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A pilot study of L-arginine supplementation on functional capacity in peripheral arterial disease

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Abstract: Peripheral arterial disease (PAD) impairs walking capacity and is often associated with a profound endothelial vasodilator dysfunction, characterized by reduced bioactivity and/or synthesis of endothelium-derived nitric oxide (NO). Previous studies have suggested that dietary supplementation of L-arginine, the precursor of NO, improves endothelium-dependent vasodilation, limb blood flow and walking distance. However, these studies have been small, and have used large intravenous doses of L-arginine. The optimal dose of L-arginine has not been determined. Accordingly, this pilot study was conducted to establish the lowest effective oral dose of L-arginine to improve walking distance in preparation for the definitive study. Patients with PAD and intermittent claudication ($n = 80$) participated in this study. Eligibility criteria included: (1) ankle–brachial index (ABI) at rest $\leq$0.90; (2) post-exercise reduction in ABI $\geq$25%; and (3) difference in absolute claudication distance of $\leq$25% between two consecutive treadmill tests. Treadmill testing was performed using the Skinner–Gardner protocol and community-based walking was assessed using the walking impairment questionnaire. Patients were randomly assigned to oral doses of 0, 3, 6 or 9 g of L-arginine daily in three divided doses for 12 weeks. Treadmill testing was performed prior to administration of the study drug and again after 12 weeks of treatment. The study drug was well tolerated, with no significant adverse effects of L-arginine therapy. The safety laboratory studies were unremarkable, except for a statistically significant reduction in hematocrit in the L-arginine–treated groups. There was no significant difference observed in absolute claudication distance between the groups. However, a trend was observed for a greater increase in walking distance in the group treated with 3 g L-arginine daily, and there was a trend for an improvement in walking speed in patients treated with L-arginine. This pilot study provided data for safety, for power calculation and for dosing for the larger definitive trial that is now underway.

Key words: functional capacity; L-arginine; vascular disease

Introduction

Patients with peripheral arterial disease (PAD) suffer from functional limitations that include leg pain with exertion, i.e. intermittent claudication.[1] The severity of symptoms is determined in part by the degree of conduit vessel obstruction, the development of collateral vessels and the derangement of skeletal muscle metabolism.[2] Another potential determinant is the impairment of endothelial vasodilator function associated with PAD, particularly if it affects collateral vessel reactivity. The impairment of endothelial vasodilator function in patients with atherosclerosis is in large part due to reduced bioactivity and/or synthesis of endothelium-derived nitric oxide (NO).

Endogenous inhibitors of NO synthase (NOS) may contribute to the impairment. Monomethylarginine (NMA) and asymmetric dimethylarginine (ADMA) are competitive inhibitors of NO synthase. ADMA is the more prevalent species, and most attention has focused on it. Evidence suggests that the effect of ADMA may be reversed by administration of supplemental L-arginine (the precursor to NO). Previous studies have demonstrated that administration of L-arginine improves limb blood flow and walking distance in patients with PAD.[3–8] However, these studies were small, of short duration, and/or used high

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doses of intravenous L-arginine. A dose-ranging assessment of oral arginine therapy has not been performed. The purpose of this study was to obtain data on dose-dependent actions of oral L-arginine in patients with PAD in preparation for a definitive trial.

Methods

The Nitric Oxide for Peripheral Arterial Insufficiency (I), or NO PAIN I study, is a randomized double-blind, placebo-controlled, dose-ranging trial funded by the National Heart, Lung and Blood Institute and the National Institute of Nursing Research. The study is designed to obtain pilot data for a randomized clinical trial regarding the safety and efficacy of L-arginine on functional capacity in patients with claudication and PAD. Participants were recruited via a citywide media campaign and physician referral.

Sample

Eligibility criteria for participants in this study included the following: (1) age 40 years or older; (2) PAD secondary to atherosclerosis with significant claudication (Fontaine class II, i.e. intermittent claudication, or Fontaine class III, i.e. pain at rest); (3) intermittent claudication characterized by pain, ache, cramp, numbness or severe fatigue involving muscles of one or both lower extremities, reproducibly provoked by walking and relieved by rest; (4) ankle–brachial index (ABI) $\leq$0.90 and at least a 25% decrease in ABI within 1 min during exercise recovery; (5) ability to walk at least 2 min (50 feet or 15 meters) but no more than 12 min on a treadmill using the Skinner–Gardner protocol; (6) walking limited by claudication, not coexisting conditions; and (7) difference between two consecutive baseline exercise treadmill tests of $\leq$25%.

