L-Arginine Supplementation in Peripheral Arterial Disease: No Benefit and Possible Harm

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Background—L-Arginine is the precursor of endothelium-derived nitric oxide, an endogenous vasodilator. L-Arginine supplementation improves vascular reactivity and functional capacity in peripheral arterial disease (PAD) in small, short-term studies. We aimed to determine the effects of long-term administration of L-arginine on vascular reactivity and functional capacity in patients with PAD.

Methods and Results—The Nitric Oxide in Peripheral Arterial Insufficiency (NO-PAIN) study was a randomized clinical trial of oral L-arginine (3 g/d) versus placebo for 6 months in 133 subjects with intermittent claudication due to PAD in a single-center setting. The primary end point was the change at 6 months in the absolute claudication distance as assessed by the Skinner-Gardner treadmill protocol. L-Arginine supplementation significantly increased plasma L-arginine levels. However, measures of nitric oxide availability (including flow-mediated vasodilation, vascular compliance, plasma and urinary nitrogen oxides, and plasma citrulline formation) were reduced or not improved compared with placebo. Although absolute claudication distance improved in both L-arginine– and placebo-treated patients, the improvement in the L-arginine–treated group was significantly less than that in the placebo group (28.3% versus 11.5%; $P=0.024$).

Conclusions—In patients with PAD, long-term administration of L-arginine does not increase nitric oxide synthesis or improve vascular reactivity. Furthermore, the expected placebo effect observed in studies of functional capacity was attenuated in the L-arginine–treated group. As opposed to its short-term administration, long-term administration of L-arginine is not useful in patients with intermittent claudication and PAD. **(*Circulation*. 2007;116:188-195.)**

Key Words: amino acids ■ atherosclerosis ■ endothelium ■ nitric oxide ■ peripheral vascular disease

Endothelium-derived nitric oxide (NO) is a potent endogenous vasodilator with vasoprotective properties.[1] In patients with cardiovascular risk factors or disease, inactivation or reduced synthesis of NO impairs endothelium-dependent vasodilation.[2–4] Furthermore, the vasoprotective actions of NO are attenuated by cardiovascular risk factors.[2–4]

Clinical Perspective p 195

L-Arginine is the precursor of NO. In animal models of cardiovascular disease, supplementation with L-arginine improves vasodilation and increases vascular NO synthesis.[5,6] In preclinical studies, long-term administration of L-arginine inhibits atherosclerosis and myointimal hyperplasia and enhances angiogenesis.[7–9] More importantly, short-term studies in patients with cardiovascular risk factors or disease indicated that L-arginine supplementation may increase NO synthesis and improve vasoreactivity. In patients with severe peripheral arterial disease (PAD), intravenous infusion of L-arginine (30 g over 30 minutes) increases limb blood flow 2-fold and to the same extent as prostaglandin E_1.[10] The increase in limb blood flow is associated with parallel increases in urinary nitrogen oxides, as well as plasma and urinary cGMP, consistent with increased NO synthesis.[10]

There are limited options for medical management of intermittent claudication. A nutritional adjunct to PAD therapy seems low risk, and the scientific rationale for L-arginine supplementation seems sound. Accordingly, we initiated a randomized clinical trial of L-arginine supplementation with the aim of improving vascular reactivity and functional capacity in patients with PAD.

Methods

Trial Design

The Nitric Oxide in Peripheral Arterial Insufficiency (NO-PAIN) trial was a prospective, single-center, randomized, double-blind, placebo-controlled National Heart, Lung, and Blood Institute–funded study of the efficacy and safety of oral L-arginine supplementation in patients with intermittent claudication. The trial was

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approved by the institutional review board at Stanford University; registered at www.clinicaltrials.gov (No. NCT00284076); and monitored by an external Data and Safety Monitoring Board and a National Heart, Lung, and Blood Institute program officer. Written informed consent was obtained from each patient.

