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REVIEW ARTICLE

Therapeutic Potential of Citrulline as an Arginine Supplement: A Clinical Pharmacology Review

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Abstract

Supplemental arginine has shown promise as a safe therapeutic option to improve endogenous nitric oxide (NO) regulation in cardiovascular diseases associated with endothelial dysfunction. In clinical studies in adults, L-arginine, an endogenous amino acid, was reported to improve cardiovascular function in hypertension, pulmonary hypertension, preeclampsia, angina, and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome. L-citrulline, a natural precursor of L-arginine, is more bioavailable than L-arginine because it avoids hepatic first-pass metabolism and has a longer circulation time. Although not yet well-studied, arginine/citrulline has immense therapeutic potential in some life-threatening diseases in children. However, the optimal clinical development of arginine or citrulline in children requires more information about pharmacokinetics and exposure–response relationships at appropriate ages and under relevant disease states. This article summarizes the preclinical and clinical studies of arginine/citrulline in both adults and children, including currently available pharmacokinetic information. The pharmacology of arginine/citrulline is confounded by several patient-specific factors such as variations in baseline arginine/citrulline due to developmental ages and disease states. Currently available pharmacokinetic studies are insufficient to inform the optimal design of clinical studies, especially in children. Successful bench-to-bedside clinical translation of arginine supplementation awaits information from well-designed pharmacokinetic/pharmacodynamic studies, along with pharmacometric approaches.

Key Points

Supplemental arginine therapy improves nitric oxide regulation in cardiovascular diseases associated with endothelial dysfunction.

Clinical pharmacology of arginine or citrulline, the two forms of exploratory clinical arginine supplement therapies, is not well understood.

Well-designed pharmacokinetic-pharmacodynamic studies are needed to establish exposure-response relationships of citrulline/arginine therapy for cardiovascular diseases.

1 Introduction

L-arginine is a semi-essential amino acid that plays critical physiological roles in muscle development and ammonia detoxification [1, 2]; it has been investigated for therapeutic use in a wide variety of pathological conditions [3–5] and is commonly used as an ergogenic nutrition supplement [5]. Therapeutic arginine supplementation improves endogenous nitric oxide (NO) production so has mostly been investigated for clinical use in ailments of the cardiovascular system, including hypertension, pulmonary hypertension (PH), angina, nitrate tolerance, and preeclampsia. L-arginine supplementation was reported to restore normal endothelial

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function in both normotensive and hypertensive patients with microvascular angina [6, 7]. Organic nitrates are NO donors widely used for the treatment of coronary artery diseases and chronic heart failure, but tolerance to these agents remains a long-standing therapeutic problem, and nitrate-free treatment intervals are the only way to prevent nitrate tolerance in current clinical practice [8, 9]. Arginine supplementation restores the normal function of NO, so has been useful in preventing rapid nitrate tolerance from the use of transdermal nitroglycerine in patients with stable angina [10]. Altered NO production also contributes to the pathophysiology of preeclampsia and intrauterine growth restriction [11]. Supplemental arginine during pregnancy reduced the incidence of preeclampsia in a high-risk pregnant population [12].

Arginine is also a promising therapy for PH, a devastating cardiopulmonary disease with a high mortality rate [13]. Clinical studies in adults showed that arginine supplementation, administered in the form of either arginine or its precursor, citrulline, improved pulmonary hemodynamics in adults with PH [14–16]. Although aspects of the underlying pathophysiology for PH may differ between children and adults, arginine/citrulline supplementation for idiopathic or disease-associated PH is also receiving attention in children [17–21].

Evidence also indicates that arginine may be therapeutically useful in some life-threatening inherited diseases in children that are accompanied by cardiovascular complications. MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome, a progressively fatal mitochondrial disease, is caused by genetic mutations in the mitochondrial DNA. In MELAS syndrome, uncontrolled mitochondrial proliferation causes endothelial dysfunction, which leads to alterations in cerebral hemodynamics and stroke-like episodes. The published literature indicates that supplemental arginine can replenish NO deficiency and thus improve blood circulation in the cerebral microvasculature [22, 23]. A recently published clinical study suggested that arginine supplementation, in the form of either arginine or citrulline, appears to have therapeutic benefits in children with MELAS syndrome [24].

Urea cycle disorders (UCD) are another group of diseases that affect young children. In UCD, inherited deficiencies of some key enzymes and transporters of the urea cycle pathway result in the accumulation of ammonia and some precursor metabolites, causing severe neurological damage [18, 25]. For the last two decades, supplemental arginine has been useful in improving clinical outcomes in children with UCD [26, 27]. Arginine therapy replenishes the deficient urea cycle intermediates by activating alternative enzymatic pathways [27, 28]. In clinical studies, supplemental arginine therapy has been used as either direct L-arginine or in the form of the arginine precursor, citrulline. Citrulline is an alpha amino acid that is metabolized to L-arginine, and, when administered orally, is more effective than L-arginine in improving arginine plasma concentrations because it is not the substrate of hepatic or intestinal arginases [5].

Although arginine supplementation has tremendous therapeutic potential in some life-threatening diseases in children, the clinical pharmacology of arginine/citrulline therapy—particularly the pharmacokinetics, exposure–response relationship, and safety—has not been investigated extensively in the pediatric population. In this review, we summarize the molecular basis of arginine/citrulline supplementation in different diseases and the pharmacokinetics and pharmacodynamics of arginine/citrulline in their potential therapeutic uses in adult and pediatric diseases. We discuss the relationship between pharmacokinetic exposure and therapeutic response to arginine/citrulline in preclinical studies, with its possible translation to clinical studies. This article is intended to serve as a ready reference as reviews of arginine/citrulline supplementation are lacking in terms of the pharmacokinetics and potential therapeutic uses in pediatric diseases.

