

Therapeutic Use of Citrulline in Cardiovascular Disease

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ABSTRACT

L-citrulline is the natural precursor of L-arginine, substrate for nitric oxide synthase (NOS) in the production of NO. Supplemental administration L-arginine has been shown to be effective in improving NO production and cardiovascular function in cardiovascular diseases associated with endothelial dysfunction, such as hypertension, heart failure, atherosclerosis, diabetic vascular disease and ischemia-reperfusion injury, but the beneficial actions do not endure with chronic therapy. Substantial intestinal and hepatic metabolism of L-arginine to ornithine and urea by arginase makes oral delivery very ineffective. Additionally, all of these disease states as well as supplemental L-arginine enhance arginase expression and activity, thus reducing the effectiveness of L-arginine therapy. In contrast, L-citrulline is not metabolized in the intestine or liver and does not induce tissue arginase, but rather inhibits its activity. L-citrulline entering the kidney, vascular endothelium and other tissues can be readily converted to L-arginine, thus raising plasma and tissue levels of L-arginine and enhancing NO production. Supplemental L-citrulline has promise as a therapeutic adjunct in disease states associated with L-arginine deficiencies.

INTRODUCTION

L-citrulline is a colorless, water soluble α -amino acid with an asymmetric carbon. Like other α -amino acids, L-citrulline has the capacity of forming peptide bonds, but it is not used in protein synthesis. L-citrulline was once considered mainly as a metabolic interme-

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diate in the urea cycle. However, recent studies have revealed that it plays an important role in the metabolism and regulation of NO.

Production of NO by vascular endothelial cell NOS is essential for normal cardiovascular regulation and depends critically on availability of the substrate L-arginine. Deficiencies in L-arginine supply have been strongly implicated in cardiovascular diseases, including hypertension, atherosclerosis, diabetic vascular disease, hyperhomocysteinemia, heart failure and ischemia-reperfusion injury (9,12,13,39,48,68,94). The normal range of plasma levels of L-arginine in humans is 40 to 110 μM (57). When the supply of L-arginine does not meet the needs of active NOS, NO formation is reduced and superoxide (O_2^-), a deleterious oxidant, is formed by NOS. This state of imbalance between L-arginine availability and NOS activity can occur when cellular transport of L-arginine is inhibited, as with oxidative stress associated with cardiovascular disease, with prolonged and elevated NOS activity (1,40), reduced recycling of L-citrulline back to L-arginine (51) and/or with elevated catabolism of L-arginine by arginase (4,10,96).

While the above reports strongly implicate L-arginine deficiency in endothelial cell (EC) dysfunction, measurement of L-arginine levels in ECs has shown that intracellular L-arginine concentration (0.1–1 mM) greatly exceeds eNOS's K_m for L-arginine [$\sim 3 \mu\text{M}$] (66). Accordingly, eNOS should be saturated with substrate under all but the most extreme conditions of L-arginine deficiency. This contradiction between eNOS's low K_m for L-arginine and the strong positive effects of supplemental L-arginine in acutely reversing vascular dysfunction and augmenting the actions of eNOS agonists even in normal conditions has been termed the "L-arginine paradox." This seeming conflict may be explained by experimental evidence indicating that the metabolism of L-arginine varies greatly within the EC due to intracellular compartmentalization and sequestration (16). Studies showing that a complex exists between eNOS and the major L-arginine system y^+ transporter protein (CAT1) and that both molecules are located within plasma membrane caveolae provide further evidence of L-arginine compartmentalization (50). Collectively, these data suggest that eNOS is sequestered from the intracellular L-arginine supply by being located within caveolae and that its activity depends on the directed transfer of L-arginine into this subcellular compartment by the system y^+ transporter. If transporter function is decreased as can occur with oxidative injury, L-arginine supply could immediately become limiting and be the basis of EC dysfunction. Additionally, asymmetric dimethyl arginine (ADMA), a risk factor elevated in cardiovascular diseases associated with endothelial dysfunction, inhibits L-arginine transport (system y^+) and NOS activity and is likely a participant in the "L-arginine paradox" (73). Both circumstances lead to a deficiency in L-arginine available to NOS.

Supplemental L-arginine administration has been reported to prevent EC dysfunction or restore endothelium vasodilation in various cardiovascular disease states. However, studies with rabbits suggest that with chronic therapy the benefits of supplemental L-arginine are not maintained or may convert to a negative outcome (38). Reduced plasma levels of L-arginine were observed. Most significantly, a recent clinical trial in humans showed that treatment of patients with supplemental L-arginine (9 g/day for 6 months) subsequent to myocardial infarction was associated with higher mortality (72). A drawback to administering L-arginine orally to elevate plasma levels of arginine is that a large portion of the L-arginine passing through the gastrointestinal tract and the portal system to the liver is catabolized by arginase to ornithine and urea (57). Furthermore, high

levels of dietary or circulating L-arginine can increase arginase activity in the liver, kidney, vasculature and probably other tissues, which increases rate of L-arginine catabolism (37,57,80,90).

Because L-citrulline is the natural precursor for L-arginine, it could be an important substitute for L-arginine under pathologic conditions that limit L-arginine availability. L-citrulline is converted to L-arginine in many tissues. Therefore, it plays an important role in supplying L-arginine to NOS as it bypasses metabolism in the liver and it is not a substrate or inducer of arginase (74).

PHYSICAL AND CHEMICAL PROPERTIES OF L-CITRULLINE

An extensive review of the physical and chemical properties of L-citrulline has been recently provided by Curis and colleagues (14). There is no codon for the addition of L-citrulline to growing peptide chains. However, L-citrulline, like other α -amino acids, can form peptide bonds. Proteins containing L-citrulline residues can occur, and are formed due to post-translational modifications involving activity of the enzyme protein-arginyl deiminase (PAD) in causing the hydrolytic cleavage and conversion of L-arginine to L-citrulline and an ammonium ion (36). The function of this modification is not completely understood. However, it may be involved in normal or pathophysiological functions resulting from alterations in the charge of the modified protein residues (2,19,41,87).

With respect to L-citrulline function as substrate for synthesis of L-arginine, the terminal ureido group on L-citrulline is subject to nucleophilic attack by aspartic acid in the presence of argininosuccinate (AS) synthase to yield the immediate L-arginine precursor argininosuccinate (14,33). Fumarate is removed from this intermediate compound by AS lyase to form L-arginine.