Subjects were excluded for: (1) PAD of non-atherosclerotic nature; (2) Fontaine class IV, i.e. ulcer or gangrene; (3) any type of major cardiovascular surgery during the last 3 months, i.e. aortic or lower extremity arterial surgery, angioplasty, or lumbar sympathectomy; (4) leg amputation above the ankle; (5) myocardial infarction (MI) within the past 3 months; (6) current enrollment in another clinical trial and/or ingestion of another investigational product within the past 30 days; (7) proliferative retinopathy; (8) history of disease state or surgery that affects gastrointestinal absorption; (9) serum hepatic enzymes three times normal or serum creatinine $>$3.0 mg/dl; (10) intolerance to sublingual nitroglycerin; (11) uncontrolled hypertension; (12) type I diabetes; (13) active malignancy or tumor; (14) serious infection or hypotension associated with sepsis in the last month; (15) cerebrovascular infarct in the last 3 months; (16) autoimmune disorders (systemic lupus erythematosis, ulcerative colitis); and (17) unwillingness to discontinue arginine-containing products, pentoxifylline, L-carnitine, or prostacyclin for at least 1 month prior to and during study period.

Evaluation procedures

Ankle–brachial index (ABI)

The ABI is a rapid, non-invasive and reliable measure that detects and quantifies PAD.[9] ABI is defined as the ratio of the ankle systolic blood pressure (SBP) to that in the arm. This method provides an overall assessment of cardiovascular health and identifies individuals who are at particularly high risk for morbidity and mortality.[1,10–12] The sensitivity of the ABI to detect PAD has been reported in clinical trials to be approximately 95%, with a specificity of near 100%.[13]

ABI was measured in study subjects after 5 min of supine rest. An appropriately sized cuff was placed over the brachial artery to obtain a SBP reading. Another cuff was placed around the ankle, proximal to the malleolus. The BP cuff was rapidly inflated to 20 mmHg above the audible SBP and deflated over the artery in 2 mm/s increments. Using a hand-held doppler (Imex Pocket dop-II, Golden, CO, USA) with a 5-MHz probe, blood pressures were obtained in the following sequence: right and left brachial artery followed by right then left dorsalis pedis and right and left posterior tibial pulses. The ABI was calculated for each leg by dividing the highest ankle pressure (posterior tibial or dorsalis pedis) by the higher of the two brachial pressures.[14]

Exercise performance and walking ability

1) Exercise treadmill test (ETT)

To assess walking capacity, subjects performed an ETT using the Skinner–Gardner protocol. The Skinner–Gardner protocol consists of a graded workload, with a constant speed of 2 mph (3.2 k/h) and an increase in grade of 2% every 2 min.[15] During the ETT, standardized verbal encouragement was given and all subjects were continuously monitored for hemodynamic response (heart rate, rhythm and BP) to exercise.

Initial claudication distance (ICD) was measured as the distance in meters walked on the ETT at the onset of claudication, regardless of whether this was manifested as muscle pain, ache, cramps, numbness or fatigue. The absolute claudication distance (ACD) for this study was the maximum distance walked on the ETT before stopping due to claudication.

2) Walking impairment questionnaire (WIQ)

Self-reported walking ability was assessed with the 11-item walking impairment questionnaire (WIQ) developed by Regensteiner and colleagues.[16] The WIQ is designed specifically for PAD patients to evaluate the effectiveness of various interventions, including physical training, on self-reported walking function. This questionnaire assesses a patient's

degree of difficulty with defined distances and speeds as well as the severity of claudication pain. Scores are determined for walking distance, walking speed and stair climbing. The WIQ has been validated with treadmill walking time.[16]

Biochemical analysis

All patients were on a nitrate-free diet including nitrate-free water for 24 h prior to measurement and spot urine was obtained after a 12 h fast. Urinary nitrogen oxides (NOx) were measured by fluorimetry as previously described.[17] Urinary creatinine was determined by spectrophotometry with the alkaline picric acid method in an automatic analyzer (Beckman). The urinary NOx level was normalized by urinary creatinine concentration so as to reduce variability due to differences in urine volume and renal function.[17]

Statistical analysis

Analysis of variance was used to evaluate between dose-related group differences. Medical and surgical history information was analyzed using chi-squared tests with Fischer exact test statistics. The α level was set at 0.05 using a two-tailed test of significance. Demographic and clinical characteristics of the study sample are reported as means and standard deviations. The baseline values for ICD and ACD were the average of values obtained from two consecutive treadmill tests. Subjects underwent two to four ETTs in the run-in period to obtain two consecutive ETTs where the lower of the two ACD values was within 25% of the higher value. Subjects with persistent variability in ACD greater than the acceptable range were excluded after four ETTs.

