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Restoring Vascular Nitric Oxide Formation by L-Arginine Improves the Symptoms of Intermittent Claudication in Patients With Peripheral Arterial Occlusive Disease

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Background. **Administration of L-arginine improves nitric oxide (NO) formation and endothelium-dependent vasodilation in atherosclerotic patients.**

Objectives. **We investigated in this double-blind, controlled study whether prolonged intermittent infusion therapy with L-arginine improves the clinical symptoms of patients with intermittent claudication, as compared with the endothelium-independent vasodilator prostaglandin E_1, and control patients.**

Methods. **Thirty-nine patients with intermittent claudication were randomly assigned to receive 2 × 8 g L-arginine/day, or 2 × 40 μg prostaglandin E_1 (PGE_1)/day or no hemodynamically active treatment, for 3 weeks. The pain-free and absolute walking distances were assessed on a walking treadmill at 3 km/h, 12% slope, and NO-mediated, flow-induced vasodilation of the femoral artery was assessed by ultrasonography at baseline, at 1, 2 and 3 weeks of therapy and 6 weeks after the end of treatment. Urinary nitrate and cyclic guanosine-3′, 5′-monophosphate (GMP) were assessed as indices of endogenous NO production.**

Results. **L-Arginine improved the pain-free walking distance by 230 ± 63% and the absolute walking distance by 155 ± 48% (each $p < 0.05$). Prostaglandin E_1 improved both parameters by 209 ± 63% and 144 ± 28%, respectively (each $p < 0.05$), whereas control patients experienced no significant change. L-Arginine therapy also improved endothelium-dependent vasodilation in the femoral artery, whereas PGE_1 had no such effect. There was a significant linear correlation between the L-arginine/asymmetric dimethylarginine (ADMA) ratio and the pain-free walking distance at baseline ($r = 0.359$, $p < 0.03$). L-Arginine treatment elevated the plasma L-arginine/ADMA ratio and increased urinary nitrate and cyclic GMP excretion rates, indicating normalized endogenous NO formation. Prostaglandin E_1 therapy had no significant effect on any of these parameters. Symptom scores assessed on a visual analog scale increased from 3.51 ± 0.18 to 8.3 ± 0.4 (L-arginine) and 7.0 ± 0.5 (PGE_1; each $p < 0.05$), but did not significantly change in the control group (4.3 ± 0.4).**

Conclusions. **Restoring NO formation and endothelium-dependent vasodilation by L-arginine improves the clinical symptoms of intermittent claudication in patients with peripheral arterial occlusive disease.**

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Peripheral arterial occlusive disease (PAOD) is a manifestation of generalized atherosclerosis; many PAOD patients also suffer from atherosclerotic complications at other sites that are frequently affected, like the coronary and extracranial carotid arteries (1). Recent studies on the pathophysiology of atherosclerotic vascular disease have shown that the endothelium plays a crucial role in the active regulation of local blood flow. Atherosclerosis is characterized by decreased ability of the endothelium to induce vasodilation (2). This is mainly due to impaired biological activity of the endothelium-derived vasodilators, nitric oxide (NO) and prostacyclin (3). Prostaglandin E_1 (PGE_1) activates prostacyclin receptors and thereby induces endothelium-independent vasodilation; it has been used for several years in the pharmacologic treatment of patients with severe PAOD (4,5).

Nitric oxide, which is synthesized from the amino acid precursor L-arginine, is constitutively released from the endothelium and contributes to the physiologic regulation of blood pressure and vascular tone (6). The biological activity of NO is impaired in animal models of hypercholesterolemia (7,8) and in patients with atherosclerotic vascular disease (9,10). Experimentally, chronic supplementation with L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits (8,11). In hypercholesterolemic and atherosclerotic humans, acute intravenous administration of L-arginine improves endothelium-dependent vasodilation in the coronary (9) and in the peripheral circulation (12). We have recently shown that a single intravenous infusion of L-arginine increases the perfu-

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Table 1. Baseline Characteristics of the Patients