L-CITRULLINE METABOLISM

Synthesis

L-citrulline is an amino acid product of glutamine metabolism (6) that serves as substrate in the *de novo* synthesis of L-arginine in mammals. Although L-citrulline is produced by cells in both the intestine (enterocytes) and liver (hepatocytes), only the small intestine contributes significantly to circulating L-citrulline levels. In contrast, L-citrulline produced in the liver is compartmentalized as an intermediate of the urea cycle (14). The pathway of intestinal L-citrulline synthesis begins with conversion of glutamine to glutamate and then to ornithine as catalyzed sequentially by pyrroline-5-carboxylate synthase (P-5-C-S) and ornithine aminotransferase (OAT). Ornithine is then converted to L-citrulline via ornithine carbamoyltransferase (OCT, Fig. 1). Another precursor, carbamoyl phosphate (CP), is involved in this process. This pathway involves coordinated regulation of two enzymes: N-acetylglutamate synthase to produce N-acetylglutamate, which regulates synthesis of CP by carbamoyl-phosphate synthase I (CPS-I, Fig. 2). Ornithine can also be produced from proline coming from the diet, by proline oxidase (18).

With development, intestinal synthesis of L-arginine from glutamine decreases and the small intestine gradually becomes the major site of net L-citrulline production. This

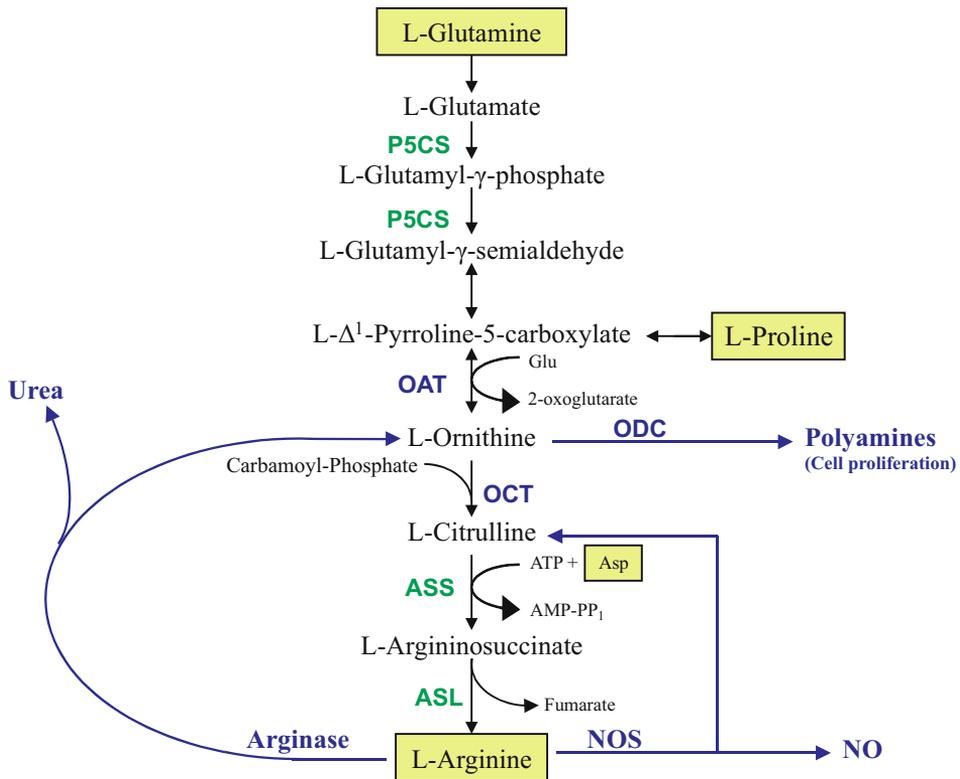


FIG. 1. Scheme of synthesis of L-citrulline and L-arginine from L-glutamine. Catabolism of L-arginine to L-ornithine/urea or L-citrulline/NO, production of polyamines, and anabolism and catabolism of proline are also shown. Abbreviations: ASL, aminosuccinate lyase; ASS, aminosuccinate synthase; Asp, aspartate; NOS, nitric oxide synthase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OCT, ornithine carbamoyl-transferase; P5CS, pyrroline-5-carboxylate synthase.

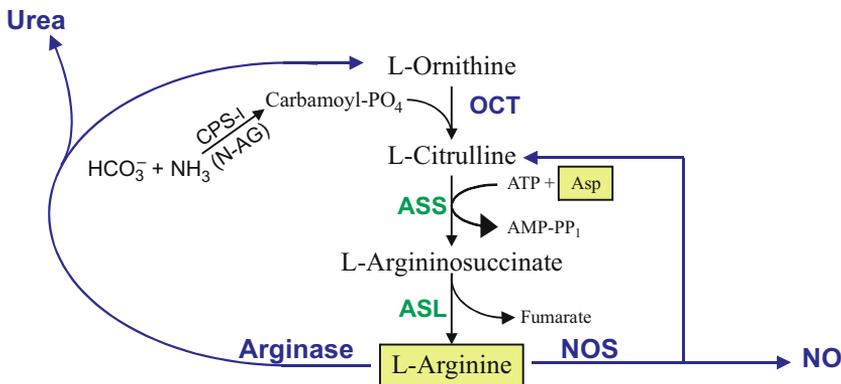


FIG. 2. Scheme of synthesis of carbamoyl-PO₄ and the incorporation of NH₃ into the urea cycle. Abbreviations: CPS-I, carbamoyl PO₄ synthase I; N-AG, N-acetylglutamate.

process is due to progressively reduced expression of the two cytosolic enzymes responsible for the conversion of citrulline into arginine, argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) (61). This transition is compensated for by a gradually increasing capacity of the kidney to convert L-citrulline to arginine via ASS and ASL. Therefore, following release from enterocytes into the portal circulation, citrulline passes through the liver without major metabolism. L-citrulline is largely taken up and metabolized by the kidney, which in turn releases arginine equivalent to ~75% of the L-citrulline taken up. Thus, much of the L-citrulline produced by enterocytes reaches the systemic circulation as L-arginine. This L-arginine/L-citrulline homeostasis allows a proper supply of L-arginine for the whole body.

About 60% of dietary L-arginine makes it into the hepatic portal circulation, while the rest is metabolized in the intestine. (58). One study using rapid injection of protein into the hepatic portal vein showed that there was an immediate uptake and metabolism of about 37% of the available L-arginine by the liver (62). Others suggest that most of the L-arginine derived from dietary protein is removed from the adult hepatic portal circulation by periportal hepatocytes for use in the liver urea cycle (14).

In the liver, much of the L-arginine entering via the portal circulation is catabolized by arginase which converts arginine to urea and ornithine. Products of ornithine metabolism in the ornithine/polyamine/proline pathway play essential roles in cellular proliferation and tissue repair and are necessary for cellular growth (46,79,83). Depending on cellular needs and the presence of carbamoyl phosphate, ornithine can be converted to L-citrulline by OCT. In the liver L-citrulline can be recycled to L-arginine through ASS and ASL. This L-arginine/L-citrulline cycle provides a continuous supply of substrate for the formation of urea and the maintenance of nitrogen homeostasis (107).