Patients

Inclusion criteria were age of at least 45 years, unilateral or bilateral PAD confirmed by a resting ankle-brachial index (ABI) <0.9, stable intermittent claudication for the previous 3 months, and the ability to walk 1 to 12 minutes on a treadmill. Variability of maximum walking distances between 2 consecutive screening treadmill tests was <25%. Exclusion criteria included ischemic rest pain, ulceration or gangrene, history in the previous 3 months of acute coronary syndrome or revascularization involving the peripheral or coronary arteries, major amputation, malignancy within the previous 5 years (except for treated nonmelanoma skin cancer), proliferative retinopathy, uncontrolled hypertension, or active inflammatory, infectious, or autoimmune diseases. A 1-month washout period was also required before enrollment for patients taking pentoxifylline, cilostazol, prostanoids, L-carnitine, or L-arginine. Patients were advised to maintain their current lifestyle habits related to diet, tobacco, and exercise during study participation.

Eligible patients were randomly assigned to either L-arginine (1 g TID with meals) or placebo. L-Arginine was manufactured by Ajinomoto Inc and encapsulated by Cosmo-Pharm Inc. Bioavailability studies before study initiation confirmed that active study drug increased plasma arginine levels. Amino acid analysis (Beckman 6300 analyzer; Beckman) at the Stanford Clinical Laboratory after the completion of the study showed 95.8% and 97.9% recovery of L-arginine in randomly selected capsules from the remaining lot of L-arginine and 0% recovery of L-arginine from randomly selected capsules from placebo.

Study Procedures

Assessment of Functional Capacity

Graded treadmill tests were performed at 3 and 6 months after randomization, according to the Skinner-Gardner protocol.[11] Initial claudication distance (ICD) and absolute claudication distance (ACD) were recorded. Two consecutive treadmill tests were performed within 1 week at baseline (before administration of study drug); 1 test was performed at 3 months; and 2 treadmill tests were performed after 6 months on study drug. Functional status was also assessed by the Walking Impairment Questionnaire and the Health Status Survey SF-36 questionnaire (SF-36).

Vascular Studies

ABI was measured after 10 minutes of supine rest with a hand-held Doppler. Systolic pressures were measured at the dorsalis pedis, posterior tibial, and brachial arteries bilaterally. The right and left ABI values were calculated by taking the higher pressure of the 2 arteries at each ankle, respectively, divided by the higher of the 2 brachial arterial systolic pressures. The index ABI was defined as the ABI of the extremity with the lowest value.

Flow-mediated vasodilation of the brachial artery was measured with the use of an Acuson Sequoia C256 high-resolution ultrasound unit with a 14-mHz probe (Siemens Inc). Vascular studies were performed in a quiet, darkened room with the patient in a fasting state. After measurement of the brachial artery diameter, a blood pressure cuff on the forearm was inflated to a pressure of 50 mm Hg above systolic pressure for 5 minutes. After deflation, measurements of the brachial artery diameter were performed at 30, 45, and 60 seconds during reactive hyperemia. Electronic calipers were used for the measurement of artery diameter. Flow-mediated vasodilation was expressed as the maximal percent change in diameter from the resting condition during the period of reactive hyperemia.

Vascular compliance was measured with the use of a CVProfilor DO 2020 (Hypertension Diagnostics Inc). A tonometer was positioned over the radial artery to obtain a stable waveform. The measurement of oscillatory compliance has been shown to be at least partially dependent on NO availability,[12] and it correlates with measures of flow-mediated vasodilation in the same subjects.[13] Coefficients of variation for these tests performed in our laboratory have been published previously.[14]

Patients ingested a low-nitrite diet for 24 hours before testing, fasted overnight, and were given low-nitrate water to consume for 12 hours before blood samples and urine were collected for safety laboratory studies; urinary and plasma nitrogen oxides; amino acid analysis; and plasma asymmetrical dimethylarginine (ADMA) levels. Venous blood was collected into EDTA-coated tubes on ice and plasma stored at −80°C. Nitrogen oxide measurements were performed by Greiss reaction with a colorimetric assay; plasma arginine, ornithine, and citrulline were measured with an amino acid analyzer; and plasma ADMA was measured by immunoassay.[15]

A questionnaire to elicit adverse events, a pill count to assess compliance, and a treadmill test were conducted at 3- and 6-month follow-up. Secondary measures such as ABI, flow-mediated vasodilation, vascular compliance, and basic clinical laboratories were repeated at 6 months.