Results

Sample

A total of 610 individuals who responded to the citywide promotion or were referred by their primary care physician were screened by telephone (Figure 1). Of these, 264 were ineligible primarily because of medical history, leg pain not of atherosclerotic origin,

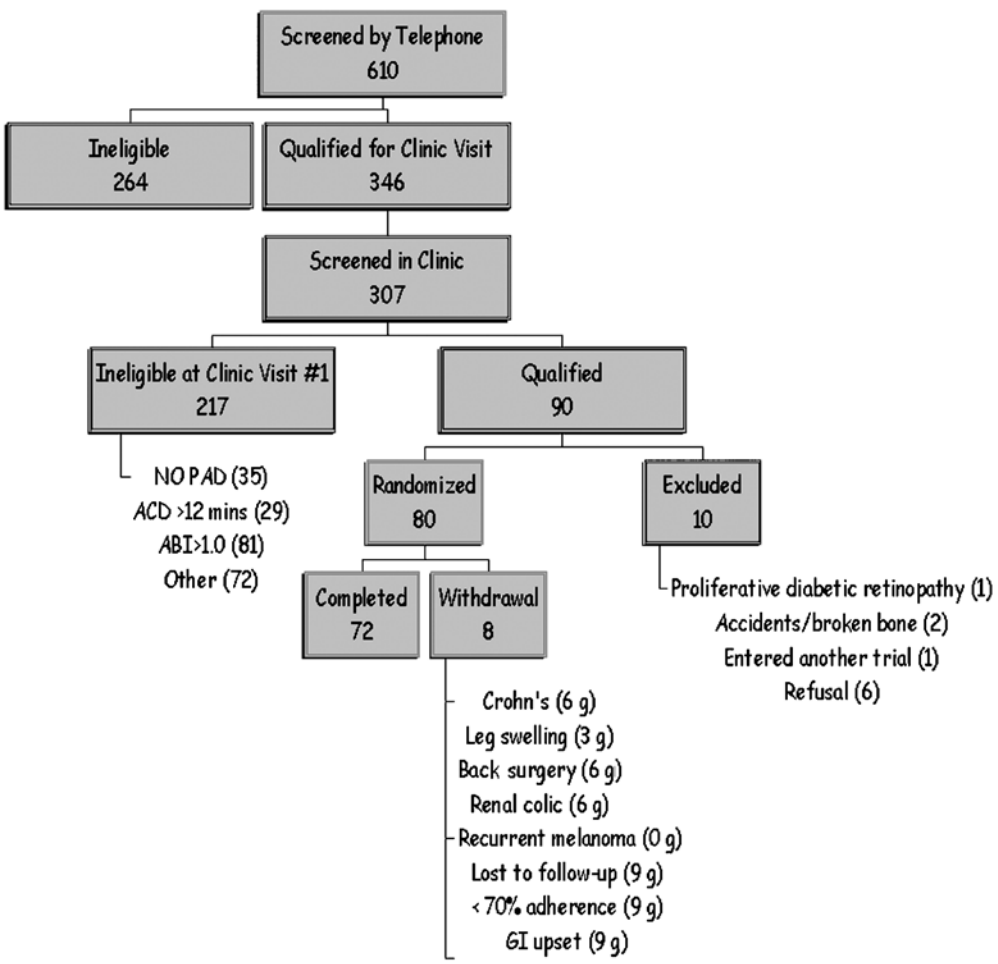

Figure 1 The screening process and the number of individuals determined to be eligible and ineligible and the reason for ineligibility after the screening at the clinic visit. Also shown are the number of individuals randomized and the number and reason for dropouts in the study.

Table 1 Clinical characteristics.