The intestinal-renal axis of L-citrulline/L-arginine synthesis becomes established post-natally. In the perinatal period L-arginine synthesis predominates within the cells of the small intestine, but as development continues, levels of the L-arginine synthesizing enzymes ASS and ASL decrease and L-citrulline/L-arginine metabolism gradually shifts towards L-citrulline production and L-arginine degradation (102). In addition to the decreases in ASS and ASL, other factors are involved in the developmental changes of L-citrulline synthesis within the small intestine. The rate of L-citrulline synthesis may be limited by P-5-C synthase activity (7). Earlier reports indicate that a low rate of L-citrulline synthesis in glutamine-treated enterocytes from pre-weaning pigs is due to limited availability of ornithine rather than to any deficiency in carbamoyl phosphate. This might result from a low rate of P-5-C synthesis from glutamate and/or from the competitive conversion of P-5-C into proline (102). Increased L-citrulline synthesis from glutamine in enterocytes from weanling pigs coincides with a 15–20 fold increase in activity of P-5-C synthase. Little is known about the molecular regulation of this pathway. However, transcription is likely to be the major control mechanism for regulating expression and activity of intestinal L-citrulline-synthetic enzymes. Weaning is associated with increased plasma concentrations of hydrocortisone, which is the major circulating glucocorticoid in pigs (100) and humans (22) and is known to enhance P-5-C synthase activity (104).

Although intestinal CPS-I and OCT are involved in L-citrulline synthesis, these enzymes are not upregulated in pig enterocytes during weaning. Similar findings have been described in rats, indicating that intestinal expression of these enzymes is not induced by glucocorticoids (70,101). These data support the notion that P5C synthase is the key regulatory enzyme in the synthesis of L-citrulline from glutamine.

This evidence certainly supports the concept that developmental changes in intestinal tissue occur in order to ensure the adequate supply of L-citrulline for L-arginine in the kidney. However, species differences in intestinal synthesis of L-citrulline and L-arginine and the lack of information about L-citrulline synthesizing enzymes in human tissues, present strong reasons for future studies. CPS-I is highly expressed in most of the small intestine during the entire lifespan in human and rat. Age-related increases in CPS-I mRNA levels have been demonstrated in humans between the embryonic period and 12 years (89).

The physiological role of CPS-I in the intestine differs from that in the liver. In the adult, cells of the small intestine use CPS-I to produce and release L-citrulline, whereas in liver CPS-I activity leads to the conversion of ammonia into urea and L-citrulline is not released in any significant amount (103). Amounts of L-arginine or protein in the diet directly influence L-citrulline synthesis in the small intestine. Prolonged administration of high-protein (L-arginine-rich) diet leads to an adaptation of the intestinal enzymes which results in decreased production of L-citrulline. Basically, this adaptation represents a downregulation of intestinal OCT and *N*-acetylglutamate synthetase, which produces *N*-acetylglutamate, the allosteric activator of CPS-I (15). On the other hand, prolonged administration of low-protein diets (low in L-arginine content) leads to upregulation of intestinal OCT and *N*-acetylglutamate synthetase, which results in formation of more L-citrulline (90).

An opposite effect is observed in liver tissue in which an increase in L-arginine uptake activates *N*-acetylglutamate synthetase (53). However, as was described earlier, L-citrulline produced in liver is compartmentalized to the urea cycle within hepatocytes, without net contribution to systemic L-citrulline flux.

L-citrulline synthesis in many tissues also occurs as a byproduct of NOS activity. Three isoforms of NOS, encoded by different genes, are found in multiple cell types throughout the body. Neuronal NOS (nNOS, NOS1), which is found mainly in neural cells, and endothelial NOS (eNOS, NOS3), which is found mainly in endothelial cells, are constitutively expressed. In contrast, the inducible isoform (iNOS, NOS2) is not normally expressed in resting cells, but is induced in macrophages, smooth muscle cells and other cell types during inflammation or upon stimulation with bacterial endotoxins or inflammatory cytokines. Although NOS is widely distributed throughout the body, its activity does not contribute substantially to whole body L-citrulline flux under normal conditions (27).

Conversion of L-Citrulline to L-Arginine

As noted above, enterocytes in the small intestine convert dietary glutamine to L-citrulline. L-citrulline released by the enterocytes, enters the portal circulation, bypasses metabolism by periportal hepatocytes and is transported to the kidneys where it is catabolized to L-arginine by cells of the proximal tubules (90). The catabolic conversion of L-citrulline to L-arginine occurs not only in the cells of the kidney proximal tubules, but in the cells of many tissues (34,97). Cells involved in the production of NO as well as cells that produce ornithine and urea by the catabolism of L-arginine also produce L-citrulline, which can then be recycled to arginine (34). The process of L-citrulline catabolism to L-arginine is a two-step enzymatic process involving the rate limiting enzyme arginosuccinate synthase (ASS) and arginosuccinate lyase (ASL). In the presence of aspartate and ATP, L-citrulline

is converted to arginosuccinate by ASS. Arginosuccinate is cleaved by ASL to form fumarate and L-arginine (24,34,97).

In cells that express NOS, L-arginine is converted to NO and L-citrulline upon NOS activation. ASS and ASL effectively convert L-citrulline back to L-arginine and largely determine the fate of L-citrulline produced intracellularly by NOS activity. Regulation of the L-citrulline-NO cycle enzymes has been associated with the same factors that regulate NOS (42). For example, increased expression of ASS, the rate limiting enzyme of L-citrulline synthesis has been reported in endothelial cells exposed to shear stress (49,63). Enhanced conversion of L-citrulline to L-arginine has been reported in macrophages stimulated with inflammatory factors that induce expression/activity of iNOS (32). However, L-citrulline supplemented in the culture medium does not increase L-arginine availability to NOS under high NO output conditions, such as iNOS activation. This could result from the reversible direct inactivation of ASS by covalent binding of NO to cysteine residues (S-nitrosylation) of ASS (28). Hypoxia reduces ASS expression and can block the effects of iNOS (82). In the liver ASS activity and expression is modulated by hormones. For example, glucocorticoids and glucagon have a generally stimulatory effect, whereas insulin suppresses glucocorticoid-mediated stimulation of ASS activity (23,67). Dietary proteins and amino acids also have a regulatory effect on these enzymes. Protein supplementation in rats is reported to increase ASS and ASL expression/activity in liver. However in the kidney, protein deficiency causes induction of these enzymes (11,56).