Statistical Analysis

The change in ACD was prespecified as the primary end point. ACD values were log transformed. Secondary efficacy end points included changes in ICD, SF-36 and Walking Impairment Questionnaire measures, ABI, flow-mediated vasodilation, vascular compliance, urinary and plasma nitrogen oxides, and plasma amino acid levels. Sample size calculation for the primary end point was based on the results of a pilot, dose-ranging study.[16] These calculations indicated that 120 patients randomized into 2 treatment groups would provide 90% power at a 1-sided α level of 0.05 to reject the null hypothesis assuming a 20% increase in ACD over placebo in the L-arginine group. Thus, we sought to enroll at least 132 patients to allow for an anticipated dropout of 10%.

Baseline characteristics in the 2 groups were compared by Student *t* test or Mann-Whitney test. The primary efficacy analysis, based on a modified intention-to-treat principle, included all patients who received at least 1 dose of blinded study treatment and had at least 1 valid postrandomization treadmill test. A last observation carried forward method was applied to missing 6-month data. Comparisons of 6-month changes from baseline in efficacy and laboratory values between the 2 groups were conducted with an ANCOVA model with terms for treatment and baseline values. All statistical comparisons between mean treatment-related changes from baseline were performed with the model-adjusted (least-squares) mean values rather than unadjusted raw means. Nonparametric tests were used to analyze nonnormally distributed data when appropriate. In all cases, $P<0.05$ was used to indicate statistical significance. All analyses were performed with SPSS software, version 12.0.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

A total of 2365 people presented for the study. After initial telephone screening, 687 provided consent and underwent further onsite screening. Most exclusions from those providing consent were due to normal ABI. Thus, 133 patients were randomized (67 subjects randomized to placebo and 66 to L-arginine). The numbers of discontinuations were similar between the groups, resulting in 61 patients in the placebo group and 58 patients in the L-arginine group. Baseline characteristics of the 2 treatment groups were not different (Tables 1 and 2).

Laboratory Measures

Plasma arginine increased in the group receiving the active study drug, as did plasma ornithine (Figure 1) and blood urea nitrogen (from 20.3±6.8 to 22.9±9.8 mg/dL at 6 months;

TABLE 1. Baseline Clinical Characteristics

Characteristic	Placebo (n=67)	L-Arginine (n=66)
Age, mean±SD, y	72±7	73±9
Women	18 (27)	14 (21)
Race		
White	52 (78)	52 (79)
Black	5 (7)	6 (9)
Asian/Pacific Islands	5 (7)	2 (3)
Hispanic or Latino	1 (1)	1 (1)
Mixed or other	4 (6)	5 (8)
Diabetes mellitus	20 (30)	20 (30)
Hypertension	60 (90)	62 (94)
Statins	42 (63)	44 (67)
Calcium channel blockers	19 (28)	21 (32)
ACEI and/or ARB	37 (55)	36 (55)
β-Blockers	30 (45)	28 (42)
Long-acting nitrates	9 (13)	5 (8)
Loop diuretics	12 (18)	6 (9)
Thiazolidinediones	2 (3)	5 (8)
Current smoking	12 (18)	8 (12)
Index leg ABI, mean±SD	0.56±0.18	0.59±0.17
Median ACD, m (IQ range)	292 (185–438)	258 (173–350)
Median ICD, m (IQ range)	94 (62–152)	75 (57–111)

Data are n (%) unless otherwise indicated. Statins indicates 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; and IQ, interquartile. No significant differences were seen between variables.

P=0.005). Surprisingly, plasma citrulline levels did not increase in the arginine group (Figure 1). In the placebo group, no changes occurred in plasma levels of arginine, ornithine, or urea.