2 L-Arginine Deficiency in Vascular Endothelial Cell Dysfunction and the Use of Citrulline as an Efficient Arginine Supplement

Regulation of vascular tone and contractility is critically dependent on the availability of L-arginine, a significant substrate for NO production in the vascular endothelium. Inadequate supply of L-arginine results in increased superoxide generation by endothelial NO synthase (eNOS) and altered L-arginine metabolism within the vascular endothelial cells. Prolonged L-arginine deficiency further elevates oxidative stress, sequestration of intracellular L-arginine, and slower recycling of L-arginine from its precursor L-citrulline. As a result, vasoconstriction and vascular remodeling occur as hyperactive eNOS fails to produce enough NO [29–31]. Published studies have shown that supplemental arginine can prevent endothelial dysfunction and thus restore the normal vasodilatory capacity of the vascular endothelium. However, the presence of arginase in intestinal enterocytes and the high first-pass metabolism of L-arginine to ornithine and urea by the liver arginases means that oral arginine supplementation cannot sufficiently elevate plasma L-arginine levels [32, 33]. Moreover, higher levels of circulating L-arginine induce arginases in most tissues, resulting in rapid L-arginine clearance. However, the arginine paradox, an observed phenomenon with arginine supplementation, suggests that exogenous L-arginine causes NO-mediated biological effects even if the NOS pathways are saturated with the substrate L-arginine. One explanation for this paradox is L-arginine-mediated displacement of asymmetric dimethylarginine, a natural byproduct and an analog of L-arginine that impairs normal endothelial function [34].

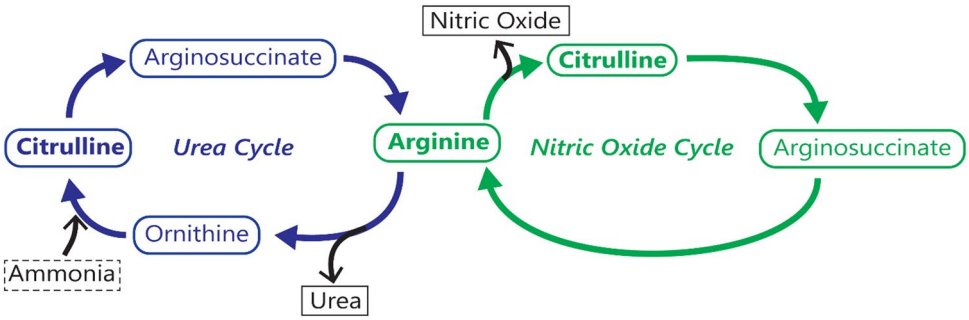

Fig. 1 Arginine and citrulline: two major players in the urea and nitric oxide cycles. As a natural precursor, citrulline engages in both nitric oxide and urea cycles. In urea cycle, ornithine carbamoyl transferase catalyzes the formation of citrulline from ammonia and ornithine, a glutamine metabolite. Citrulline is then converted to arginine which, in turn, releases nitric oxide to become citrulline. With the growth of the children, the small intestine produces more citrulline than arginine. Citrulline is mostly metabolized in the kidney

L-citrulline, a natural precursor of L-arginine, plays a role in both urea and NO cycles and can be a better substitute of L-arginine supplementation because it bypasses hepatic first-pass metabolism and can be converted to L-arginine specifically within the tissues (Fig. 1). L-citrulline is an intermediate product of the urea cycle in which L-ornithine, a metabolite of L-glutamine, is converted to L-citrulline by ornithine carbamoyltransferase. With development, L-citrulline becomes a useful product of the small intestine in humans as compared with L-arginine, and the kidney, the primary organ of L-citrulline metabolism, gradually becomes more efficient in converting L-citrulline to L-arginine in the postnatal period. In other words, oral L-citrulline can effectively improve L-arginine plasma levels as it is transported into enterocytes without metabolic loss by arginase and because it then bypasses hepatic metabolism and is transported to the kidneys where it is metabolized to L-arginine [33]. These elements of L-citrulline processing provide pharmacokinetic advantages over L-arginine. Consequently, oral L-citrulline is expected to result in comparatively higher concentrations of L-arginine and therefore bring about more significant therapeutic benefits than oral L-arginine. Thus, L-citrulline supplementation is considered a better alternative to L-arginine supplementation in a wide variety of diseases caused by L-arginine deficiency.

3 Preclinical Studies Exploring L-Arginine Supplementation in Various Diseases

Preclinical studies are necessary when investigating novel drugs/supplements to establish safety and proof of concept for efficacy before initiating clinical trials in humans, especially in vulnerable populations such as pediatric or pregnant patients. The use of in vivo (whole animal) or ex vivo (isolated organ) models for assessing safety and efficacy provide a basis for understanding the potential effect size in humans and the possible side effect profile.

Table 1 Summary of preclinical studies of arginine supplementation

Disease	Animal model	Outcome of L-arginine or citrulline supplementation
Atherosclerosis	Hypercholesterolemic rabbit LDL receptor knockout mouse	↓ Lesion surface area [35, 36, 39, 48] ↓ Intima thickness [35–37, 46] Absence of adherent monocytes or tissue macrophages [46] Prevents xanthoma formation [48]
Vascular endothelial function	Hypercholesterolemic rabbit	↑ Endothelial-dependent relaxation [35–40] ↑ NO production/superoxide radical release [35, 39, 40, 114]
PH	Hypoxia-induced PH (rat and newborn pig) Monocrotaline-induced PH [44]	↑ NO production [41–43, 45, 54–57] ↓ Pulmonary vascular resistance [41–45]
Systemic hypertension	Salt-sensitive hypertension (rats) Spontaneous hypertension (young rat)	↑ NO production [56, 57]
Preeclampsia	Insulin-induced hypertension (pregnant rat) Adriamycin-induced nephropathy (pregnant rat)	↓ Blood pressure [50–54] ↓ Urinary metabolites or nitrate [50–54] ↓ Urine protein excretion [53, 54]

LDL low-density lipoprotein, *NO* nitric oxide, PH pulmonary hypertension, ↓ indicates decrease, ↑ indicates increase

The use of citrulline and L-arginine has been investigated preclinically in some diseases and different animal models. This section focuses on atherosclerosis (vascular wall and vascular function), systemic hypertension, PH, and preeclampsia. Table 1 summarizes the preclinical studies conducted in these diseases as well as outcomes from arginine or citrulline supplementation.

