the rate of epithelialization of a superficial skin defect, indicating that the predominant effect is on wound collagen deposition [71].

One important potential clinical application of arginine and its beneficial effects on wound healing is in the area of diabetes. In diabetic patients, the inflammatory reaction to injury is impaired, as demonstrated by delayed neutrophil chemotaxis and impaired phagocytosis. Growth factors and NO in wounds of diabetic patients are decreased compared to those of healthy patients, and collagen synthesis is impaired as well. This results in impaired wound healing and a greater propensity toward wound infections. In animal models of diabetes, however, arginine supplementation has been shown to partially reverse these effects, as demonstrated by increased hydroxyproline and collagen levels and greater wound breaking strength [72, 73].

THE EFFECT OF ARGININE ON CARCINOGENESIS AND TUMOR GROWTH

In contrast to the data regarding arginine and wound healing, the role of arginine in carcinogenesis and tumor progression remains somewhat controversial. While some studies have demonstrated beneficial effects of arginine and NO in delaying progression of malignant disease, others have shown that arginine and NO may actually promote tumorigenesis and tumor growth. To some degree, these effects depend on the tumor type and specific properties of the tissues involved, as well as interactions between arginine, NO and various components of the immune system with respect to tumor biology.

The role of arginine and NO in tumor biology is complex. When given as a dietary supplement, arginine provides some protection against tumorigenesis. In an experimental model of colon cancer in mice, dietary supplementation with L-arginine decreased tumor production and crypt cell hyperproliferation when administered during the initiation stage of carcinogenesis. However, when supplemented during the promotion stage, the mice exhibited enhanced tumor growth [74]. In another model of murine colorectal cancer, dietary arginine supplementation resulted in significantly decreased tumor incidence and overall tumor burden; again, the effects were more pronounced when the supplementation was provided earlier in the disease course [75].

In contrast, NO has been directly linked to carcinogenesis through a number of mechanisms. As a free radical, both NO and its reactive products may induce DNA damage by causing G:C $\rightarrow$ A:T transitions. NO and other reactive species have also been shown to impair or inactivate DNA repair enzymes. Finally, inactivation of the tumor suppressor p53 by NO may further contribute to a state that promotes tumorigenesis [71, 72].

The influence of arginine and NO on tumor cell growth and progression is even less clear. Some *in vitro* studies have demonstrated that arginine is necessary for the growth of tumor cell lines. As a precursor for polyamine synthesis, arginine provides substrate for cellular proliferation. When tumor cell lines are cultured in various concentrations of arginine, higher concentrations of arginine are associated with increased protein synthesis [77]. However, while *in vitro* studies show positive effects on tumor growth, *in vivo* effects may differ, possibly due to the presence of other cells and regulatory processes. For example, tumor infiltrating macrophages (TIM) have a high content of arginase and may regulate the availability of arginine in the tumor microenvironment. Also, decreased growth of immunogenic tumors with arginine supplementation is likely due to positive effects of arginine on the immune system, especially on T-cell cytotoxicity, natural killer cells and macrophages.

The seemingly tumor-enhancing effects of arginine may be used for a treatment advantage with regard to adjuvant chemotherapy and/or radiation. In order to be effective, these methods of treatment depend on a relatively increased rate of cell cycling. When administered prior to chemotherapy cycles, supplemental arginine effectively increases tumor sensitivity against chemotherapy and enhances the overall response.

In contrast to the tumorigenic association between NO and p53 previously described, NO at high concentrations may cause p53-dependent cytostasis as well as apoptosis [78]. This appears to be regulated by iNOS activity, as cells are protected from apoptosis in the presence of NO at low concentrations such as those produced by nNOS and eNOS. Other dose-dependent effects of NO include increased lactate dehydrogenase activity, which may result in cell death by necrosis [76].

The role of NOS in human cancers and the implication of variable NOS levels within a given tumor is an area of current research. Comparisons of benign and malignant tissues of the breast, stomach, cervix and ovary have demonstrated higher NOS activity in areas of malignant phenotypes [79]. Furthermore, in the case of breast cancer, areas of higher NOS activity correlate with those of higher pathologic tumor grade [80]. In general, it appears that increased levels of NOS in tumor areas with significantly higher activity appears to be due to stimulation of iNOS; this is thought to be a result of macrophage activation due to cytokines in the intratumoral environment.

Finally, another important aspect of tumor growth and progression is angiogenesis, which enables tumors larger than 1 mm in size to provide oxygen and nutrients to cancer cells in the center of the growing mass. A substantial body of evidence points to an enhancing effect of tumor-derived NO in angiogenesis and tumor invasion. Much of this has been demonstrated in mouse models, in which transduction of iNOS resulted in increased vascularity and growth of colon cancers. Also, eNOS knockout mice demonstrated impaired angiogenesis in ischemic hind limbs, with the

impairment being less pronounced when mice were provided dietary supplementation with L-arginine [76]. Overall, while much has been done to elucidate the respective roles of arginine, NO and NOS in the initiation and progression of human cancer, current research continues to further characterize these complex interactions.