Plasma and urinary nitrogen oxides rose slightly in the placebo group but not with L-arginine treatment (Figure 2 and Table 3). Plasma ADMA levels were high in both groups at baseline (Table 3) but did not change significantly over 6 months, nor were there any significant changes in clinical chemistry, hematology, lipid measures, glucose, or insulin in either group (data not shown).

TABLE 2. Baseline Clinical Laboratory Measures

	Placebo (n=67)	L-Arginine (n=66)
Total cholesterol, mg/dL	184±139.7	180.5±47.9
HDL cholesterol, mg/dL	49.7±16.8	49.8±15.3
LDL cholesterol, mg/dL	103.7±32.5	99.1±38.1
Triglycerides, mg/dL	158.1±113.1	163.6±143.1
Hematocrit, %	41.8±4.1	40.2±4.6
Platelets, $\times 10^3/\mu L$	232.8±60.7	225.5±51.0
BUN, mg/dL	20.5±8.4	21.1±7.7
Creatinine, mg/dL	1.2±0.4	1.2±0.4
Fasting glucose, mg/dL	113.2±33.7	116±50.1

Data are mean±SD unless otherwise indicated. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and BUN, blood urea nitrogen. No significant differences were seen between variables.

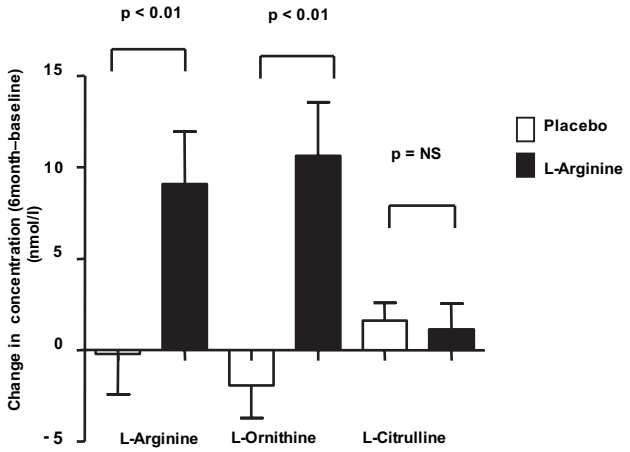

Figure 1. Changes in serum concentration of L-arginine and metabolites. Oral administration of L-arginine for 6 months increased plasma levels of arginine and ornithine but not citrulline. Values are mean±SEM.

Vascular Studies

No group differences existed for ABI or vascular compliance measurements (Table 4). ABI increased slightly in both groups (Table 4). No correlation existed between change in ABI and ACD (r=−0.116, P=0.23). Surprisingly, mean flow-mediated vasodilation in the L-arginine group decreased after 6 months, whereas flow-mediated vasodilation in the placebo group increased. This resulted in a significant difference in the mean change of flow-mediated vasodilation between the 2 groups (Figure 2), although the changes within the groups did not reach statistical significance (Figure 2).

Functional Capacity

Walking distances (ICD and ACD) increased in both groups from baseline to last observation carried forward through 6 months (Table 5). In the placebo group, ACD increased significantly from baseline to 3 months and baseline to 6 months, whereas no significant change

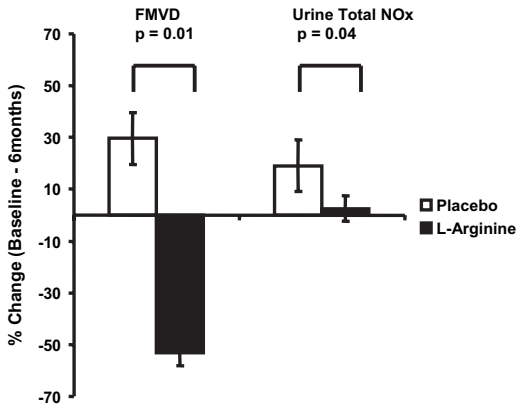

Figure 2. Change in flow-mediated vasodilation and urinary nitrogen oxide (NOx) during study. Oral administration of L-arginine for 6 months was associated with a decline in flow-mediated vasodilation and urinary nitrogen oxides. Values are mean±SEM percent change from baseline.