As described in the previous section, NO is vital to maintain vascular endothelial function. As such, the significant effect of arginine/citrulline supplementation is to improve endothelial function in both systemic [35–40] and pulmonary [41–45] vasculature. Notably, the improvement in endothelial function results in downstream benefits such as decreased vascular/pulmonary pressure or normalization of vascular wall intimal thickness [35–37, 46]. For example, Preli et al. [47] summarized the vascular wall effects of L-arginine supplementation. The dominant animal model used in this setting was the hypercholesteremic rabbit (high-cholesterol chow). Briefly, L-arginine supplementation reduced the surface area of atherosclerotic lesions and the intima thickness compared with untreated animals (Table 1). Another study used low-density lipoprotein receptor knockout mice to show that L-arginine prevented the formation of xanthomas and concluded that L-arginine supplementation might be of benefit to patients with familial hypercholesterolemia [48]. L-arginine supplementation also reduced systemic blood pressure in both salt-sensitive hypertension [49] and spontaneous hypertension rat models [50] as well as in different animal models for preeclampsia, including insulin-induced hypertension [51, 52] and adriamycin-induced nephropathy models [53, 54]. The link between arginine/citrulline supplementation, improved NO production, and vascular function has been shown in several animal models investigating PH. Both intact animal and ex vivo models for PH were developed with either environmental injury (hypoxia) [41–43, 55] or drug-toxin-induced (monocrotaline) injury [45]. In these models, L-arginine supplementation increased NO production [41–43, 45, 54–57], ultimately resulting in reduced pulmonary vascular resistance [41–45] (Table 1). Similarly, L-citrulline supplementation was effective in improving pulmonary vascular NO production and ameliorating PH when investigated in newborn/juvenile animal models, for example, newborn pigs [41, 42, 55] and young rats [57].

The effects of arginine supplementation were also evaluated in some preclinical models representing systemic hypertension. L-arginine or L-citrulline intake improved NO synthesis and prevented salt-sensitive hypertension in rats [56]. A recently published study showed that L-citrulline supplementation prevented spontaneous hypertension in rats [57].

These preclinical studies have demonstrated the efficacy of arginine/citrulline supplementation in a wide variety of pathologic conditions but do have limitations. One limitation is the dearth of studies using age or physiologically appropriate models (i.e., pediatric/juvenile or pregnant animal). Many of the studies also involved short-term exposures and did not sufficiently address safety concerns. One animal study investigated the toxicity of L-arginine over 13 weeks in rats and reported that, overall, the side effects were minimal and generally transient [58]. Theoretically, an overdose of arginine/citrulline may result in hypotension because of its vasodilatory effects. Published articles show that large doses of L-arginine are used in preclinical models to induce acute pancreatitis [59], and an accidental overdose of arginine hydrochloride was fatal [60]. No adverse side effects have been reported in studies with citrulline, but only a limited range of doses have been used. Thus, the safety of arginine supplementation remains of concern and mandates that safety issues be carefully considered and adverse effects closely monitored when clinical translation occurs.

4 Clinical Use of Arginine Supplementation

The therapeutic effects of L-citrulline mostly depend on the action of L-arginine. L-arginine is generated from L-citrulline by arginine-succinate synthase as part of the urea cycle (Fig. 1). As a substrate for NOS, L-arginine is integral to the formation of NO and reformation of the precursor L-citrulline. NO is a cofactor for soluble guanylate cyclase, catalyzing the formation of cyclic guanosine monophosphate (cGMP), a regulator, mediator, and messenger in the nervous, immunologic, and cardiovascular systems. In the vasculature, cGMP is a regulator for vasodilation. As dysfunctional NO signaling causes a wide variety of diseases, supplemental arginine in the form of L-arginine or L-citrulline has been evaluated in different clinical studies, as summarized in Table 2.

4.1 Supplemental Arginine/Citrulline in Adult Clinical Studies

Clinical studies in adults have evaluated the effect of dietary supplementation with citrulline or citrulline-rich watermelon on exercise outcomes [61, 62] and cardiovascular health, including PH [62–81]. Although clinical studies in the mid-1990s were not in agreement on the benefits of arginine supplementation in PH [81–85], recent studies have more consistently supported the beneficial effects of arginine/citrulline in adults with cardiovascular problems [80, 86]. However, a longer treatment period (12 weeks) with oral L-arginine 6 g/day brought about this benefit [80]. Administration of oral L-citrulline 3 g daily for 2 weeks was associated with improved quality of life in patients with idiopathic PH [15]. Three studies assessed the effect of arginine