Many NO producing cells, including vascular endothelial cells and activated macrophages, use L-citrulline as substrate to form L-arginine. However, this may not be true of all NO producing cells. In vascular smooth muscle cells, ASL is constitutively expressed but ASS is not and L-citrulline has been considered a nonfunctional byproduct of NOS function. It is assumed that uptake of extracellular L-arginine is necessary for iNOS function. Recent studies, however, have shown that when iNOS is induced in these cells by bacterial endotoxin, ASS is expressed and L-citrulline is recycled to L-arginine (30). In the brain, there appears to be a complex and incompletely understood compartmentalization of L-arginine synthesis and function of the L-citrulline/NO cycle. Neurons exhibit expression of all enzymes of the L-citrulline/NO cycle. However, these enzymes are localized in different cells. Studies suggest that astrocytes may serve as storage sites for L-arginine which can be released for use by adjacent neurons (97). In neurons outside the brain, such as the inhibitory non-adrenergic non-cholinergic (NANC) neurons which mediate relaxation of airway and intestinal smooth muscle, NO production from supplemental L-citrulline through the ASS/ASL pathway has been demonstrated (47,69). Whether intercellular compartmentalization and transport of components of the L-citrulline/NO cycle occurs in these cells, is unclear.

As previously stated, arginase has a primary role in the catabolism of L-arginine to urea and ornithine and the ornithine/polyamine/proline pathway is important in cellular proliferation, growth and tissue repair. Recent studies have shown that a number of normal and transformed cell lines are incapable of using L-citrulline as a source of L-arginine synthesis. While fibroblasts and many other cell types can use L-citrulline as a source of L-arginine, human umbilical vein endothelial cells and some microvascular endothelial cells and tumor cells appear to be completely dependent on arginine for cell growth (95). As the possible therapeutic uses of L-citrulline supplementation expand, more studies are needed to determine the capacity of each cell type to use L-citrulline for L-arginine synthesis.

L-CITRULLINE TRANSPORT

Intestine

The optimal site of L-citrulline absorption is the middle to lower ileum of the small intestine. At the brush border L-citrulline shares the Na⁺-dependent active transport system for neutral aminoacids that has been previously described for the mucosal brush border membranes of the rabbit jejunum (88). L-citrulline can also be transported by a system analogous to system ASC described for nonepithelial cells and for basolateral membranes of certain epithelia (88).

Kinetic study of L-citrulline transport has revealed an apparent K_m of 4.10 ± 0.86 mM and a V_{max} of 18.7 ± 1.66 mmol/g wet weight tissue/30 min (88). Transport of amino acids and di-tripeptides is regulated mainly by the amount of substrate at the mucosal membrane with higher substrate amounts yielding higher absorption (35). However, there is no evidence for an increased or decreased uptake of L-citrulline by enterocytes under low or high protein or L-citrulline intake, as has been reported for L-alanine during starvation (59).

Kidney

L-citrulline absorption occurs mainly in the proximal convoluted tubules of the kidney. L-citrulline delivery to the kidney has been suggested to occur via reabsorption from the glomerular filtrate (8). Neutral amino acids are co-transported with Na⁺ ions via system B^o. Because system B^o is the main neutral-amino-acid transporter of renal brush-border membranes, it is feasible that L-citrulline uptake in the kidney occurs via this broad specificity system. This transporter is also capable of catalyzing the Na⁺-dependent transport of branched-chain, aromatic and small neutral amino acids (65).

This localization of L-citrulline transport in the proximal convoluted tubules, enriched by ASS and ASL, is very convenient, since it ensures proper substrate supply for L-arginine synthesizing enzymes. L-arginine synthesis is mainly dependent on the delivery of L-citrulline to the kidney. The kidney has a high capacity for the conversion of L-citrulline to L-arginine, but this process is limited *in vivo* by the rate of delivery of L-citrulline. This means that the coordinated interorgan amino acid exchange between the intestine and the kidney is relevant to L-arginine flux, in which the synthesis of L-citrulline by the intestine is the crucial regulatory event (17).

Endothelial Cells and Other Cells

Endothelial cells synthesize NO, which is a potent vasodilator and a critical regulator of blood flow and blood pressure. NO also maintains vascular wall integrity due to its antiinflammatory, antiproliferative and antiplatelet aggregatory properties. In this context, L-citrulline can supply L-arginine substrate for eNOS as it is recycled by ASS and AL (31). L-arginine synthesis, as indicated previously in this review, may occur from endogenous NO-derived L-citrulline or from L-citrulline transported into the cell from the extracellular space. L-citrulline transport into endothelial cells has been suggested to be mediated by the neutral amino acid system N transporter 1 (SN1) (77) since it can be inhibited by glutamine, histidine, and asparagine, which are substrates of the system N transporter.

L-citrulline transport under conditions of L-arginine deficiency in endothelial cells has not been examined. However, our laboratory has demonstrated that L-citrulline supplied to cultured endothelial cells provides sufficient substrate to sustain NOS activity and NO production under L-arginine-deficient conditions (80).

Other cell types have also been reported to transport and recycle L-citrulline to L-arginine (3,98). Transport of L-citrulline into smooth muscle cells (SMC) is slow. Unlike L-arginine, kinetic analysis revealed that transport is mediated via a low-affinity carrier ($K_m \approx 1.6$ mM), which may limit uptake from plasma in the presence of circulating competitor amino acids. Transport of L-citrulline in SMC appears to be mediated predominantly via a carrier with characteristics of the Na⁺-independent system L. This may not be the only system involved. A partially Na⁺-dependent mechanism of L-citrulline entry is mediated via system N (98).

PHARMACOLOGY OF L-CITRULLINE

L-citrulline as a Means of Preventing/Reversing Vascular Dysfunction

Endothelial cell (EC) dysfunction is a primary cause of disability and death associated with cardiovascular disease. EC dysfunction is defined as impaired endothelium-dependent relaxation due to a decrease in bioavailability of NO. Reactive oxygen species are involved with this dysfunction. Production of NO by eNOS is critically involved in maintaining integrity and stability of the vascular endothelium as well as in regulating blood flow and organ circulation. L-arginine is the substrate required by eNOS along with O₂ to produce NO. Thus, maintaining an adequate cellular supply of L-arginine is critical for normal vascular function. Deficiencies in L-arginine supply have been strongly implicated in cardiovascular diseases, including diabetes, hypertension, atherosclerosis, hyperhomocysteinemia, heart failure and reperfusion injury (9,12,13,39,48,68,94). The intracellular supply of L-arginine for NOS depends on L-arginine transport, level of NOS activity, recycling of L-citrulline back to L-arginine, proteolysis and metabolism of L-arginine by arginase. Supplemental L-arginine given orally has been reported to prevent EC dysfunction and restore endothelium-dependent vasodilation in diabetes, hypertension (16,71), reperfusion injury (94) and heart failure (85). However, relatively large doses (50 mg/kg/day or more) appear to be required for this effect (72,84). Moreover, a number of studies in experimental models and a recent clinical trial have reported no benefit with chronic administration of supplemental L-arginine (38,72,84,85,106).