TABLE 3. Total Nitrogen Oxide and ADMA Measurements in Urine and Plasma

	Baseline	Follow-Up	P
Placebo			
Urinary total nitrogen oxide, μmol/L	396.7±320.7	472.6±339.0	0.04*
Plasma ADMA, μmol/L	0.77±0.17	0.67±0.16	0.47
L-Arginine			
Urinary total nitrogen oxide, μmol/L	384.5±362.3	376.5±250.2	0.71
Plasma ADMA, μmol/L	0.78±0.15	0.68±0.17	0.47

Values are median±interquartile range compared by Wilcoxon signed-rank test.
*Significant *P* value.

TABLE 5. Walking Distance (Intention-to-Treat)

	Placebo (n=61)	L-Arginine (n=58)	P
ACD, m			
Baseline	310±19	280±16	
6 Month	392±28	314±25	
Absolute change from baseline (least-squares mean)	78±16	36±17	0.09
ICD, m			
Baseline	105±7.0	91.0±7.1	
6 Month	146±12	110±11	
Absolute change from baseline (least-squares mean)	41±8.9	19.0±9.2	0.07

Values are mean±SD.

occurred in the L-arginine group between baseline and 3 months (Figure 3). The change in baseline to 6 months reached significance ($P=0.04$). No significant intragroup differences existed between 3- and 6-month time points. Subjects receiving L-arginine manifested significantly less improvement in ACD (mean improvement of 11.5% in the L-arginine group versus 28.3% in the placebo group) (Figure 4). The change in ICD in the L-arginine group tended to be less than in the placebo group (39.6% versus 47.1%; $P=0.06$). Quantitative composite measures of quality of life with the use of the Walking Impairment Questionnaire or SF-36 were similar between the treatment groups (data not shown).

TABLE 4. Vascular Studies (Intention-to-Treat)

	Placebo	L-Arginine	P
ABI			
No.	56	52	
Baseline	0.56±0.02	0.60±0.02	
6 Month	0.67±0.03	0.71±0.03	
Change from baseline (least-squares mean)	0.12±0.02	0.11±0.02	0.67
Flow-mediated vasodilation			
No.	52	44	
Baseline, % vasodilation	3.7±0.8	3.2±0.8	
6 Month, % vasodilation	4.7±0.9	1.6±0.7	
Change from baseline (least-squares mean)	1.1±0.8	−1.7±0.8	0.01
C1 vascular compliance			
No.	53	45	
Baseline, log [(mL/mm Hg)×10]	1.06±0.03	1.02±0.023	
6 Month, log [(mL/mm Hg)×10]	1.04±0.02	1.03±0.022	
Change from baseline (least-squares mean)	−0.02±0.02	0.01±0.018	0.38
C2 vascular compliance, log [(mL/mm Hg)×100]			
No.	53	45	
Baseline	0.41±0.03	0.49±0.03	
6 Month	0.44±0.03	0.47±0.03	
Change from baseline (least squares mean)	0.01±0.02	0.00±0.02	0.78

Values are mean±SD.

Adverse Events

The adverse event profile for the safety population is summarized in Table 6. Of these, 9 in each group were considered serious events. No group differences existed in serious or total adverse events.

Discussion

The major observation of the present study is that long-term administration of L-arginine does not improve walking distance in patients with PAD. Furthermore, the data indicate that long-term administration of L-arginine may even impair functional capacity, perhaps through an adverse effect on vascular reactivity. Specifically, significant group differences were observed in flow-mediated vasodilation and in plasma and urinary nitrogen oxide elaboration.