Table 2 Clinical studies of arginine supplementation in different diseases

Disease	Study population			Clinical outcome
	Age (y)	*n*	Dosing	
Inborn errors of ureagenesis [26]	< 0.5	4	ARG/CIT 0.2 mmol/kg/d	ARG supplementation improved symptomatic hyperammonemia
Angina pectoris [6]	Angina 57 ± 9 Control 59 ± 8	16; microvascular angina 8, control 8	ARG 500 mg as 10-minute infusion	ARG supplementation improved endothelium-dependent vasodilation in coronary arteries
Angina pectoris [7]	Angina 57 ± 9 Control 59 ± 8	13	Oral ARG 2 g TID	ARG supplementation improved endothelial function in hypertensive pts with microvascular angina
Preeclampsia [12]	PL 28.2 ± 5.1 ARG 28.0 ± 6.1	450: PL 222, ARG 228	Oral ARG 6.6 g/d	ARG supplementation prolonged latency to development of preeclampsia
Nitrate tolerance in pts with angina [10]	14: ARG 7, PL 7		Oral ARG 700 mg	ARG supplementation modified or prevented development of nitrate tolerance during continuous transdermal nitroglycerine therapy
Sickle cell disease [91]	10–18	5	Oral CIT 0.1 g/kg BID	↓ Total leukocyte and segmental neutrophil count Symptomatic improvement No controls used
Postoperative PH [92]	< 6	40: PL 20, CIT 20	5 postoperative doses of CIT 1 g/m^2	CIT supplementation elevated plasma ARG/CIT concentration Elevated CIT in plasma prevented postoperative PH
MELAS syndrome [24]	6–14	10: PL 5, CIT/ARG 5	CIT/ARG 3 g/kg/d	CIT supplementation was more effective than ARG in improving NO production
MELAS syndrome [23]	20–46	20: PL 10, CIT/ARG 10	CIT/ARG 0.8 g/kg/d	CIT supplementation substantially increased NO production vs. ARG supplementation
Acute MI [89]	> 30	153: PL 75, CIT/ARG 78	1–3 g TID for 6 mo	No improvement in vascular stiffness or ejection fraction Might be associated with higher postinfarction mortality L-ARG supplementation should not be recommended in acute MI
Tuberculosis [90]	15–73	200: ARG + vit D 50, ARG + PL 49, PL + vit D 51, all PL 50	Oral tablets: ARG 6 g daily, vit D_3 1250 µg	L-ARG supplementation had no effect on tuberculosis outcome
PH [83]	52 ± 5	PH 10, heart failure without PH 5	30-minute infusion of ARG 500 mg/kg	Exogenous L-ARG was beneficial in some pts with PH in short term
CF-associated airway obstruction [88]	14–45	13 pts with CF	18 mL of 7% ARG HCl solution via electronic nebulizer	Single inhalation of ARG resulted in acute and transient improvement of pulmonary function and oxygenation

ARG arginine, *BID* twice daily, *CF* cystic fibrosis, *CIT* citrulline, *d* day(s), *L-ARG* L-arginine, *MELAS* mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, *MI* myocardial infarction, *mo* month(s), *NO* nitric oxide, *PH* pulmonary hypertension, *PL* placebo, *pt(s)* patient(s), *TID* three times daily, *vit* vitamin, *y* year(s), ↓ indicates decrease

supplementation in complications associated with angina pectoris. Egashira et al. [6] showed that intracoronary infusion of L-arginine 500 mg over 10 min improved coronary microcirculation in patients with angina pectoris but not in control subjects who had atypical chest pain and normal coronary angiogram. In this study, L-arginine supplementation was associated with improved endothelium-dependent vasodilation. Another clinical study 8 years later [7] reported that oral L-arginine supplementation 6 g daily for 4 weeks was associated with improved resting systolic blood pressure and quality of life in hypertensive patients with microvascular angina. In this study, treatment-associated increases in plasma cGMP also suggested that L-arginine supplement improved endothelial function in hypertensive patients [7]. A randomized, placebo-controlled double-blind study [10] found that supplemental oral L-arginine 2.8 g daily effectively prevented nitrate tolerance, a significant limitation of organic nitrates used as first-line therapies for angina pectoris. In this study, oral L-arginine therapy showed beneficial effects in preventing nitrate tolerance during continuous transdermal nitroglycerin administration [10]. Supplementation with oral L-arginine (10 g per squared meter of body surface area) was effective in managing symptoms associated with MELAS syndrome [87], and a single dose of inhaled L-arginine was also effective when used in cystic fibrosis-associated airway obstruction [88]. However, arginine supplementation was not effective in improving acute myocardial infarction or tuberculosis outcomes [89, 90].

4.2 Supplemental Arginine/Citrulline in Pediatric Clinical Studies

Brusilow [26] first showed that arginine supplementation could ameliorate symptomatic hyperammonemia in a pediatric patient with inborn errors in urea synthesis. In 2001, Waugh et al. [91] reported a pilot phase II clinical trial with five pediatric participants (aged 10–18 years) with sickle cell disease. This study found that administration of 0.09–0.13 g/kg of oral L-citrulline twice daily for 4 weeks increased plasma arginine concentrations by 65% and normalized leukocyte and neutrophil counts. Based on these results and positive perceptions by the patients, the authors suggested L-citrulline be used as palliative therapy; however, the pharmacokinetic exposure of L-citrulline was not investigated. Unfortunately, despite these encouraging results, this pilot study had significant limitations, including a small sample size, the absence of controls and definitive dose, and measured outcomes within the designated 4-week period. In recognition of these limitations, the authors recommended additional well-controlled, long-term studies be conducted [91].

In 2006, Smith et al. [92] reported a randomized, placebo-controlled trial of 40 children undergoing cardiopulmonary bypass who received oral L-citrulline supplementation for prevention of postoperative PH. Perioperative L-citrulline 9.5 g/m^2 was associated with ~85% increases in mean arginine and L-citrulline plasma concentrations 12 h after surgery. This is the largest of the reported pediatric studies. One study observation (that citrulline concentrations were below the age-specific norms for participants who developed postoperative PH) suggested a correlation between low citrulline concentration and postoperative PH [92]. Supplemental oral citrulline in this study was well-tolerated and increased plasma citrulline and arginine levels, which may prevent postoperative PH.