A drawback to administering L-arginine orally to elevate plasma levels of L-arginine is that a large portion of the L-arginine passes through the gastrointestinal tract and the hepatic portal system where it is catabolized by arginase I to ornithine and urea (57). Furthermore, high levels of dietary or circulating L-arginine can induce cytoplasmic arginase (I) and mitochondrial arginase (II) in the liver, kidney, vasculature and probably other tissues which increases the rate of this L-arginine catabolism (37,57,90). In addition, disease conditions such as diabetes, trauma, pulmonary hypertension, sickle cell, heart failure and even aging increase arginase levels and activity (4–6,52,81,86,105) which can be associated with decreases in plasma levels of L-arginine (26,55,64). Thus, doses of L-arginine necessary to produce desired L-arginine blood levels have to be determined keeping in mind a considerable catabolic loss via arginase and may not be clinically at-

tainable. Moreover, chronic L-arginine treatment may have adverse effects on cardiovascular function (72).

L-citrulline supplementation could be an important substitute for L-arginine supply under pathologic conditions that increase arginase activity and/or limit L-arginine availability. L-citrulline, through its conversion to L-arginine, plays an important role supplying L-arginine to NOS. Unlike L-arginine, it bypasses hepatic metabolism and it is not a substrate of arginase. Furthermore, there is no evidence of transporter dysfunction for L-citrulline under pathological conditions, such as oxidative stress, that can occur for L-arginine transport. It also has been demonstrated that L-citrulline cannot sustain NO production by iNOS, but that it can support NO production with eNOS activation (75). L-citrulline supplementation to patients with sickle cell disease, in which elevated arginase activity is manifest (54), raised plasma L-arginine levels and reduced their symptoms (93). Our own studies in EC exposed to L-citrulline (1 mM) for 5 days revealed a reduction in basal arginase activity vs. control. Further, supplemental oral treatment of normal rabbits with L-citrulline for 3 days improved acetylcholine-induced NO production (80). L-citrulline also has been reported to be a noncompetitive inhibitor of arginase (25,74).

Even though most L-arginine administered enterally is metabolized to ornithine and urea during passage through the gut and liver (~80%), levels that reach the systemic circulation are sufficient to augment vascular arginase activity. L-citrulline, on the other hand, is not taken up by the liver, but is transformed to L-arginine in other tissues (10,44). The ability of L-citrulline to increase or restore blood L-arginine levels was first reported by Hartman and colleagues (29). Waugh et al. have also reported that ingested L-citrulline is more effective than L-arginine in raising plasma arginine levels (92,93). Thus, systemic administration of L-citrulline appears to be a more efficient way to elevate extracellular levels of L-arginine than L-arginine itself. We have found that supplemental L-citrulline does not activate arginase activity in liver or blood vessels, and is able to enhance acetylcholine-induced NO production and hypotension in rabbits. Our findings suggest that long term supplemental therapy to support NOS function in cardiovascular diseases should involve administration of L-citrulline (80), possibly also given in combination with L-arginine in amounts and schedules that do not enhance vascular and hepatic arginase activity.

L-citrulline as Therapy for Cardiovascular and Other Disease States

L-citrulline supplementation should be considered for use in all circumstances or disease states in which L-arginine has been reported to have beneficial effects. As stated above, these conditions include systemic hypertension, heart failure, diabetes, and ischemia-reperfusion injury. Other states of cardiovascular dysfunction have already been reported to be reversed by supplemental L-citrulline as well as L-arginine. Treatment of sickle cell disease with L-arginine as well as L-citrulline has been shown to reduce levels of plasma peroxidase and activated leukocytes, RBC sludging, vaso-occlusion and pulmonary hypertension and to improve sensory motor deficits (20,55,60,91,93). Primary pulmonary hypertension as well that resulting from emboli or cardiopulmonary bypass can also be reversed by supplemental oral treatment with L-arginine or L-citrulline (78). The fatality of cerebral malaria has also been associated with low plasma L-arginine levels and decreased NO production. Supplemental L-arginine (or L-citrulline) therapy is being considered (45).

L-citrulline has also been used to reverse the L-arginine deficiency which occurs with disease or resection of the small bowel (61). Gastrointestinal absorption of L-citrulline occurs predominately in the small bowel. Treatment of burns and general wounds with L-citrulline or L-arginine is also effective in improving wound healing (21,99). Resultant increases in polyamines and proline levels and in NO synthesis are involved with this restorative process.

PHARMACOKINETICS

A study in humans has shown that oral administration of L-citrulline at 3.8 g/m² body surface, resulted in a 227% peak increase in plasma L-arginine levels at 4 h, compared with a 90% peak increase with the same dose of L-arginine (43). Furthermore, the area under the curve plot of L-arginine plasma concentration vs. time was 3 fold larger for L-citrulline, and the elevation in L-arginine levels was more persistent following L-citrulline administration. Thus, acute oral administration of L-citrulline appears to be considerably more efficient raising plasma levels of L-arginine than L-arginine itself. Additionally, a recent study in children and young adults showed that five oral doses of L-citrulline every 12 hours (1.9 g/m²/dose) for a total dose of 9.5 g/m² resulted in 57 and 85% increases in mean plasma levels of L-arginine and L-citrulline, respectively (78).

In young healthy humans, dietary levels of L-arginine in fasting or fed states have not been reported to affect plasma flux of L-citrulline, an index of rate of formation and degradation of L-citrulline, or the conversion rate of L-citrulline to L-arginine. In contrast, plasma flux of L-arginine is substantially reduced with fasting or L-arginine free diets. Thus, effectiveness of supplemental L-citrulline therapy is expected to be maintained in different dietary states.

TOXICOLOGY

L-citrulline is a naturally occurring amino acid for which widely different plasma and tissue levels may exist. Few studies have examined or reported on the toxicology of supplemental L-citrulline administration. L-citrulline is generally recognized as safe for oral consumption (78,93). In fact, L-citrulline can prevent some of the untoward effects of L-arginine supplementation. For example, L-arginine is likely to cause excessive urea genesis, whereas L-citrulline administration would not (14,76).

Hypercitrullinemia may occur with dysfunction of the ASS gene or aspartate transport for that enzyme. However, the pathology of these disease states appears to be related to build-up of compounds involved in L-citrulline synthesis and disruption of the urea cycle and elevated ammonia levels.

CONCLUSIONS

Supplemental administration of L-citrulline, which is converted to L-arginine in the kidney, vascular endothelium and other cells, is an effective means of elevating plasma

and tissue levels of L-arginine. When given orally, supplemental L-citrulline is more effective than L-arginine in raising circulating L-arginine levels because it bypasses metabolism in the gastrointestinal tract and liver. Also, it is not a substrate for arginase and does not induce arginase expression or activity. Therefore, use of L-citrulline supplements is a promising treatment of cardiovascular disease involving L-arginine deficiencies, reduced NO availability and vascular dysfunction. L-citrulline is a natural and apparently safe means of providing L-arginine for constitutive NOS production of NO.