Rationale for L-Arginine Supplementation

These findings were unexpected. In previous studies, we and others have shown that short-term administration of L-arginine (the NO precursor) improves endothelial vasodilator function of coronary or peripheral arteries in patients

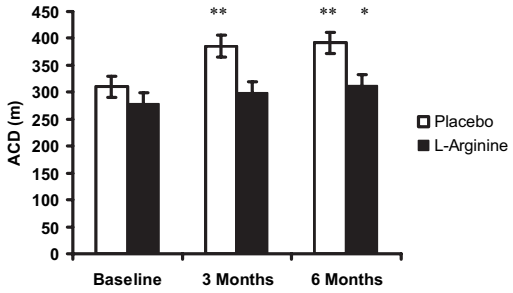

Figure 3. ACD at baseline, 3 months, and 6 months. ACD improved in both groups over time. **Placebo group: baseline to 3 months, $P<0.001$; baseline to 6 months, $P=0.001$. *L-Arginine group: baseline to 3 months, $P=0.1$; baseline to 6 months, $P=0.04$. No significant difference was seen between 3- and 6-month values in either group. Values are mean±SEM.

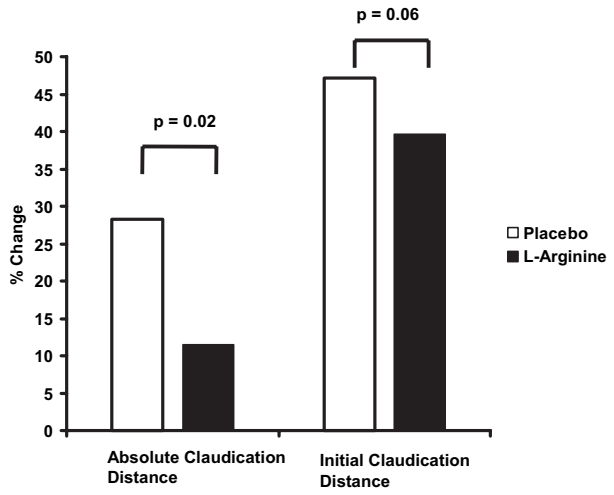

Figure 4. Change in walking distances. Oral administration of L-arginine for 6 months was associated with significantly less improvement in ICD and ACD. Values are mean±SE percent change from baseline.

with cardiovascular disease.[17] The improvement in vascular reactivity is likely due to the metabolism of L-arginine to NO because it is associated with increases in urinary or plasma nitrogen oxides and cGMP.[18]

TABLE 6. Adverse Events

	Placebo (n=67)	L-Arginine (n=66)
Serious adverse events, n	9	9
Acute coronary syndrome	0	1
Abdominal aortic aneurysm	0	1
Aortoenteric fistula	1	0
Critical limb ischemia	2	3
Proliferative retinopathy	1	0
Cancer diagnosis	2	0
Gastrointestinal hemorrhage	2	1
Major infection	0	2
Death	1	1
Other adverse events, n	35	46
General condition		
Abdominal discomfort	3	3
Diarrhea	3	1
Fall	4	4
Myalgia	1	3
Nausea	1	2
Infection		
Influenza	2	1
Upper respiratory infection	2	3
Cardiovascular		
Peripheral edema	3	1
Transient ischemic attack	0	2
Other*	16	26
Total adverse events, n	44	55

*Adverse events (excluding serious events) are listed only if they occurred in ≥2 patients in either group.

We hypothesized that administration of L-arginine would enhance NO synthesis and, by so doing, improve calf blood flow and functional capacity in patients with PAD. This hypothesis was based on the observation that patients with PAD have elevated plasma levels of ADMA.[18,19] ADMA is a methylated arginine analogue and an endogenous competitive inhibitor of NO synthase.[20] ADMA is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). In conditions in which DDAH is impaired or deficient, ADMA accumulates.[21] Vascular DDAH is impaired by the oxidative stress associated with elevated levels of cholesterol, blood glucose, triglycerides, or homocysteine.[19,22,23] Preclinical and human studies indicate that ADMA adversely affects angiogenesis and vascular regeneration and accelerates vascular disease.[9,24–26] ADMA correlates with the severity of arterial disease[27] and is predictive of cardiovascular events.[27,28] Infusion of ADMA increases systemic resistance, reduces cardiac output and vascular compliance, and lessens cerebral blood flow in humans.[29,30]