In 2016, El-Hattab et al. [24] reported a controlled crossover study in pediatric patients (five with MELAS syndrome, five healthy controls) who received citrulline and arginine. Participants with a body weight of ≥ 20 kg received 10 g/m^2/day of the supplement, and those weighing < 20 kg received 3 g/kg/day. Compared with arginine supplementation, L-citrulline supplementation resulted in greater increases in NO production rate, arginine and citrulline flux, plasma arginine and citrulline concentrations, and de novo arginine synthesis rates [24].

In 2017, Stepanova et al. [93] showed that treatment with an unreported dose of citrulline malate improved serum concentrations of L-arginine, nitrite, and NO metabolites in pediatric patients with signs of endothelial dysfunction.

Overall, recent clinical studies in both adult and pediatric populations provide evidence for supplemental arginine/citrulline-associated improvement in efficacy outcomes, although the exposure–response correlation and dose-ranging effects are yet to be well-evaluated. These studies create the basis for future well-designed, randomized clinical trials to establish efficacy and inform dosing decisions for arginine supplementation in both pediatric and adult diseases. However, the optimal design of future clinical trials depends on accurate information about the pharmacokinetics of arginine/citrulline.

5 Evaluation of Arginine and Citrulline Pharmacokinetics in Preclinical Studies

The pharmacokinetics of supplemental arginine are complex because basal levels of this endogenous molecule vary and it contributes to multiple biochemical pathways. Arginine exposure and clearance after supplementation are influenced by the patients' age and the types and stages of the targeted diseases. Wu et al. [94] evaluated the pharmacokinetics of arginine in different animal models. Regardless of the physiologic, pathologic, and species differences in these animals, intravenous supplementation of arginine increased plasma arginine levels, but they returned to baseline with 4–5 h of administration. Higher clearance of arginine with

intravenous administration was observed in pregnant, neonatal, and lean animals compared with those in nonpregnant, adult, and obese animals, respectively. Oral arginine supplementation resulted in variable absorption kinetics because of the interplay of intestinal absorption and extensive gut metabolism [95]. However, clearance of arginine followed a trend similar to that observed with intravenous dosing. Circulating arginine undergoes rapid clearance in animals of different stages of physiological maturation.

Disease states can influence the basal levels of arginine and citrulline. The effect of arginine supplementation, either by arginine or citrulline, on its pharmacokinetics, was evaluated in different animal disease models. Alloxan-induced diabetic rats showed reduced basal levels of arginine that were restored to normal levels with arginine supplementation [96]. To evaluate the effects of NO in different central nervous system (CNS) disorders, Heinzen et al. [97] reported a pharmacokinetic/pharmacodynamic (PK/PD) model of exogenous arginine supplements that successfully predicted arginine levels in the brain. The PK/PD model obtained from this study suggested that exogenous arginine supplementation increased hippocampal arginine concentrations in a dose-dependent fashion. Another study evaluated the effect of oral arginine plus citrulline therapy in augmenting NO-dependent responses in animal models of cardiovascular diseases [98]. Oral combinations of arginine and citrulline showed a shorter time to reach maximum plasma arginine concentration (t_{max}) than did single L-citrulline therapy in humans, suggesting an advantage of rapid-acting effects that could be useful in managing anginal attacks provided that interspecies absorption differences between rats and humans are negligible. While citrulline-only supplements support the long-acting enhancement of arginine availability, the benefits of citrulline plus arginine therapy as a rapid-acting arginine supplement requires validation in human studies. Choice of supplements can also depend on the pathologic conditions. Infectious conditions such as endotoxemic sepsis can severely reduce the bioavailability of citrulline, although arginine pharmacokinetics remain largely unaltered [99].

Although preclinical research is generating impressive evidence to support the therapeutic efficacy of supplemental arginine/citrulline in a wide variety of pathological conditions, studies reporting arginine pharmacokinetics are few, sporadic regarding study objectives, and difficult to translate to humans. Significant challenges in studying arginine exposure after supplementation include the estimation of highly variable baselines and the prediction of disease-associated alterations in arginine biosynthesis. Ultimately, for accurate translation to clinical trials, pharmacokinetic information must be generated in humans.

6 Pharmacokinetics of Arginine and Citrulline in Humans

To date, most of the clinical studies exploring the pharmacokinetics of supplemental arginine have been conducted in healthy adult populations. Table 3 summarizes the clinical studies that have evaluated the pharmacokinetics of arginine or citrulline.

6.1 Arginine/Citrulline Supplementation in Healthy Adult Populations

Tangphao et al. [100] tested exogenous arginine in healthy humans as an initial effort to understand the pharmacokinetics of L-arginine in patients with cardiovascular diseases [100]. Basal L-arginine levels in untreated healthy adults showed substantial diurnal variations. Baseline corrected plasma L-arginine concentrations measured after oral or intravenous L-arginine administration showed a biphasic elimination pattern [100]. Intravenous L-arginine administration triggered an initial concentration-dependent rapid renal elimination followed by a slower elimination mainly guided by nonrenal elimination. A single dose of oral L-arginine 10 g with ~20% bioavailability caused a threefold increase in plasma L-arginine concentration from baseline [100]. Schwedhelm et al. [95] reported that chronic oral administration of citrulline dose dependently increased the concentration of L-arginine in healthy adults. In this randomized, double-blind, placebo-controlled study, different doses of L-citrulline were compared with two different formulations of L-arginine. L-citrulline at a one-half dosage strength of L-arginine was associated with plasma exposure similar to that of L-arginine, suggesting an efficient L-arginine supplementation method. Like in previous studies, these authors were unable to determine the plasma L-arginine half-life because of irregular baseline values. They reported t_{max} and maximum plasma concentration (C_{max}) for both citrulline and arginine after citrulline 3 g: t_{max} 0.7 ± 0.1 h and 1.4 ± 0.1 h and C_{max} 846 ± 45 and 149 ± 42 µmol/L, respectively [95]. Another study characterized the pharmacokinetics of citrulline in healthy adults for four oral doses (2, 5, 10, and 15 g). A dose-dependent significant decrease in clearance was observed for doses of 2, 5, and 10 g, although mean distribution volumes for each dose was similar [101]. L-citrulline doses of ≥ 10 g were associated with ~50% decrease in clearance compared with the 2-g dose. Plasma half-life and exposure of citrulline also increased dose dependently over a dose range of 2–15 g, and the t_{max} and C_{max} were determined for both citrulline (t_{max} 0.72 ± 0.08 h, C_{max} 2756 ± 70 µmol/L) and arginine (t_{max} 1.67 ± 0.05 h, C_{max} 280 ± 1043 µmol/L) [101]. In 2007, Rouge et al. [102] reported results from a randomized study