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REFERENCES

1. Abou-Mohamed G, Johnson JA, Jin L, et al. Roles of superoxide, peroxynitrite, and protein kinase C in the development of tolerance to nitroglycerin. *J Pharmacol Exp Ther* 2004;308:289–299.
2. Asaga H, Ishigami A. Protein deimination in the rat brain after kainate administration: Citrulline-containing proteins as a novel marker of neurodegeneration. *Neurosci Lett* 2001;299:5–8.
3. Baydoun AR, Bogle RG, Pearson JD, Mann GE. Discrimination between citrulline and arginine transport in activated murine macrophages: Inefficient synthesis of NO from recycling of citrulline to arginine. *Br J Pharmacol* 1994;112:487–492.
4. Berkowitz DE, White R, Li D, et al. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* 2003;108:2000–2006.
5. Bivalacqua TJ, Hellstrom WJ, Kadowitz PJ, Champion HC. Increased expression of arginase II in human diabetic corpus cavernosum: In diabetic-associated erectile dysfunction. *Biochem Biophys Res Commun* 2001;283:923–927.
6. Boelens PG, van Leeuwen PA, Dejong CH, Deutz NE. Intestinal renal metabolism of L-citrulline and L-arginine following enteral or parenteral infusion of L-alanyl-L-[2,15N]glutamine or L-[2,15N]glutamine in mice. *Am J Physiol Gastrointest Liver Physiol* 2005;289:G679–685.
7. Brosnan JT. Interorgan amino acid transport and its regulation. *J Nutr* 2003;133:2068S–2072S.
8. Brosnan ME, Brosnan JT. Renal arginine metabolism. *J Nutr* 2004;134:2791S–2795S; discussion 2796S–2797S.
9. Chen PY, Sanders PW. L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J Clin Invest* 1991;88:1559–1567.
10. Chicoine LG, Paffett ML, Young TL, Nelin LD. Arginase inhibition increases nitric oxide production in bovine pulmonary arterial endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L60–68.
11. Christowitz D, Mattheyse FJ, Balinsky JB. Dietary and hormonal regulation of urea cycle enzymes in rat liver. *Enzyme* 1981;26:113–121.
12. Cooke JP, Andon NA, Girerd XJ, Hirsch AT, Creager MA. Arginine restores cholinergic relaxation of hypercholesterolemic rabbit thoracic aorta. *Circulation* 1991;83:1057–1062.
13. Creager MA, Cooke JP, Mendelsohn ME, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990;86:228–234.
14. Curis E, Nicolis I, Moinard C, et al. Almost all about citrulline in mammals. *Amino Acids* 2005;29:177–205.
15. Cynober L. Can arginine and ornithine support gut functions? *Gut* 1994;35:S42–45.
16. Cynober L, Le Boucher J, Vasson M. Arginine metabolism in mammals. *J Nutr Biochem* 1995;6:402–413.
17. Dhanakoti SN, Brosnan JT, Herzberg GR, Brosnan ME. Renal arginine synthesis: Studies *in vitro* and *in vivo*. *Am J Physiol* 1990;259:E437–442.
18. Dillon EL, Knabe DA, Wu G. Lactate inhibits citrulline and arginine synthesis from proline in pig enterocytes. *Am J Physiol* 1999;276:G1079–1086.
19. Dong S, Kojima T, Shiraiwa M, et al. Regulation of the expression of peptidylarginine deiminase type II gene (PADI2) in human keratinocytes involves Sp1 and Sp3 transcription factors. *J Invest Dermatol* 2005;124:1026–1033.
20. Fasipe FR, Ubawike AE, Eva R, Fabry ME. Arginine supplementation improves rotorod performance in sickle transgenic mice. *Hematology* 2004;9:301–305.

21. Flynn NE, Meininger CJ, Haynes TE, Wu G. The metabolic basis of arginine nutrition and pharmacotherapy. *Biomed Pharmacother* 2002;56:427–438.
22. Ganong WF. *Review of Medical Physiology*. Los Altos, CA: Lange Medical Publications 1991;340.
23. Gebhardt R, Mecke D. Permissive effect of dexamethasone on glucagon induction of urea-cycle enzymes in perfused primary monolayer cultures of rat hepatocytes. *Eur J Biochem* 1979;97:29–35.
24. Goodwin BL, Solomonson LP, Eichler DC. Argininosuccinate synthase expression is required to maintain nitric oxide production and cell viability in aortic endothelial cells. *J Biol Chem* 2004;279:18353–18360.
25. Greenberg D. Arginase. In: *The Enzymes*. Boyer PD, Lardy H, Myrback K, Eds. New York: Academic Press 1960;4:257.
26. Hagenfeldt L, Dahlquist G, Persson B. Plasma amino acids in relation to metabolic control in insulin-dependent diabetic children. *Acta Paediatr Scand* 1989;78:278–282.
27. Hallemeesch MM, Janssen BJ, de Jonge WJ, Soeters PB, Lamers WH, Deutz NE. NO production by cNOS and iNOS reflects blood pressure changes in LPS-challenged mice. *Am J Physiol Endocrinol Metab* 2003;285:E871–875.
28. Hao G, Xie L, Gross SS. Argininosuccinate synthetase is reversibly inactivated by S-nitrosylation *in vitro* and *in vivo*. *J Biol Chem* 2004;279:36192–36200.
29. Hartman WJ, Torre PM, Prior RL. Dietary citrulline but not ornithine counteracts dietary arginine deficiency in rats by increasing splanchnic release of citrulline. *J Nutr* 1994;124:1950–1960.
30. Hattori Y, Campbell EB, Gross SS. Argininosuccinate synthetase mRNA and activity are induced by immunostimulants in vascular smooth muscle. Role in the regeneration or arginine for nitric oxide synthesis. *J Biol Chem* 1994;269:9405–9408.
31. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: Cultured endothelial cells recycle L-citrulline to L-arginine. *Proc Natl Acad Sci USA* 1990;87:8612–8616.
32. Heneka MT, Schmidlin A, Wiesinger H. Induction of argininosuccinate synthetase in rat brain glial cells after striatal microinjection of immunostimulants. *J Cereb Blood Flow Metab* 1999;19:898–907.
33. Hilscher LW, Hanson CD, Russell DH, Raushel FM. Measurement of positional isotope exchange rates in enzyme-catalyzed reactions by fast atom bombardment mass spectrometry: Application to argininosuccinate synthetase. *Biochemistry* 1985;24:5888–5893.
34. Husson A, Brasse-Lagnel C, Fairand A, Renouf S, Lavoinin A. Argininosuccinate synthetase from the urea cycle to the citrulline-NO cycle. *Eur J Biochem* 2003;270:1887–1899.
35. Inoue Y, Copeland EM, Souba WW. Growth hormone enhances amino acid uptake by the human small intestine. *Ann Surg* 1994;219:715–722; discussion 722–714.
36. Ishigami A, Ohsawa T, Asaga H, Akiyama K, Kuramoto M, Maruyama N. Human peptidylarginine deiminase type II: Molecular cloning, gene organization, and expression in human skin. *Arch Biochem Biophys* 2002;407:25–31.
37. Jean C, Rome S, Mathe V, et al. Metabolic evidence for adaptation to a high protein diet in rats. *J Nutr* 2001;131:91–98.
38. Jeremy RW, McCarron H, Sullivan D. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilatation in the hypercholesterolemic rabbit. Response according to treatment duration, anatomic site, and sex. *Circulation* 1996;94:498–506.
39. Jin L, Abou-Mohamed G, Caldwell RB, Caldwell RW. Endothelial cell dysfunction in a model of oxidative stress. *Med Sci Monit* 2001;7:585–591.
40. Kaesemeyer WH, Ogonowski AA, Jin L, Caldwell RB, Caldwell RW. Endothelial nitric oxide synthase is a site of superoxide synthesis in endothelial cells treated with glyceryl trinitrate. *Br J Pharmacol* 2000;131:1019–1023.
41. Kinloch A, Tatzer V, Wait R, et al. Identification of citrullinated alpha-enolase as a candidate autoantigen in rheumatoid arthritis. *Arthritis Res Ther* 2005;7:R1421–1429.
42. Koga T, Zhang WY, Gotoh T, Oyadomari S, Tanihara H, Mori M. Induction of citrulline-nitric oxide (NO) cycle enzymes and NO production in immunostimulated rat RPE-J cells. *Exp Eye Res* 2003;76:15–21.
43. Kuhn KP, Harris PA, Cunningham GR, Robbins IM, Summar ML, Christman BW. Oral citrulline effectively elevates plasma arginine levels for 24 h in normal volunteers. *Circulation AHA Scientific Sessions* 2006; abstract 1692, p. II-1339.
44. Lerman A, Burnett JC, Jr., Higano ST, McKinley LJ, Holmes DR, Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation* 1998;97:2123–2128.
45. Lopansri BK, Anstey NM, Weinberg JB, et al. Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet* 2003;361:676–678.