	L-Arginine	PGE_1	Control
Patients (n)	13	13	13
Sex (M/F)	6/4	9/4	9/4
Age (yr)	70.1 ± 2.8	66.1 ± 3.6	66.8 ± 2.4
(range)	49–87	48–89	53–82
Height (cm)	169.8 ± 2.4	168.2 ± 2.7	170.2 ± 1.9
Weight (kg)	72.7 ± 2.6	74.0 ± 3.5	77.1 ± 4.0
Systolic blood pressure (mm Hg)	153.1 ± 6.2	150.4 ± 5.6	152.7 ± 5.4
Diastolic blood pressure (mm Hg)	78.1 ± 2.7	77.7 ± 4.2	82.3 ± 3.6
Plasma total cholesterol (mmol/L)	5.8 ± 0.3	6.2 ± 0.4	5.9 ± 0.3
Classification of PAOD			
Pain-free walking distance (m)	53.0 ± 9.0	56.3 ± 12.3	53.6 ± 4.8
Absolute walking distance (m)	93.7 ± 12.9	98.3 ± 19.8	99.6 ± 9.7
Ankle-arm blood pressure index	0.53 ± 0.06	0.55 ± 0.04	0.59 ± 0.06
Occlusion type			
Femoral (n)	8	8	8
Multisegmental (n)	4	5	4
Tibioperoneal (n)	1	0	1
Cardiovascular risk factors (n)			
Hypercholesterolemia	8	9	8
Hypertension	8	9	8
History of smoking	7	7	7
Diabetes mellitus	4 (type II)	3 (type II)	3 (type II)
Coronary heart disease	7	7	7
Carotid artery stenosis	6	6	6
Comedication (n)			
Acetylsalicylic acid (100 mg/d)	10	10	9
Calcium channel blockers	9	7	5
Lipid-lowering drugs	2	2	2
Nitroglycerine spray	4	4	3
Oral antidiabetics	2	2	1
Insulin	2	1	1
Other (miscellaneous)	5	6	5

Data are given as mean ± SEM. There were no statistically significant differences between the groups in any of the parameters. See text for abbreviations.

sion of the diseased leg in patients with critical limb ischemia (13). However, it has not been investigated in these studies whether the hemodynamic effects of L-arginine are maintained during an intermittent infusion therapy lasting for several weeks, and whether improved endothelial function is associated with improved clinical symptoms of atherosclerotic vascular disease. Therefore, the present study was performed to investigate whether intermittent intravenous infusion therapy with L-arginine improves the symptoms of intermittent claudication in PAOD patients as compared with the endothelium-independent peripheral vasodilator, PGE_1. A third group of patients was studied who did not receive a peripheral vasodilator (control group) in order to allow for fluctuations in the natural course of disease activity.

Methods

Patients and study design. Thirty-nine patients with PAOD (Fontaine stage IIb) were included into this study after they had given their informed consent. The clinical characteristics of the patients are given in Table 1. The study protocol had previously been approved by the local Institutional Review Board for Studies in Humans; it conformed with recent national and international guidelines for therapeutic studies in patients with peripheral arterial disease (14–16). Inclusion criteria were: the presence of stable intermittent claudication due to chronic PAOD Fontaine stage IIb, defined as the presence of stenoses in the arterial vessels of the lower limbs as confirmed by angiography (within 3 months before inclusion into the study, n = 18) or duplex ultrasonography (at the time of inclusion into the study, n = 21); symptoms of intermittent claudication with no rest pain for at least 12 months; and no major change (≥20%) in pain-free walking distance on two different occasions within 4 weeks before the beginning of the study. Exclusion criteria were the presence of systemic inflammatory, renal or liver diseases, recent progression of PAOD, indication for peripheral angioplasty or bypass surgery, concomitant diseases associated with reduced walking distance (arthrosis, arthritis, diseases of the spinal column, venous diseases, cardiopulmonary insufficiency with or without coronary disease, neurologic diseases) or conditions interfering with a normal conduction of the trial (seriously impaired

cerebral function). All analgesic medication was omitted during the entire study period; all other medication was kept constant during the study. The comedication of the patients is listed in Table 1.

At the time of inclusion into the study, the pain-free and total walking distances were assessed on a walking treadmill (3 km/h, 12% slope). Systemic arterial blood pressure and systolic ankle pressures in the dorsal foot artery and the posterior tibial artery were measured before and after the treadmill test. Blood flow in the femoral artery and femoral artery diameter were determined by duplex ultrasonography at baseline and during the hyperemic reaction induced by 3 min of suprasystolic occlusion using an adequately sized blood pressure cuff around the thigh. A 24 h urine and a venous plasma sample were collected for biochemical analyses.