Table 3 Clinical studies reporting the pharmacokinetics of arginine

Study type	Subjects	Dosage	PK outcome
PK study after watermelon ingestion [103]	6 healthy adults	3.3 kg wet weight red fruit of ripe watermelon	CIT: increase from 22 to 593 µmol/L (range 386–1069) 1 h after ingestion ARG: increase from 65 to 199 µmol/L (range 128–251) 2 h after ingestion
PK study after watermelon ingestion [104]	23 healthy adults	780 g (i.e., CIT 1 g), 1560 g (i.e., CIT 2 g) watermelon juice for 3 wk	Low ingestion of watermelon juice: 12% increase in plasma ARG High ingestion of watermelon juice: 22% increase in plasma ARG, 18% increase in plasma ornithine
Single-blind crossover study [115]	8 healthy adults	CIT 2, 5, 10, or 15 g	After ingestion of CIT 10 g: CIT: t_{max} 0.72 ± 0.08 h, C_{max} 2756 ± 70 µmol/L ARG: t_{max} 1.67 ± 0.05 h, C_{max} 280 ± 1043 µmol/l
Randomized study [102]	10 healthy volunteers	0.18 g/kg/d	CIT: increase of 448 ± 92% (from 39 ± 4 to 225 ± 44) ARG: increase of 92 ± 57% (from 134 ± 33 to 247 ± 62) Increase in urine and RBC CIT No change in urinary ARG, plasma urea, urinary urea nitrogen excretion, thus enhanced nitrogen balance
Double-blind randomized placebo-controlled crossover study [116]	20 healthy volunteers	CIT: 0.75, 1.5, and 3 g BID ARG: immediate release 1 g TID, sustained release 1.6 BID for 1 wk	After load of CIT 3 g: CIT: t_{max} 0.7 ± 0.1 h, C_{max} 846 ± 45 µmol/L ARG: t_{max} 1.4 ± 0.1 h, C_{max} 149 ± 42 µmol/L
Randomized placebo-controlled trial [117]	40 children undergoing surgery to correct congenital heart lesions	CIT 1.9 g/m^2/dose × 5 (total dose 9.5 g/m^2)	12 h postoperative: CIT: 37 (18–83) vs. 20 (15–29) (placebo) ARG: 36 ± 24 vs. 23 ± 1357 and 85% increases in mean plasma levels of ARG and CIT, respectively
Pilot phase II clinical trial [91]	5 pts with sickle cell disease (aged 10–18)	0.09–0.13 g/kg BID for 4 wk	Increase of 65% (from 77 ± 9.1 to 127 ± 18) in plasma ARG
Controlled crossover study [24]	5 pediatric pts with MELAS syndrome; 5 healthy children as controls	CIT/ARG dose: Pts weighing < 20 kg: 500 mg/kg/dose orally every 4 h for 48 h Patients weighing ≥ 20 kg: 10 g/m^2/d	CIT supplementation increased plasma ARG concentration from 64 ± 5.7 to 257 ± 21 µmol/L ARG supplementation increased plasma ARG concentration from 59 ± 5 to 184 ± 14 µmol/L
Phase I dose escalation [19]	5 infants and children undergoing cardiac surgery for congenital problems	Stepwise dose escalation: 50, 100, and 150 mg/kg; two doses administered: one before and one immediately after surgery	CIT plasma clearance 0.6 L/h/kg Distribution volume 0.9 L/kg Half-life = 1 h
Randomized placebo-controlled study [14]	19 subjects with precapillary PH	Oral L-ARG 50 mg/kg	Plasma L-ARG: from 90 ± 4 µmol/L (baseline) to 274 ± 34 µmol/L (statistically significant change from baseline)

Table 3 (continued)