46. Lux GD, Marton LJ, Baylin SB. Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. *Science* 1980;210:195–198.
47. Maarsingh H, Leusink J, Zaagsma J, Meurs H. Role of the l-citrulline/l-arginine cycle in iNANC nerve-mediated nitric oxide production and airway smooth muscle relaxation in allergic asthma. *Eur J Pharmacol* 2006;546:171–176.
48. Mayhan WG, Patel KP, Sharpe GM. Effect of L-arginine on reactivity of hamster cheek pouch arterioles during diabetes mellitus. *Int J Microcirc Clin Exp* 1997;17:107–112.
49. McCormick SM, Eskin SG, McIntire LV, et al. DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc Natl Acad Sci USA* 2001;98:8955–8960.
50. McDonald KK, Zharikov S, Block ER, Kilberg MS. A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the “arginine paradox.” *J Biol Chem* 1997;272:31213–31216.
51. Meininger CJ, Wu G. L-glutamine inhibits nitric oxide synthesis in bovine venular endothelial cells. *J Pharmacol Exp Ther* 1997;281:448–453.
52. Ming XF, Barandier C, Viswambharan H, et al. Thrombin stimulates human endothelial arginase enzymatic activity via RhoA/ROCK pathway: Implications for atherosclerotic endothelial dysfunction. *Circulation* 2004;110:3708–3714.
53. Morimoto BH, Brady JF, Atkinson DE. Effect of level of dietary protein on arginine-stimulated citrulline synthesis. Correlation with mitochondrial N-acetylglutamate concentrations. *Biochem J* 1990;272:671–675.
54. Morris CR, Morris SM, Jr., Hagar W, et al. Arginine therapy: A new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med* 2003;168:63–69.
55. Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005;294:81–90.
56. Morris SM, Jr., Moncman CL, Holub JS, Hod Y. Nutritional and hormonal regulation of mRNA abundance for arginine biosynthetic enzymes in kidney. *Arch Biochem Biophys* 1989;273:230–237.
57. Morris SM, Jr. Regulation of enzymes of urea and arginine synthesis. *Annu Rev Nutr* 1992;12:81–101.
58. Morris SM, Jr. Arginine: Beyond protein. *Am J Clin Nutr* 2006;83:508S–512S.
59. Muniz R, Burguillo L, del Castillo JR. Effect of starvation on neutral amino acid transport in isolated small-intestinal cells from guinea pigs. *Pflügers Arch* 1993;423:59–66.
60. Okpala IE. New therapies for sickle cell disease. *Hematol Oncol Clin North Am* 2005;19:975–987,ix.
61. Osowska S, Moinard C, Neveux N, Loi C, Cynober L. Citrulline increases arginine pools and restores nitrogen balance after massive intestinal resection. *Gut* 2004;53:1781–1786.
62. Pardridge WM, Jefferson LS. Liver uptake of amino acids and carbohydrates during a single circulatory passage. *Am J Physiol* 1975;228:1155–1161.
63. Parmar K, Larman H, Dai G, et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest* 2006;116:49–58.
64. Pieper GM, Dondlinger LA. Plasma and vascular tissue arginine are decreased in diabetes: Acute arginine supplementation restores endothelium-dependent relaxation by augmenting cGMP production. *J Pharmacol Exp Ther* 1997;283:684–691.
65. Plakidou-Dymock S, Tanner MJ, McGivan JD. Regulation of System B0 amino-acid-transport activity in the renal epithelial cell line NBL-1 and concomitant changes in SAAT1 hybridizing transcripts. *Biochem J* 1994;301(Pt 2):399–405.
66. Pollock JS, Forstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, Murad F. Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. *Proc Natl Acad Sci USA* 1991;88:10480–10484.
67. Raiha NC, Edkins E. Insulin antagonism of dexamethasone-induced increase or argininosuccinate synthetase and argininosuccinate lyase activities in cultured fetal rat liver. *Biol Neonate* 1977;31:266–270.
68. Rector TS, Bank AJ, Mullen KA, et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. *Circulation* 1996;93:2135–2141.
69. Ricciardolo FL. Multiple roles of nitric oxide in the airways. *Thorax* 2003;58:175–182.
70. Ryall JC, Quantz MA, Shore GC. Rat liver and intestinal mucosa differ in the developmental pattern and hormonal regulation of carbamoyl-phosphate synthetase I and ornithine carbamoyl transferase gene expression. *Eur J Biochem* 1986;156:453–458.
71. Schlaich MP, Parnell MM, Ahlers BA, et al. Impaired L-arginine transport and endothelial function in hypertensive and genetically predisposed normotensive subjects. *Circulation* 2004;110:3680–3686.