Thereafter, the patients were randomly assigned to one of three groups. The first group received two intravenous infusions daily of 8 g of L-arginine dissolved in 50 ml of physiologic saline, for 3 weeks (L-arginine group, n = 13). The second group received two intravenous infusions daily of 40 μg of PGE_1 dissolved in 50 ml of physiologic saline, for 3 weeks (PGE_1 group, n = 13). Patients in these two groups were additionally asked to maintain a walking training (three times daily, 15 min of walking up to the onset of claudication pain). Patients in the third group were outpatients; they were asked to maintain the same level of walking exercise for 3 weeks as the first two groups, and did not receive any peripheral vasodilators throughout the study period (control group, n = 13). Patients in the first two groups were blinded as to which of the medications they received, as were the investigators performing the analyses. The walking training was explained to all patients with the help of a formalized instruction sheet, and the training was supervised by one of the investigators once a week. During the entire study period patients were asked to assess claudication-associated pain every day using a subjective analog scale graded from 0 (maximal pain even during rest) to 10 (no pain at all during everyday exercise intensity).

After 1, 2 and 3 weeks of treatment and 6 weeks after the end of the treatment period, the hemodynamic and biochemical measurements were repeated. Plasma samples were always drawn in the morning before the infusions. All investigators performing the treadmill tests and the hemodynamic and biochemical measurements were blinded to treatment assignment.

Hemodynamic measurements. Blood pressure and heart rate were measured by the standard sphygmomanometric method after the patients had rested in the supine position for at least 20 min.

Femoral arterial blood flow velocity and diameter were measured by image-directed duplex ultrasonography in a segment of the superficial femoral artery with a longitudinal cross-section. Measurements were made in the more severely affected leg using a DF 400 image-directed duplex ultrasound system (Diasonics-Sonotron, Cologne, Germany) with a transducer combining 7.5-MHz B-mode imaging and 3-MHz pulsed Doppler beams, as described previously (13), according to the procedure described by Sorensen et al. (17). All studies were performed in a temperature-controlled room (20 to 23°C). The subjects rested in the supine position for 15 min before the first scan, and remained supine until the last recording was finished. The vessel was scanned just below the bifurcation into the superficial and profound femoral arteries, and the transducer was adjusted in the position that produced the clearest images of the anterior and posterior wall of the superficial femoral artery. Vessel diameter was calculated using the electronic caliper of the ultrasound system as the mean value of at least seven repeated measurements. After each determination of vessel diameter, blood flow volume was automatically calculated as the product of the cross-sectional area and the time-averaged blood flow velocity (17,18). Interassay variability was previously determined as 3.2% with our system. The optimal position of the transducer was marked on the skin at the first investigation using a permanent marker, and the same position and scanning orientation were relocated at the following investigations. Hyperemia was induced by inflating an adequately sized blood pressure cuff around the thigh to suprasystolic pressure for 3 min. The scanning position of the ultrasound transducer was fixed throughout this procedure. Immediately after releasing pressure, repeated measurements were performed until blood flow had returned to baseline.

Systolic ankle blood pressures were measured by directional Doppler sonography before and after a constant load treadmill test at a velocity of 3 km/h and a slope of 12%. Systemic arterial blood pressure was measured simultaneously, and the ankle-arm pressure indices were calculated for each leg. Values are given for the clinically predominant leg.

Biochemical analyses. Urinary nitrate excretion was determined as its pentafluorobenzyl derivative by gas chromatography-mass spectrometry, as described previously (13,19). The detection limit of the method was 20 fmol/ml. Intraassay variability was below 3.8%. Cyclic guanosine-3′, 5′-monophosphate (cGMP) concentrations in urine samples were measured by radioimmunoassay using [^{125}I]-cGMP as a tracer after acetylation of the samples, as described previously (13). The detection limit of the assay was 83 fmol/ml. Urinary creatinine was determined spectrophotometrically with the alkaline picric acid method in an automatic analyzer (Beckman, Galway, Ireland). The urinary excretion rates of nitrate and cGMP were corrected by urinary creatinine concentration in order to reduce the variability due to differences in renal excretory function (20).

Plasma L-arginine and dimethylarginine concentrations were determined by high performance liquid chromatography using precolumn derivatization with *o*-phthalaldehyde, as described previously (21). The variability of the method was below 5.5%; the detection limit of the assay was 0.1 μmol/L.