Study type	Subjects	Dosage	PK outcome
Controlled study in subjects with MELAS syndrome [23]	10 pts with MELAS syndrome, 10 healthy subjects	L-ARG or L-CIT: 10 g/m^2 BSA per day for 2 days	ARG supplementation: plasma ARG increased from 57.1 ± 3.2 µM (baseline) to 143.8 ± 9.9 µM (statistically significant change from baseline) CIT supplementation: plasma ARG increased from 57.6 ± 2.1 µM (baseline) to 182 ± 14.4 µM (statistically significant change from baseline)
Pilot study in pts with PH [83]	10 pts with PH	L-ARG 500 mg/kg as 30-minute infusion	Plasma ARG increased from 59 ± 6 to 10,726 ± 868 µmol/L (statistically significant change)
Randomized controlled trial of oral CIT [92]	40 children undergoing cardiopulmonary bypass	5 doses (1.9 g/m^2 BSA)	Plasma L-ARG with CIT was significantly higher than with PL (36 vs. 26 µmol/L immediately after operation; 37 vs. 20 µmol/L 12 h after operation)
Pilot study in pts with pulmonary hypercholesterolemia [105]	10 pts	Oral L-ARG 5 or 7 g followed by IV L-ARG 10 or 30 g after 8 h at each of the four different visits	Mean C_{max} range after oral dose: 42.8–46.5 µg/mL Mean AUC range after oral dose: 651–894 µg/min/ml/g Mean AUC range after IV dose: 1762–1926 µg/min/ml/g Mean nonrenal clearance: 564.4–586 mL/min Mean bioavailability: 0.37–0.52
Randomized pilot study in pts with moderately severe malaria [107]	78 subjects: 48 in observational group, 30 received L-ARG	L-ARG 3, 6, or 12 g infused over 30 min	Follows a two-compartment PK model Clearance from central compartment: 31 L/hr Clearance from peripheral compartment: 74 L/hr Distribution volume (central): 27 L Distribution volume (peripheral): 21 L
Randomized pilot study in pts with severe malaria [108]	8 subjects: 2 received saline, 6 received L-ARG	L-ARG 12 g infused over 8 h	Follows a two-compartment PK model Clearance from central compartment: 61.8 L/hr Clearance from peripheral compartment: 21.4 L/hr Distribution volume (central): 115 L Distribution volume (peripheral): 70.9 L
Phase I dose-finding study in pts with sickle cell disease [109]	8 subjects	Step 1: Bolus L-CIT 20 mg/kg Step 2: L-CIT 7 mg/kg/h continuous infusion	L-CIT C_{max} after bolus dose: 257 µmol/L Clearance and distribution volume after bolus administration: 0.52 ± 0.13 L/h/kg and 0.50 ± 0.14 L/kg, respectively Clearance and distribution volume after continuous infusion: 0.52 ± 0.17 L/h/kg and 0.41 ± 0.02 L/kg, respectively

ARG arginine, *AUC* area under the plasma concentration–time curve, *BID* twice daily, *BSA* body surface area, *CIT* citrulline, C_{max} maximum plasma concentration, *d* day, *h* hour, *IV* intravenous, *L-ARG* L-arginine, *L-CIT* L-citrulline, *MELAS* mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, *PH* pulmonary hypertension, *PK* pharmacokinetics, *PL* placebo, *pt(s)* patient(s), *RBC* red blood cells, *TID* three times daily, t_{max} time after dose to observe maximum plasma concentration, *vit* vitamin, *wk* week(s)

of ten healthy volunteers. Supplementation with citrulline 0.18 g/kg/day resulted in increased citrulline and arginine plasma concentrations. Plasma concentrations of citrulline increased from 39 ± 4 to 225 ± 44 μmol/L, and those of arginine increased from 134 ± 33 to 247 ± 62 μmol/L. This supplementation enhanced nitrogen balance and increased levels of red blood cell (RBC)-bound citrulline and the renal clearance of citrulline [102]. Few studies have evaluated whether citrulline-rich dietary intervention can improve plasma citrulline/arginine levels. Mandel et al. [103] observed significant increases in both arginine and citrulline in six healthy adults within the first 2 h after ingestion of 3.3 kg (wet weight) of watermelon. Citrulline increased from 22 to 593 μmol/L (range 386–1069) 1 h after ingestion, and arginine increased from 65 to 199 μmol/L (range 128–251) 2 h after ingestion [103]. In 2007, Collins et al. [104] also reported increases in plasma arginine in 23 healthy adults who ingested watermelon juice (780 or 1560 g) for 3 weeks, resulting in 12 and 22% increases in plasma arginine concentrations, respectively.

6.2 Arginine/Citrulline Supplementation in Adult Patients with Disease States

As a step toward their previous study in healthy adults, Tangphao et al. [105] evaluated the pharmacokinetics of L-arginine in hypercholesterolemic patients reported to have impaired NO signaling in the endothelium and platelets. Long-term (~ 12 weeks) administration of oral or intravenous L-arginine was associated with a significant increase in plasma L-arginine level in patients with hypercholesterolemia. A previous study in rabbits suggested that long-term treatment with exogenous arginine caused a gradual decline in plasma L-arginine levels [106]. However, this study showed a sustained increase in plasma L-arginine after oral or intravenous administration, although the authors were unable to calculate two key parameters (distribution volume and elimination half-life) of L-arginine because of diurnal variations in basal L-arginine levels. Exogenous L-arginine treatment did not show pharmacodynamic tolerance to plasma L-arginine-induced growth hormone release [105].

The pharmacokinetics of L-arginine were also evaluated in patients with severe malaria who had lower than normal plasma L-arginine levels because of malaria-associated endothelial dysfunction [107]. In a prospective observational study in healthy subjects and patients with malaria, the dose-ranging effect of intravenous L-arginine infusion in replenishing plasma L-arginine levels was determined using a population pharmacokinetic approach. Plasma concentration–time profiles with a 30-minute infusion, for doses of 3, 6, or 12 g, supported a two-compartment linear model with first-order elimination. With an estimated clearance of 733 mL/min, exogenous L-arginine had a slightly shorter half-life in patients with malaria than in healthy subjects. NO production depends on the intracellular movement of L-arginine from extracellular space driven by cationic amino acid transporter protein-1 (CAT-1). The half-saturation concentration of extracellular L-arginine for CAT-1 is estimated at 100–150 μmol/L [107]. Simulations of plasma profiles for L-arginine 12 g at different dosing frequencies (6, 8, and 12 h) predicted a 60, 75, and 90% increase, respectively, above the required half-maximal saturating concentration at L-arginine transporter for optimal NO production [107]. In a separate open-label pilot study, L-arginine 12 g every 8 h was associated with concentrations lower than those predicted in the previous model and 40% increased L-arginine clearance in patients with severe malaria than in patients with moderately severe malaria [107, 108].

In 2012, El-Hattab et al. [23] reported a controlled crossover study of ten patients with MELAS syndrome and ten healthy participants who received oral citrulline or oral arginine 10 g/m^2/day. Supplementation with citrulline resulted in more significant increases in NO production rate, arginine, citrulline flux, and plasma arginine and citrulline concentrations. Citrulline supplementation increased plasma arginine concentrations from 57.6 ± 2.1 to 182.0 ± 14.4 μmol/L; arginine supplementation increased plasma arginine concentrations from 57.1 ± 3.2 to 143.8 ± 9.9 μmol/L. De novo arginine synthesis decreased with arginine supplementation and increased with citrulline supplementation [23].