72. Schulman SP, Becker LC, Kass DA, et al. L-arginine therapy in acute myocardial infarction: The Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA* 2006;295:58–64.
73. Schulze F, Lenzen H, Hanefeld C, et al. Asymmetric dimethylarginine is an independent risk factor for coronary heart disease: Results from the multicenter Coronary Artery Risk Determination investigating the Influence of ADMA Concentration (CARDIAC) study. *Am Heart J* 2006;152:491–498.
74. Shearer JD, Richards JR, Mills CD, Caldwell MD. Differential regulation of macrophage arginine metabolism: A proposed role in wound healing. *Am J Physiol* 1997;272:E181–190.
75. Shen LJ, Lin WC, Beloussow K, et al. Recombinant arginine deiminase as a differential modulator of inducible (iNOS) and endothelial (eNOS) nitric oxide synthetase activity in cultured endothelial cells. *Biochem Pharmacol* 2003;66:1945–1952.
76. Shigesada K, Tatibana M. N-Acetylglutamate synthetase from rat-liver mitochondria. Partial purification and catalytic properties. *Eur J Biochem* 1978;84:285–291.
77. Simon A, Plies L, Habermeier A, Martine U, Reining M, Closs EI. Role of neutral amino acid transport and protein breakdown for substrate supply of nitric oxide synthase in human endothelial cells. *Circ Res* 2003;93:813–820.
78. Smith HA CJ, Christian KG, Drinkwater DC, et al. Nitric oxide precursors and congenital heart surgery: A randomized controlled trial of oral citrulline. *J Thorac Cardiovasc Surg* 2006;132:58–65.
79. Smith RJ, Phang JM. The importance of ornithine as a precursor for proline in mammalian cells. *J Cell Physiol* 1979;98:475–481.
80. Socha HM, Romero MJ, Caldwell RB, Caldwell RW. Oral citrulline administration enhances NO-dependent vasodilation. *FASEB Exp Biol* 2006;Abs 703.4.
81. Spolarics Z, Bond JS. Comparison of biochemical properties of liver arginase from streptozocin-induced diabetic and control mice. *Arch Biochem Biophys* 1989;274:426–433.
82. Su Y, Block E. Hypoxia inhibits the induction of argininosuccinate synthetase by endotoxin in lung endothelial cells. *Am J Physiol* 1997;272:L934–938.
83. Sunkara PS, Rao PN, Nishioka K, Brinkley BR. Role of polyamines in cytokinesis of mammalian cells. *Exp Cell Res* 1979;119:63–68.
84. Susic D, Francischetti A, Frohlich ED. Prolonged L-arginine on cardiovascular mass and myocardial hemodynamics and collagen in aged spontaneously hypertensive rats and normal rats. *Hypertension* 1999;33:451–455.
85. Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation* 1996;94:1298–1303.
86. Tsuei BJ, Bernard AC, Barksdale AR, Rockich AK, Meier CF, Kearney PA. Supplemental enteral arginine is metabolized to ornithine in injured patients. *J Surg Res* 2005;123:17–24.
87. Tsuji Y, Akiyama M, Arita K, Senshu T, Shimizu H. Changing pattern of deiminated proteins in developing human epidermis. *J Invest Dermatol* 2003;120:817–822.
88. Vadgama JV, Evered DF. Characteristics of L-citrulline transport across rat small intestine *in vitro*. *Pediatr Res* 1992;32:472–478.
89. Van Beers EH, Rings EH, Posthuma G, et al. Intestinal carbamoyl phosphate synthase I in human and rat. Expression during development shows species differences and mosaic expression in duodenum of both species. *J Histochem Cytochem* 1998;46:231–240.
90. van de Poll MC, Soeters PB, Deutz NE, Fearon KC, Dejong CH. Renal metabolism of amino acids: Its role in interorgan amino acid exchange. *Am J Clin Nutr* 2004;79:185–197.
91. Vichinsky E. New therapies in sickle cell disease. *Lancet* 2002;360:629–631.
92. Waugh WH, Mhupp CL, Liles DR. Undetectable plasma L-arginine level before visible hemolysis in thrombocytic thrombocytopenic purpura. *Am J Haematol* 1999;61:216–216.
93. Waugh WH, Daeschner CW, 3rd, Files BA, McConnell ME, Strandjord SE. Oral citrulline as arginine precursor may be beneficial in sickle cell disease: Early phase two results. *J Natl Med Ass* 2001;93:363–371.
94. Weyrich AS, Ma XL, Lefer AM. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* 1992;86:279–288.
95. Wheatley DN, Kilfeather R, Stitt A, Campbell E. Integrity and stability of the citrulline-arginine pathway in normal and tumour cell lines. *Cancer Lett* 2005;227:141–152.
96. White AR, Ryoo S, Li D, Champion HC, et al. Knockdown of arginase I restores NO signaling in the vasculature of old rats. *Hypertension* 2006;47:245–251.
97. Wiesinger H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog Neurobiol* 2001;64:365–391.

98. Wileman SM, Mann GE, Pearson JD, Baydoun AR. Role of L-citrulline transport in nitric oxide synthesis in rat aortic smooth muscle cells activated with LPS and interferon-gamma. *Br J Pharmacol* 2003;140:179–185.
99. Witte MB, Thornton FJ, Tantry U, Barbul A. L-Arginine supplementation enhances diabetic wound healing: Involvement of the nitric oxide synthase and arginase pathways. *Metabolism* 2002;51:1269–1273.
100. Worsaae H, Schmidt M. Plasma cortisol and behaviour in early weaned piglets. *Acta Vet Scand* 1980;21:640–657.
101. Wraight C, Lingelbach K, Hoogenraad N. Comparison of ornithine transcarbamylase from rat liver and intestine. Evidence for differential regulation of enzyme levels. *Eur J Biochem* 1985;153:239–242.
102. Wu G, Knabe DA, Flynn NE. Synthesis of citrulline from glutamine in pig enterocytes. *Biochem J* 1994;299(Pt 1):115–121.
103. Wu G. Urea synthesis in enterocytes of developing pigs. *Biochem J* 1995;312(Pt 3):717–723.
104. Wu G, Meininger CJ, Kelly K, Watford M, Morris SM, Jr. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J Nutr* 2000;130:1914–1919.
105. Xu W, Kaneko FT, Zheng S, et al. Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. *FASEB J* 2004;18:1746–1748.
106. Yoon YS, Uchida S, Masuo O, et al. Progressive attenuation of myocardial vascular endothelial growth factor expression is a seminal event in diabetic cardiomyopathy: Restoration of microvascular homeostasis and recovery of cardiac function in diabetic cardiomyopathy after replenishment of local vascular endothelial growth factor. *Circulation* 2005;111:2073–2085.
107. Zieve L. Conditional deficiencies of ornithine or arginine. *J Am Coll Nutr* 1986;5:167–176.