Serum total cholesterol and triglyceride concentrations were measured by standard spectrophotometric methods.

Calculations and statistical analysis. Data are given as mean ± SEM. Statistical significance of treatment effects on pain-free and absolute walking distance and on biochemical parameters was tested using repeated measurements analysis

of variance (ANOVA) followed by the Scheffé F test. Single comparisons between the groups were tested for statistical significance using ANOVA followed by Fisher's protected least-significant difference test. Linear regression curves and correlation coefficients were calculated according to the least squares method. Statistical significance was accepted for $p < 0.05$.

Results

Clinical observations and side effects. Infusion of L-arginine was generally well tolerated by the patients, except in one subject who experienced a mild allergic skin reaction of arms and legs, which was relieved using an oral antihistaminic without interrupting L-arginine treatment. Prostaglandin E_1 infusion caused local pain and skin reddening at the site of the infusion in four patients, which usually relented within 1 h after the end of the infusion; treatment was continued in all patients. In the control group one patient dropped out of the study after week 2 because he was admitted for angioplasty due to an acute worsening of the symptoms of PAOD. None of the patients from the L-arginine or PGE_1 groups had to undergo additional therapy for peripheral vascular disease until at least 6 weeks after the end of the active treatment period.

Walking distances. All patients had similar pain-free and absolute walking distances at entry into the study (51.7 ± 5.1 and 93.2 ± 8.0 m, respectively), and all were graded as Fontaine stage IIb (i.e., absolute walking distance <200 m). Administration of L-arginine resulted in a continuous increase in the pain-free and the absolute walking distances (Fig. 1). After 3 weeks of treatment, the mean pain-free walking distance in this group was 147.2 ± 33.2 m and the absolute walking distance was 216.1 ± 40.3 m (each $p < 0.05$ vs. baseline). A similar therapeutic effect was also induced by intermittent PGE_1 infusions (127.7 ± 24.9 and 199.1 ± 35.4 m for the pain-free and absolute walking distances, respectively; each $p < 0.05$ vs. baseline). There was no significant difference between both active treatments. At the end of the infusion therapy, six patients in the L-arginine group and five patients in the PGE_1 group had absolute walking distances ≥200m, that is, they were now graded as Fontaine stage IIa. In the control group only a slight improvement of the pain-free and the absolute walking distances was observed (65.8 ± 9.0 and 130.4 ± 14.6 m, p = NS), and only one patient was graded as Fontaine stage IIa in this group after 3 weeks. Six weeks after the end of the active treatment period, both the pain-free and the absolute walking distances showed a slight trend to further increase in the L-arginine group (to 176.3 ± 44.6 and 271.7 ± 51.5 m, respectively, n = 8), with five patients graded as Fontaine stage IIa at this time point. In contrast, no further increase of the walking distances was noted in the PGE_1 group (123.6 ± 26.1 and 191.4 ± 26.8 m, n = 11; six patients graded as Fontaine stage IIa) nor in the control group (70.0 ± 10.3 and 146.9 ± 24.3 m, n = 8; two patients graded as Fontaine stage IIa).