EFFECTS OF ARGININE ON THE ENDOCRINE SYSTEM

Arginine has stimulatory activities on several endocrine glands. It increases pituitary growth hormone and prolactin secretion. It may also enhance the release of insulin, glucagon, somatostatin, pancreatic polypeptide and adrenal catecholamines. While several other amino acids may stimulate the release of these hormones, arginine is the most potent [3].

The strong endocrine secretagogue action of arginine occurs via several mechanisms. Arginine stimulates release of growth hormone mainly by inhibiting the release of somatostatin, which itself is an inhibitory peptide of growth hormone secretion. Insulin secretion is potentiated in the pancreatic β-cell by mimicking cell membrane depolarization and increasing intracellular calcium [81]. In both *in vitro* and *in vivo* studies, the stimulation of insulin secretion by arginine is dependent upon glucose concentrations. Higher glucose concentrations potentiate the insulin response, which helps to explain differences in response to arginine between diabetic and normal subjects [82]. In other studies involving diabetic subjects, arginine infusion has been shown to transiently decrease plasma leptin concentrations. Leptin concentrations are directly associated with body mass index (BMI) and other indicators of obesity, and the effects of arginine seem to be direct rather than through insulin secretion or other intermediaries [83].

Demonstration of NOS immunoreactivity and functional activity in several secretory organs lends evidence that NO is involved in arginine-induced hormone secretion. By modulating adrenal medullary vasodilatation, NO may increase the release of adrenal hormones. In addition, NO increases levels of cGMP, which also can stimulate catecholamine release [82]. Together, arginine and NO may influence a number of endocrine functions to produce a wide range of effects.

EFFECTS OF ARGININE ON NITROGEN BALANCE

In general, arginine is not considered to be an essential amino acid for adult humans and animals. However, arginine deficiency in young animals impairs normal growth. Also, following traumatic injury, nutritional and metabolic requirements of both humans and animals approximate those of growing organisms, and demand for arginine exceeds endogenous supplies. Even mildly injured rats require arginine for survival and weight gain. Experimental animals subjected to either partial starvation or bilateral closed femoral fractures demonstrated improved nitrogen retention when given arginine supplementation [66]. In a tracer infusion study of septic pediatric patients, negative arginine balance occurred as a result of increased arginine utilization as demonstrated by arginine oxidation, with unchanged net arginine synthesis [55]. Because it becomes essential during times of stress and in the neonatal period, arginine is classified as a semi-essential amino acid.

Following promising results in experimental animal studies, the effect of arginine on nitrogen metabolism and balance has been investigated in humans. Healthy elderly human volunteers exhibited increased nitrogen retention following dietary supplementation with 30 g/d of arginine aspartate for two weeks [84]. After injury or wounding, dietary supplementation with arginine also helps to reduce nitrogen losses when compared to isonitrogenous diets. In a clinical study of post-cholecystectomy patients, arginine supplementation reduced urinary nitrogen excretion by 60% when administered for 3 days in the post-operative period. Another study investigated the role of supplemental arginine on patients undergoing abdominal surgery for gastrointestinal malignancies. When given in the first post-operative week, supplemental arginine was associated with positive nitrogen balance and enhanced T-lymphocyte response [85].

Other investigations have been less promising. In a study of malnourished head and neck cancer patients, supplemental arginine failed to provide any benefit in clinical outcomes. Nutritional status did not change significantly, nor were improvements in surgery-induced immune suppression demonstrated [86]. As this could be explained by a relatively short period of supplementation or patients' profound nutritional depletion at baseline, optimal timing and protocols for aggressive nutritional support in surgical patients remain to be defined.

Current recommendations for perioperative nutritional supplementation are relatively limited. A number of studies have investigated the effects of immunonutrition, with most benefits arising from decreased septic and infectious complications [43]. However, few have provided clear evidence for decreased mortality and increased patient survival. These studies have recently been reviewed and at present, recommendations for consideration of immunonutrition are as follows: patients undergoing abdominal surgery for cancer, especially those who are malnourished; patients who have sustained multiple trauma; and ICU patients with illness of moderate severity (APACHE scores 10-20) [87]. As the experience with immunonutrition grows, guidelines for maximal patient benefit are likely to become better defined.

CONCLUSIONS

Arginine has a significant role in nutrition due to its multiple physiological and pharmacological activities. While it is classified as a nonessential amino acid in unstressed animals and humans, it becomes an indispensable in times of trauma and severe stress. Arginine is one of the most versatile amino acids, functioning as precursor for nitric oxide, urea, ornithine, proline, polyamines, agmatine, creatine and several other body proteins. While much remains unclear with regard to arginine synthesis and catabolism at the cellular and organ level both in health and disease states, many current research efforts are focused on elucidating these mechanisms.

Dietary arginine supplementation is safe and well tolerated with minimal side effects. It has been used in a wide range

of clinical situations from hypertension to sepsis, and studies have demonstrated beneficial effects of arginine in the treatment of various illnesses ranging from viral myocarditis to necrotizing enterocolitis. As the role of arginine in various physiologic and pathologic states becomes more clearly defined, additional targets for disease therapy are sure to be identified.

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