The pharmacokinetics and safety profile of intravenous L-citrulline were recently evaluated in adults aged 18–24 years with sickle cell disease [109]. In this dose-finding study, a bolus dose of citrulline 20 mg/kg followed by a 7 mg/kg/h continuous infusion maintained a trough plasma arginine concentration of 100 μmol/L. Intravenous bolus administration of citrulline resulted in a mean arginine C_{max} of 259 μmol/L [109].

6.3 Arginine/Citrulline Supplementation in Pediatric Patients

The pharmacokinetics of intravenous L-citrulline was evaluated in children (aged 0–6 years) undergoing congenital cardiac surgery [19]. In the first group of patients, intravenous bolus doses ranging between 50 and 150 mg/kg were implemented to achieve a 4-h trough citrulline plasma concentration of 80–100 μmol/L. Pharmacokinetic simulations based on these data suggested a bolus dose of 150 mg/kg followed by an intravenous infusion of 9 mg/kg/h after 4 h would maintain optimum citrulline levels. The half-life of citrulline was approximately 60 min, with a distribution volume ranging between 0.8 and 1.0 L/kg [19]. However, this study did not measure the effect of citrulline administration on plasma levels of the active compound L-arginine. In pediatric patients with MELAS, oral citrulline supplementation (10 g/

m^2 body surface area for body weight ≥ 20 kg or 500 mg/kg for body weight < 20 kg) six times daily showed a four-fold increase in plasma arginine concentration (64 ± 5.7 to 257 ± 21 μmol/L) from baseline. Oral arginine supplementation, at the same dosing regimen as citrulline, also showed a modest increase in plasma arginine concentration (59 ± 5–184 ± 14 μmol/L) [24].

All the clinical studies that reported the pharmacokinetics of arginine/citrulline after exogenous arginine supplementation in the form of arginine or citrulline or a citrulline-rich diet showed an associated increase in plasma arginine. Clearance of arginine/citrulline depends on the dose of citrulline/arginine, patient age, and disease state, although wide variations in baseline arginine/citrulline preclude the accurate estimation of distribution volumes and clearances. These variables must be considered when designing clinical trials to evaluate the efficacy of arginine/citrulline in different age groups with different disease states.

7 Pharmacometric Approaches to Evaluating Arginine Pharmacokinetics

Published work assessing the population pharmacokinetics of arginine and citrulline is sparse [107]. Yeo et al. [107] investigated the pharmacokinetics of L-arginine in adult patients with moderately severe malaria and developed a two-compartment model to describe the concentration–time profiles. Body weight and ethnicity influenced the pharmacokinetics in patients with malaria, although these covariates were not identified in previous analyses with healthy volunteers. Additionally, an empirical second-order polynomial equation was used to describe the increase in endogenous L-arginine levels over time in patients who did not receive exogenous arginine. An alternative turnover model parameterizing the endogenous production of arginine was also evaluated for baseline arginine descriptions but not retained because of the model's sensitivity to initial estimates. In another study with healthy Chinese volunteers, Wang et al. [110] applied a similar two-compartment model structure to describe the pharmacokinetics of L-arginine. The final parameter estimates were comparable between the two modeling attempts. However, the later study identified weight as a significant covariate on peripheral volume, and the baseline was not specifically described in the model. At the time of writing this review, no studies on the population pharmacokinetics of citrulline had been published.

The limited number of publications on arginine and citrulline population modeling and the inconsistency in published models has indicated gaps in the understanding of the exposure–response relationship of these drugs in patients. More studies are needed to establish the PK/PD characteristics of arginine and citrulline, their population variability, and potentially influential patient features. In particular, given the endogenous functionality of these molecules, study designs should accommodate appropriate sampling strategies to describe the natural progression of baseline arginine/citrulline levels in patients. The metabolism of arginine and the production of NO is influenced by disease status [110–113]. Therefore, when developing quantitative models for these molecules, disease status should also be evaluated as potential covariates for both exogenous and endogenous molecule kinetics.

8 Conclusions

Supplemental arginine has shown potential for therapeutic use in different diseases in both adult and pediatric age groups, and in both preclinical and clinical studies, largely because of its crucial role as an endogenous amino acid substrate in NO regulation. It is currently available in intravenous or oral dosage forms. The arginine precursor, citrulline, is available in oral forms; an intravenous preparation is being investigated in clinical trials in children undergoing cardiopulmonary bypass surgery but is not yet available. Ingestion of watermelon, a citrulline-rich fruit, has also been used in clinical studies to improve arginine levels. Citrulline appears to be more efficient than arginine in enhancing systemic arginine concentration. In several pilot clinical studies, arginine/citrulline supplementation improved conditions in some life-threatening diseases in children, including inborn errors of ureagenesis, MELAS syndrome, and PH. However, widespread clinical application is limited because the pharmacokinetics and the dose–response or exposure–response relationships of arginine/citrulline are yet to be characterized. Arginine follows complex pharmacology that depends on several confounding factors, such as baseline variations of arginine due to developmental and disease state, age, disease condition, pregnancy, etc. Pharmacokinetic studies of arginine/citrulline in diseased or special populations are not adequate to inform appropriate dosing guidance. Research directed toward understanding the PK/PD relationship of arginine, given either as arginine or citrulline supplements, in different age groups and disease conditions is critical for its translation from bench to bedside.

Compliance with Ethical Standards

Conflicts of interest JR, SSK, KMJ, XL, CDF, and CMS have no conflicts of interest that are directly relevant to the content of this review.

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