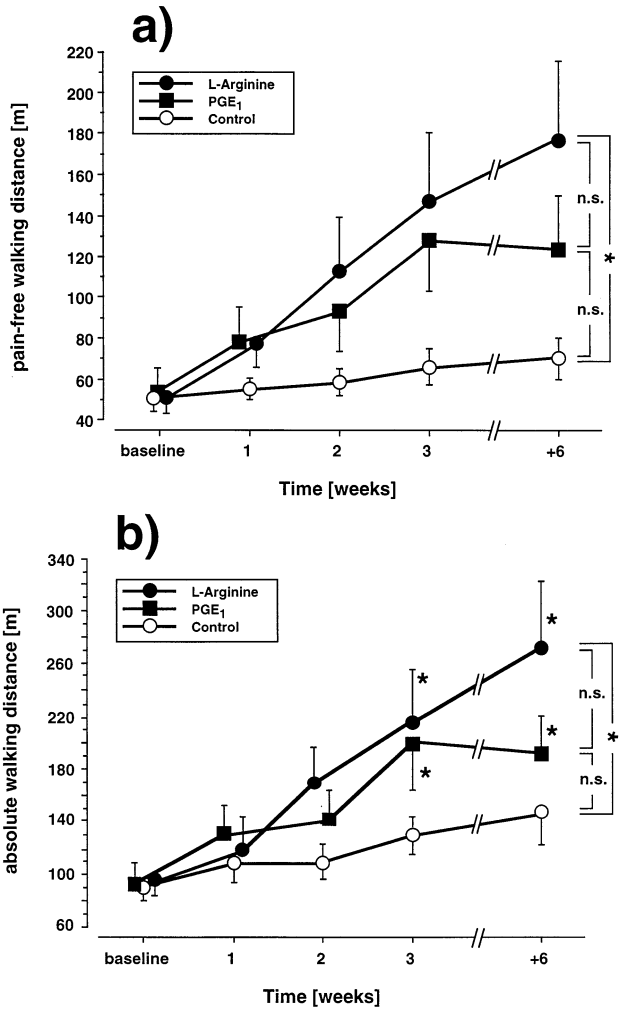

Figure 1. Effect of intravenous infusion therapy with L-arginine or PGE_1 on the pain-free **(a)** and absolute walking distance **(b)** in patients with chronic stable intermittent claudication. Data are mean ± SEM. *$p < 0.05$.

Hemodynamic measurements. There was no significant difference in systemic blood pressures at baseline or during the treatment between any of the groups. At baseline, mean hyperemia-induced femoral artery dilation after 3 min of suprasystolic occlusion was 3.6 ± 0.8%. Flow-mediated femoral artery dilation was progressively increased during intermittent L-arginine infusion therapy, reaching a maximal flow-mediated vasodilation of 7.2 ± 1.1% and 6.9 ± 1.3% at 2 and 3 weeks, respectively ($p < 0.05$ vs. baseline in weeks 2 and 3; Fig. 2a). Six weeks after the end of the active treatment period, flow-mediated vasodilation was still significantly greater than at baseline in this group (5.7 ± 1.1%). In contrast, PGE_1 only slightly enhanced flow-mediated femoral artery dilation (to 4.0 ± 0.7% in week 3, p = NS; Fig. 2b), and in the control group flow-induced dilation was unchanged (Fig. 2c). These differences in flow-induced femoral artery dilation could not be accounted for by differences in basal diameter (L-arginine group, 5.8 ± 0.3 mm; PGE_1 group, 5.8 ± 0.3 mm; control group 5.9 ± 0.2 mm). Despite an