Variable	0 g ($n = 18$)	3 g ($n = 18$)	6 g ($n = 17$)	9 g ($n = 19$)
Age (years)	72 ± 9	75 ± 9	76 ± 6	73 ± 6
Education (years)	15 ± 3	15 ± 3	16 ± 3	14 ± 2
BMI (hight/m^2)	27.1 ± 3.3	28.2 ± 4.4	28.8 ± 6.1	27.4 ± 8.0
ABI (mmHg)	0.62 ± 0.14	0.69 ± 0.19	0.65 ± 0.14	0.62 ± 0.14
SBP (mmHg)	147 ± 17	150 ± 14	153 ± 19	149 ± 21
DBP (mmHg)	70 ± 11	72 ± 8	74 ± 9	73 ± 11

Data given as mean ± SD.
BMI, body mass index; ABI, ankle–brachial index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

inadequate transportation, and refusal. Of 307 subjects screened in clinic, 217 were ineligible after one clinic visit because (1) they did not have evidence for PAD, e.g. ABI > 1.0 (37%); (2) other reasons (33%) including refusal, termination of treadmill test for symptoms other than claudication, no decrease in ABI after exercise test; and (3) walking distances >12 min (13%). Ninety subjects were eligible to be randomized; 10 were excluded because of refusal or drop-out ($n = 7$), unexpected injury ($n = 2$) and diagnosis of proliferative retinopathy ($n = 1$). Eighty subjects were randomized to the study. Of these, 10% ($n = 8$) dropped out during the course of the study. The final sample consisted of 72 PAD patients with claudication, of which 50 (69.4%) were men and 22 (30.6%) were women with a mean age of 74 years (SD = 7.7, range = 52 to 90). Characteristics of the sample are shown in Tables 1 and 2. The majority were Caucasian, men, married, well educated and retired. The majority of the study population consisted of current non-smokers, a majority of whom were ex-smokers with a mean quit time of 20 years (SD = 20) and who also participated in a regular exercise regimen and followed a dietary regimen (Table 2). No significant differences were found between groups in demographic or clinical variables (Tables 1 and 2).

Comorbidities for this study population included hypertension, hypercholesterolemia, coronary artery disease (CAD), angina, type II diabetes, arthritis, carotid artery stenosis, transient ischemic attacks (TIA), congestive heart failure (CHF), stroke, and renal disease (Table 3). Past surgical history included a remote history of coronary bypass surgery (CABG), leg bypass, carotid artery surgery, percutaneous

Table 2 Characteristics of the sample.

Variable	0 g ($n = 18$)	3 g ($n = 18$)	6 g ($n = 17$)	9 g ($n = 19$)
Gender (*n*,%)				
M/F	83%/17%	67%/33%	65%/35%	63%/37%
Ethnicity (*n*,%)				
Asian	–	–	–	5%
Caucasian	4%	88%	94%	90%
Latino	6%	–	6%	5%
Mixed ethnic	–	6%	–	–
Other	–	6%	–	–
Marital status				
Married	94%	61%	71%	68%
Widowed	6%	33%	23%	5%
Divorced	–	6%	6%	16%
Never married	–	–	–	11%
Current smoker				
Yes	11%	11%	12%	5%
No	89%	89%	88%	95%
Ever smoked				
Yes	70%	60%	50%	65%
No	30%	40%	50%	35%
Dietary program				
Yes	22%	50%	53%	56%
No	78%	50%	47%	44%
Exercise program				
Yes	56%	44%	53%	47%
No	44%	56%	47%	53%

Unless otherwise indicated, p = NS.

Table 3 Medical history.

Variable	0 g (n = 18)	3 g (n = 18)	6 g (n = 17)	9 g (n = 19)
Hypertension	72%	72%	65%	79%
Hypercholesterolemia	78%	61%	65%	74%
Angina	44%	50%	35%	39%
CAD	56%	56%	5%	53%
CHF	6%	6%	13%	32%
Stroke	17%	17%	12%	11%
Carotid artery stenosis	11%	18%	29%	28%
TIA	6%	24%	24%	22%
Diabetes	6%	61%	38%	35%
DJD	0%	17%	6%	21%
Renal disease	6%	0%	18%	21%

Unless otherwise indicated, p = NS.
Data given as mean SD.
CAD, coronary artery disease; CHF, congestive heart failure; TIA, transient ischemic attacks; DJD, degenerative joint disease.

transluminal coronary angioplasty (PTCA), abdominal aortic aneurysm (AAA) repair or peripheral angioplasty (Table 4). No differences were found between groups in medical and surgical history or medication usage (Table 5). Overall adherence to study supplementation was high in all groups (placebo = 95%, 3 g = 97%, 6 g = 95% and 9 g = 94%).