Other Studies of L-Arginine Supplementation and Functional Capacity

This is the largest randomized clinical trial, of the longest duration, to assess the effect of L-arginine on functional capacity. Several small studies of relatively brief duration have shown a beneficial effect of L-arginine on functional capacity. In patients with PAD (n=39), Boger and colleagues[18] showed that intravenous administration of L-arginine (8 g BID) for 2 weeks improved femoral endothelium-dependent vasodilation, increased urinary nitrogen oxides and cGMP, and increased walking distance compared with placebo. Maxwell and colleagues[31] reported that oral L-arginine supplementation (3 g BID, as part of a nutraceutical supplement) for 2 weeks improved ACD in patients with PAD (n=41). Previously, we performed a pilot study to establish the lowest effective oral dose of L-arginine to improve walking distance.[16] Patients with PAD and intermittent claudication (n=80) were randomly assigned to oral doses of 0, 3, 6, or 9 g of L-arginine daily in 3 divided doses for 12 weeks. We observed a trend for a greater increase in walking distance in the group treated with 3 g L-arginine daily and a trend for an improvement in walking speed in patients treated with L-arginine. Similar observations related to the beneficial effects on functional capacity of L-arginine have been made in patients with coronary artery disease[32–34] and in patients with congestive heart failure.[35]

Although these studies were typically small and of short duration, they are consistent with mechanistic preclinical studies. In hypercholesterolemic mice, plasma ADMA levels are elevated, and endothelial vasodilator function is impaired. In these animals, L-arginine supplementation increases exercise capacity in association with an increase in limb blood flow and urinary nitrogen oxides.[36] In addition to its action as a vasodilator, NO mediates angiogenesis. In the NO synthase–deficient mouse, angiogenesis in response to limb ischemia is impaired, as demonstrated by capillary densitometry.[37] By contrast, in the DDAH transgenic mouse, plasma ADMA levels are

low, NO synthesis is increased, and the angiogenic response to limb ischemia is augmented.[38] In New Zealand White rabbits, L-arginine enhances the angiogenic response to surgically induced limb ischemia.[37] Thus, by improving vascular reactivity and/or vascular regeneration, augmenting the synthesis of endothelial NO improves limb blood flow and exercise capacity.

L-Arginine Tolerance?

The discordance between the results of the present study and those of previous work might be explained by the fact that the present study was of longer duration. Perhaps counterregulatory mechanisms are activated in response to prolonged administration of the NO precursor. This is true of sustained administration of exogenous NO donors such as nitroglycerin, which causes nitrate tolerance. Multiple mechanisms may be involved in nitrate tolerance, including neurohormonal adjustments, oxidative stress, or inhibition of nitroglycerin bioactivation.[39] Could similar mechanisms exist to counter an upregulated synthesis of endogenous NO? Of relevance to this question, Moncada and coworkers[40] found that NO synthase inhibitors enhanced vascular responsiveness to exogenous nitrates. They suggested that removal of endogenous NO induced a supersensitivity to nitrovasodilators at the level of soluble guanylate cyclase.

Induction of arginase expression is a mechanism that could be involved in "arginine tolerance." Arginase metabolizes L-arginine to ornithine and urea. Arginase in the intestine and liver is responsible for extensive first-pass metabolism of L-arginine.[41] In our study, ornithine and urea levels were increased in the arginine-treated individuals, consistent with the action of arginase. However, we also observed a significant elevation of plasma arginine levels, and therefore arginase cannot be invoked as the sole cause of counterregulation.

We had anticipated that arginine administration would increase both NO and citrulline levels by the action of NO synthase. We observed no increase in either. Perhaps L-arginine administration triggers a mechanism to inhibit NO synthase. A transient increase in vascular NO levels could inhibit DDAH and cause ADMA to increase.[42] However, we did not observe an increase in plasma ADMA levels in the arginine-treated patients. Alternatively, it is possible that a transient increase in NO levels could inhibit NO synthase activity by nitrosylation of NO synthase itself[43] or the arginine transporter to counter any arginine-induced increase in NO production.