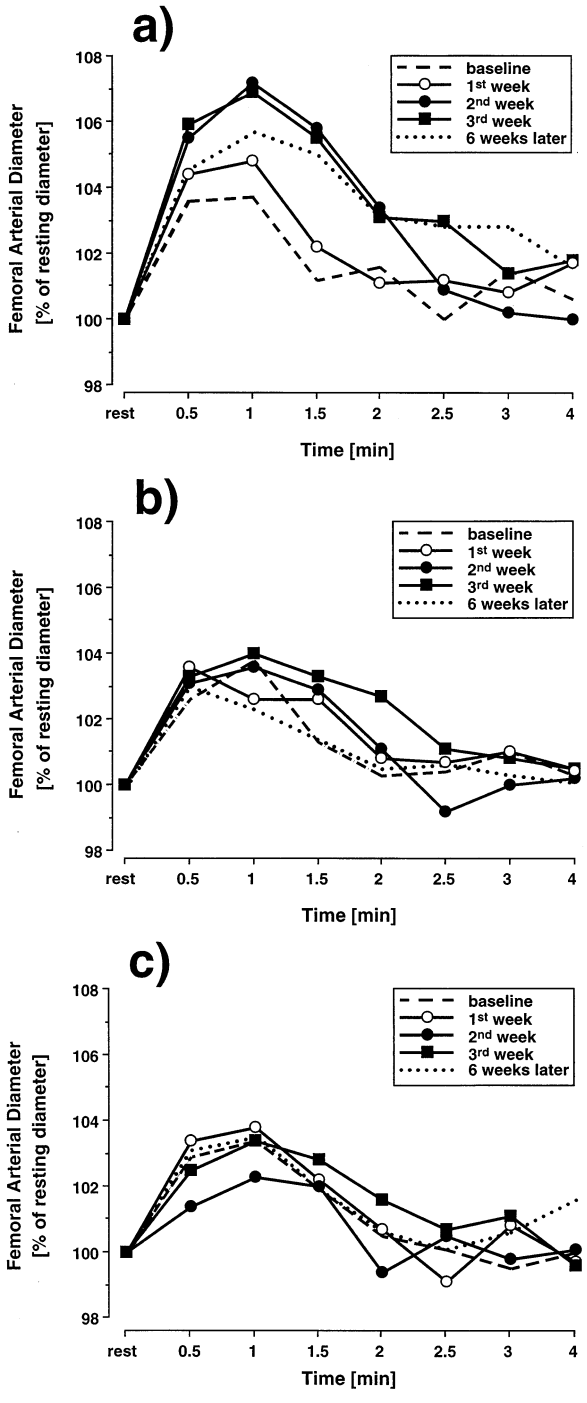

Figure 2. Changes of femoral artery diameter during the hyperemic reaction induced by 3 min of suprasystolic occlusion before, during and after 3 weeks of infusion therapy with L-arginine **(a),** PGE_1 **(b),** or in control patients **(c),** as well as 6 weeks after the end of the active treatment period. Data are mean ± SEM.

unchanged peak femoral arterial flow, L-arginine treatment significantly increased the duration of the hyperemic response, as assessed by the area under the blood flow time curve (area under the curve [AUC]), and by the time elapsing between peak hyperemia and return to 50% of peak flow ($t_{1/2}$; Fig. 3). In the PGE_1 and control groups, no significant changes in peak flow, AUC or $t_{1/2}$ were observed (Fig. 3).

Baseline ankle-arm blood pressure indices were 0.64 ± 0.03 at rest; they decreased to 0.38 ± 0.02 after treadmill exercise (mean difference preexercise vs. postexercise, −39.3 ± 3.4%). In the L-arginine group, ankle-arm blood pressure indices at rest and after exercise increased during treatment; postexercise values in week 3 were 0.48 ± 0.06 and further increased to 0.55 ± 0.07 at 6 weeks. In the PGE_1 group this reaction was similar (postexercise ankle-arm blood pressure index in week 3, 0.50 ± 0.06), but systolic ankle blood pressures showed no further increase 6 weeks after the end of the active treatment period (0.51 ± 0.04). No consistent change was observed in the control group at rest or after exercise.

Pain scale. Self-assessment of the patients on a visual analog pain scale showed that all patients were suffering from severe claudication-associated pain during everyday activities (mean pain rating at baseline, 3.51 ± 0.18 on a scale defined as: maximal pain = 0 and no pain at all = 10). Both active treatments induced a statistically significant increase in the score, indicating an improvement of claudication-associated pain. This increase was more pronounced in the L-arginine group (to 8.3 ± 0.4) than in the PGE_1 group (to 7.0 ± 0.5, $p < 0.05$ between both groups). In the control group a small increase of the score was also observed (to 4.3 ± 0.4), which, however, did not reach statistical significance.

Biochemical analyses. Baseline plasma L-arginine concentration was 90.3 ± 6.2 μmol/L with no significant difference between the groups. Intermittent infusion therapy with L-arginine resulted in about a twofold increase of plasma L-arginine concentrations (Table 2). Neither in the PGE_1 group nor in the control group were any significant changes in plasma L-arginine concentrations observed.

The plasma concentrations of asymmetric dimethylarginine (ADMA) (3.49 ± 0.32 μmol/L; age-adjusted normal range, 1.0 ± 0.1 μmol/L [10]) and symmetric dimethylarginine (2.90 ± 0.44 μmol/L; age-adjusted normal range, 0.8 ± 0.1 μmol/L [10]) were elevated above the normal range at baseline and remained unchanged during the entire study in all three groups (Table 2). However, the elevation of L-arginine plasma concentrations in the L-arginine group resulted in significantly increased L-arginine/ADMA ratios in this group during weeks 1 to 3 (Table 2). There was a significant linear correlation between the L-arginine/ADMA ratio and the pain-free walking distance at baseline ($r = 0.359$, $p < 0.03$); the increase in L-arginine/ADMA ratio during L-arginine treatment was also significantly correlated with the increase in pain-free walking distance in this group ($r = 0.385$, $p < 0.02$).