Functional capacity

Exercise treadmill testing

Prior to entry into the study, pain-free or ICD and maximal walking distance or ACD were similar in all four groups (Table 6). After the 12-week intervention period, ICD and ACD tended to improve in all groups. Improvement in ICD was greatest in individuals receiving 6 g of L-arginine (Figures 2 and 3). The largest effect on ACD was observed in the group receiving 3 g of L-arginine, although this effect did not reach statistical significance.

Walking impairment questionnaire

Results from the self-administered community-based walking questionnaire showed that at baseline all subjects reported impaired walking ability. After the

Table 4 Surgical history.

Variable procedure	0 g (n = 18)	3 g (n = 18)	6 g (n = 17)	9 g (n = 19)
CABG	50%	33%	24%	42%
PTCA	14%	47%	22%	23%
Peripheral bypass	22%	33%	6%	37%
PTCA leg	11%	24%	40%	26%
AAA repair	––	6%	19%	5%
Carotid surgery	11%	11%	31%	21%

Unless otherwise indicated, p = NS.
CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; AAA, abdominal aortic aneurysm.

Table 5 Current medications.

Currently taking?	0 g (n = 18)	3 g (n = 18)	6 g (n = 17)	9 g (n = 19)
Statins	72%	50%	65%	63%
Beta-blockers	28%	39%	47%	47%
Calcium channel blockers	56%	61%	41%	32%
ACE-inhibitors	39%	33%	53%	53%
Alpha receptor blockers	22%	28%	24%	21%
Diuretics	39%	50%	47%	42%
Other antilipids	20%	10%	5%	10%
Anticoagulants	22%	17%	18%	5%
Insulin	11%	17%	12%	21%
Biguanides	–	6%	12%	–
Sulfonylurea	–	28%	6%	11%

Unless otherwise indicated, p = NS.

Table 6 Functional capacity.

Variable	0 g (n = 18)	3 g (n = 18)	6 g (n = 17)	9 g (n = 19)
ICD				
before	121.3 ± 61.5	150.0 ± 96.9	113.2 ± 73.7	123.4 ± 61.2
after	152.6 ± 101.8	191.6 ± 114.4	179.2 ± 115.6	198.0 ± 181.6
% change in ICD	40.7 ± 90.8	41.7 ± 78.2	62.0 ± 85.0	21.4 ± 30.0
ACD				
before	299.0 ± 115.6	297.1 ± 139.8	306.3 ± 151.9	299.7 ± 131.3
after	352.9 ± 152.0	398.5 ± 208.8	371.4 ± 188.9	372.1 ± 222.1
% change in ACD	24.4 ± 52.0	45.1 ± 75.2	35.1 ± 62.9	21.4 ± 30.0
WIQ Speed %				
before	33.6 ± 27.5	24.7 ± 14.2*	24.2 ± 17.8	29.2 ± 17.1
after	42.1 ± 27.6	29.6 ± 27.6	32.1 ± 22.2	39.3 ± 25.0

Unless otherwise indicated, p = NS; *p = 0.04.
ICD, initial claudication distance; ACD, absolute claudication distance.

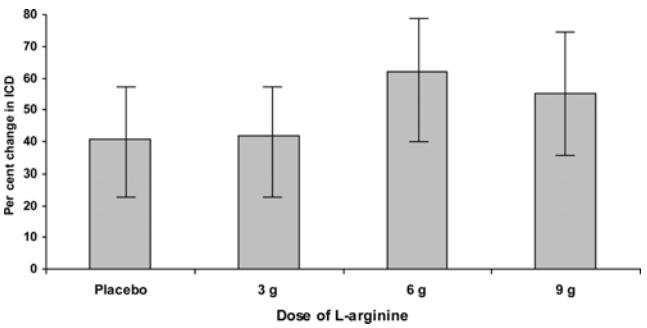

Figure 2 The per cent change in initial claudication distance from baseline to 12 weeks in all groups. No statistically significant group differences were noted using analysis of covariance.