Potential Adverse Effects of Long-Term L-Arginine Therapy

In our study, the improvement in ACD in the placebo-treated patients was 28.3%, similar to the placebo effect in other studies.[44] By contrast, long-term L-arginine therapy was associated with only an 11.5% increase in ACD. The improvement in ACD was significantly different between the 2 groups and might indicate an adverse effect of L-arginine on functional capacity. The reduced improvement might be due to an arginine-induced derangement of the NO synthase pathway, with a paradoxical reduction in NO production.

In a recent randomized clinical trial, long-term therapy with oral L-arginine supplementation in patients after myocardial infarction (6 g/d for 6 months; n=150) did not improve ejection fraction or vascular compliance, and the study was terminated prematurely for safety concerns.[45] By contrast, in a larger study of shorter duration (6 g/d for 30 days; n=750), L-arginine tended to reduce major adverse cardiovascular events.[46] Taken together, these studies are also consistent with a benefit of short-term administration, which is lost with long-term administration (ie, arginine tolerance).

The present study was based on the hypothesis that L-arginine would reverse the competitive inhibition of NO synthase by ADMA. Accumulating evidence indicates that ADMA contributes to endothelial dysfunction in cardiovascular disease and may be an independent predictor of cardiovascular events. In mice, overexpression of DDAH reduces plasma ADMA, increases NO synthesis, and reduces systemic vascular resistance.[47] The reduction in ADMA is associated with vascular protection, as shown by a greater capacity for angiogenesis, and a resistance to vascular lesion formation.[24,38,48] Agents that increase DDAH expression or activity may be useful in the treatment of cardiovascular disease. Indeed, there are currently several disease-modifying drugs that reduce ADMA, including metformin, rosiglitazone, converting enzyme inhibitors, and angiotensin receptor antagonists.[21] Of course, each of these agents has other metabolic and hemodynamic effects that are antiatherogenic.

Conclusion

In a randomized, double-blind, placebo-controlled trial in patients with intermittent claudication, oral L-arginine supplementation (3 g/d for 6 months) was less effective than placebo in improving measures of endothelial function and treadmill exercise. L-Arginine is not a useful nutritional adjunct in patients with intermittent claudication and PAD.

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Disclosures

Dr Cooke is the inventor of patents owned by Stanford University for diagnostic and therapeutic applications of the NO synthase pathway from which he receives royalties. Dr Cooke is a consultant to Ajinomoto and United Therapeutics. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

L-Arginine is a semiessential amino acid and is the precursor for nitric oxide (NO). The vasodilator action of NO resembles that of nitroglycerin. Its vasodilator and vasoprotective effects are attenuated in patients with cardiovascular disease or risk factors. Previous studies in cardiovascular patients reveal that short-term administration of L-arginine restores endothelium-dependent, NO-mediated vasodilation. Small pilot studies of brief duration indicate that L-arginine supplementation improves vascular function as well as symptoms in patients with peripheral arterial disease. Accordingly, we performed a randomized clinical trial to determine whether long-term L-arginine supplementation (1 g TID for 6 months) is useful in patients with symptomatic peripheral arterial disease. The primary end point was walking distance by treadmill testing. Secondary end points included measures of vascular function, including flow-mediated vasodilation and vascular compliance, and biochemical measures of NO synthase activity. L-Arginine supplementation increased plasma L-arginine levels but did not increase metabolites of NO synthase (plasma citrulline and nitrogen oxides). Furthermore, L-arginine supplementation did not improve measures of vascular function. Surprisingly, the increment in walking distance in the placebo group was greater than that in the group treated with L-arginine. As opposed to its short-term use, long-term L-arginine supplementation does not improve vascular function or symptoms in patients with peripheral arterial disease. The absence of a long-term benefit might be explained by "arginine tolerance."