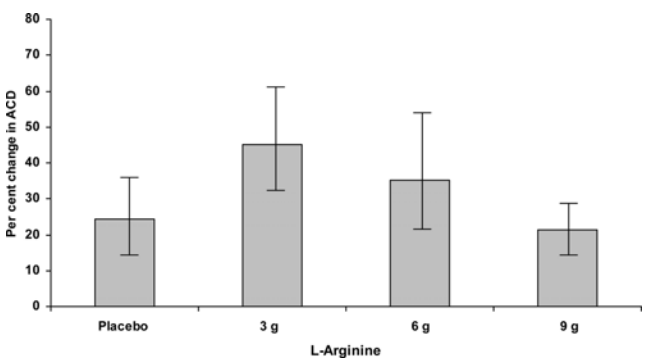

Figure 3 The per cent change in absolute claudication distance from baseline to 12 weeks in all groups. No statistically significant group differences were noted using analysis of covariance.

12-week intervention period, a dose-related trend toward improvement in the walking speed subscale was observed (p = NS). However, there were no differences between groups in the overall walking score.

Biochemical analysis

The apparent improvement in walking distance induced by L-arginine (3 g daily) was associated with an increase in plasma L-arginine and urinary NOx (Figure 4). However, the increase in plasma L-arginine or urinary NOx was not dose-dependent (Table 7).

Safety

All adverse events were reported to the appropriate institutional review panel, National Institutes of Health, and the data safety monitoring board (DSMB). Over the 12-week intervention period, eight subjects withdrew from the study. The reasons for withdrawal were: gastrointestinal discomfort (n = 1); recurrence of melanoma (n = 1); back surgery (n = 1); Crohn's disease (n = 1); renal colic (n = 1); non-adherence to study supplement (n = 1) and leg swelling (n = 1). One patient was lost to follow-up. The DSMB requested unblinding of the study drug code for the subject with recurrence of melanoma; this subject was receiving placebo. The DSMB reviewed all adverse events, and felt that only one event (gastrointestinal discomfort) was possibly related to the study drug. All study subjects were monitored for renal, liver and hematopoetic changes; no clinically relevant changes in mean values were observed in any of the groups. However, in combining the values from subjects treated with L-arginine, there were statistically significant reductions in hematocrit (p = 0.01).

Discussion

This pilot study indicates that supplementation with oral L-arginine for 12 weeks is safe and well tolerated. Overall adherence to study supplementation was high in all groups (≥95%). This pilot study suggests that L-arginine supplementation may have a modest benefit on functional capacity as determined by initial and absolute claudication distance and self-reported walking speed. In the current study, a dose of 3 g daily increased plasma L-arginine and urinary nitrogen oxides. The average intake of dietary L-arginine in the

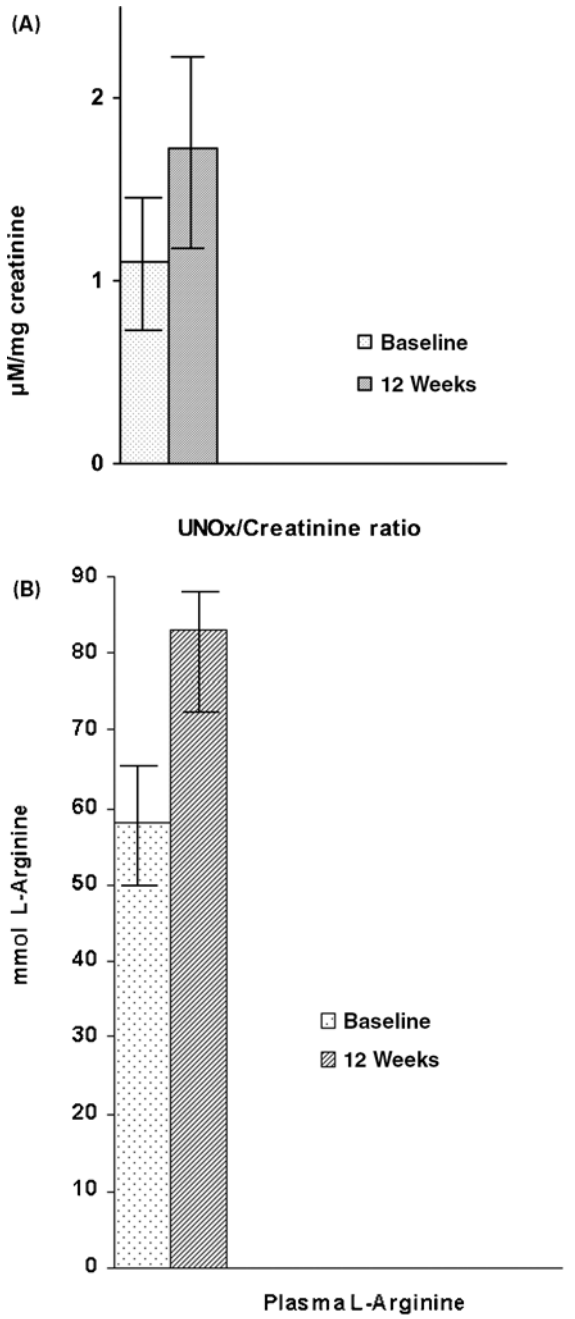

Figure 4 The increase in (A) urine nitric oxide/creatinine ratio and (B) L-arginine levels from baseline to 12 weeks in individuals randomized to 3 g of oral L-arginine. Increases in urine nitric oxide/creatinine and L-arginine levels were observed although not statistically significant.

USA is about 3 g daily.[18] Therefore, a supplementary dose of 3 g doubles daily L-arginine intake. Higher doses of L-arginine did not appear to result in a greater effect on walking capacity or urinary nitrogen oxides. Accordingly, in the definitive trial to follow, a dose of L-arginine of 3 g daily will be used. The intent of L-arginine supplementation in PAD is to enhance the synthesis of endothelium-derived NO, which in addition to its vasodilator action, has anti-atherogenic and

Table 7 Urine nitrite/creatinine and L-arginine at baseline and 12 weeks by dose.

	Placebo	3 g	6 g	9 g
Urine nitrate/creatinine (µmol/mg creatinine)				
Baseline	0.92	1.11	2.45	1.16
12 weeks	1.23	1.73	1.17	1.56
L-arginine				
Baseline	51.1	58.2	83.3	59.3
12 weeks	72.8	83.1	87.4	93.7

pro-angiogenic properties that may benefit these patients. The response to L-arginine supplementation is likely to be heterogeneous, as the impaired endothelial function in these patients is multi-factorial and dependent upon the vascular bed; the stage of atherosclerosis; and the associated metabolic disorders.[19–22] Mechanism(s) of impairment may include endothelial generation of superoxide anion and increased degradation of NO; elaboration of vasoconstrictor prostanoids and endothelin; reduced elaboration of prostacyclin; and/or impaired biosynthesis of NO.[23–28] Impaired biosynthesis of NO may be due to alterations in NOS affinity for L-arginine; to lipid-induced impairment of the high-affinity cationic amino acid transporter; to reduced availability of the cofactor tetrahydrobiopterin; or to increased levels of ADMA, the competitive inhibitor of NOS.[29–33]

ADMA is generated from post-translational modification of arginine residues within a variety of specific proteins that are predominantly found in the cell nucleus (for review, see Tran et al).[34] Methylation of arginine residues is catalyzed by a group of enzymes termed protein arginine *N*-methyltransferases. When the proteins undergo proteolysis, free methylarginines are released. Degradation of ADMA and NMA (but not SDMA (symmetric dimethylarginine)) is mediated largely by dimethylarginine dimethylaminohydrolase (DDAH).[35,36] We have shown that impaired DDAH activity is a central mechanism by which cardiovascular risk factors disrupt the NOS pathway.[37] The activity of DDAH is impaired by oxidative stress, permitting ADMA to accumulate. A wide range of pathological stimuli induces endothelial oxidative stress such as oxidized LDL cholesterol, inflammatory cytokines, hyperhomocystinemia, hyperglycemia, and infectious agents. Each of these insults attenuates DDAH activity in vitro and in vivo,[37–40] which allows ADMA to accumulate and to block NO synthesis.

The elevation of plasma ADMA provides a possible explanation for previous studies that have documented an improvement in endothelium-dependent vasodilation and NO synthesis with administration of L-arginine.[41–48] Boger and colleagues[41] measured urinary nitrate and cGMP excretion rates, and plasma

concentrations of L-arginine and ADMA, in patients with PAD ($n = 77$, Fontaine stages IIb through IV) and in young ($n = 47$) and elderly healthy subjects ($n = 37$). The excretion rates of urinary nitrate and cGMP (cyclic guanosine monophasphate) (which are indirect measures of systemic NO synthesis) were reduced in the PAD patients, and were inversely related to the severity of disease. Plasma L-arginine levels were not different, but the ADMA levels were increased in PAD, and related to the severity of disease. There was a significant negative linear correlation between plasma ADMA concentrations and urinary nitrate excretion.[49] These findings suggested that elevated levels of plasma ADMA may reflect systemic inhibition of NO synthesis in patients with PAD. This study provided the rationale for the investigators to determine whether L-arginine supplementation, by reversing the effects of the endogenous NOS inhibitor, could improve NO synthesis and functional capacity.

Accordingly, in a double-blind, randomized study, Boger and colleagues compared the effect on functional capacity of intermittent infusion therapy using L-arginine or the vasodilator prostaglandin E1 versus no active therapy. Patients with intermittent claudication ($n = 39$) were randomly assigned to receive L-arginine infusion (8 g twice daily), or prostaglandin E1 (PGE1; 40 μg twice daily) or no active treatment, for 3 weeks. L-Arginine infusion improved the pain-free walking distance by 2.3-fold and the absolute walking distance by 1.6-fold, whereas PGE1 improved both parameters by 2.1-fold and 1.4-fold, respectively. Patients receiving no active therapy experienced no significant change. L-Arginine treatment elevated the plasma L-arginine/ADMA ratio; increased urinary nitrate and cyclic GMP excretion rates; and improved endothelium-dependent flow-mediated vasodilation in the femoral artery. PGE1 therapy had no significant effect on any of these parameters. Symptom scores also improved in the L-arginine and the PGE1 groups, but did not significantly change in the control group.[8]

Maxwell and colleagues found that L-arginine administered in a medical food bar at a dose of 6 g daily for 2 weeks increased the pain-free walking distance and quality of life in PAD patients.[3] However, this medical food bar also contained other potentially vasoactive agents including the antioxidant vitamins C and E, folate and vitamin B6, and soy phytoestrogens. A subsequent study of longer duration showed no benefit of this product on maximal walking distance. It is not surprising that the response to L-arginine administration is heterogeneous. In some cases, the endothelial vasodilator dysfunction may be due to deficiency or reduced activity of tetrahydrobiopterin, heat shock protein 90, or specific tyrosine kinases; such abnormalities would probably not be addressed by supplementation with L-arginine. [50-52] Furthermore, L-Arginine may not be useful in the later stages of atherosclerosis, in which cytokine-or lipid-induced instability and/or reduced transcription of NOS may decrease its expression.[53] Endothelial dysfunction secondary to certain NOS gene polymorphisms also might be unresponsive to supplemental L-arginine.

Although the safety studies did not reveal any clinically significant changes in mean values, there was a statistically significant reduction in hematocrit. It is possible that this was a type I statistical error. However, given the slight anti-platelet effect of L-arginine that we previously documented in hypercholesterolemic humans,[54] it is possible that the combination of L-arginine enhanced the activity of other anti-platelet agents. This possible effect will be examined in the larger ongoing study. In addition, we observed a slight reduction in the plasma uric acid levels of our arginine-treated patients. The hypouricemic effect of L-arginine administration was previously observed by Maxwell and Bruinsma.[55] In their study, L-arginine administration (6 g daily) was associated with an 8% reduction in serum uric acid levels. Serum uric acid levels are thought to increase in response to oxidative stress.[56] In this regard, it is notable that nitric oxide inhibits oxidative enzyme activity.[57] The reduction in uric acid levels could thus be in response to a reduction in oxidative stress in the arginine-treated individuals.

To conclude, in patients with PAD, L-arginine supplementation was well tolerated, with no significant adverse effects. A trend for improvement was observed with a greater increase in walking distance in the group treated with 3 g L-arginine daily. There was also a trend for an improvement in self-reported walking speed (manifested in the WIQ) in patients treated with L-arginine. This pilot study provided data for safety, for power calculation and for dosing for the larger definitive trial that is now underway.

Acknowledgements

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Stanford University owns patents on the therapeutic use of L-arginine, ADMA assays, and modulators of DDAH expression or activity. Dr Cooks is an inventor on these patents, and receives royalties.

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