The urinary excretion rates of nitrate and cGMP were below the normal range at the beginning of the study (nitrate, 120.3 ± 7.4 μmol/mmol creatinine; age-adjusted normal range, 156.0 ± 7.8 μmol/mmol creatinine [10]; cGMP, 109.7 ± 16.2 nmol/mmol creatinine; age-adjusted normal range, 150.0 ± 8.3 nmol/mmol creatinine [10]). Infusion therapy with L-arginine increased urinary nitrate and cGMP excretion rates (each $p < 0.05$ vs. control;

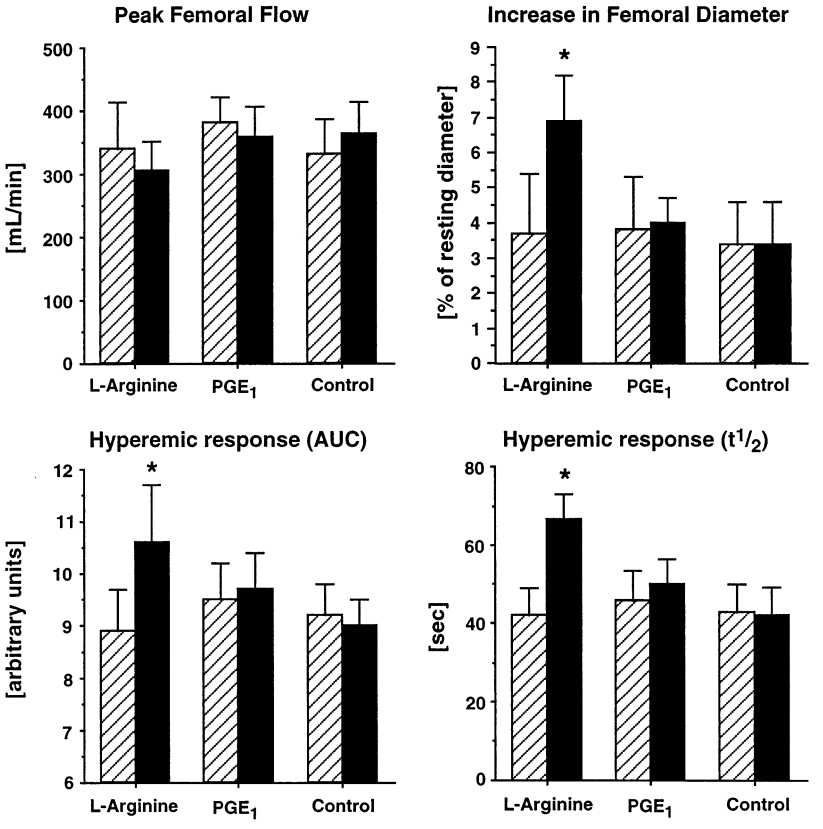

Figure 3. Bar graphs showing peak femoral artery flow (ml/min), increase in femoral artry diameter (as percent of baseline diameter) during reactive hyperemia before and after 3 weeks of intermittent infusion therapy with L-arginine or PGE_1, and in control patients. The area under the curve (AUC, arbitrary units) of femoral artery flow during hyperemia and the time elapsing between peak hyperemia and return to 50% of this peak ($t_{1/2}$, s) are also shown. Data are mean ± SEM. *$p < 0.05$ vs. baseline. **Striped columns** = baseline; **solid columns** = 3 weeks.

$p < 0.05$ for nitrate in L-arginine vs. PGE_1 group; Fig. 4). There was no significant change in urinary nitrate or cGMP excretion rates in either the PGE_1 or in the control group. There was a significant linear correlation between urinary nitrate and cGMP excretion rates ($r = 0.472$, $p < 0.01$). Urinary nitrate excretion rates were significantly correlated with the pain-free ($r = 0.356$, $p < 0.01$) and absolute walking distances ($r = 0.318$, $p < 0.01$).

Plasma total cholesterol and triglyceride levels and plasma creatinine concentrations were not statistically significantly different between either of the groups at any time point.

Discussion

The present study is the first to demonstrate that restoration of vascular NO formation with L-arginine improves the clinical symptoms of atherosclerotic vascular disease in humans. An intermittent intravenous infusion therapy with L-arginine significantly increased the pain-free and absolute walking distances of patients with chronic stable intermittent claudication. This effect was associated with a significant improvement of flow-induced, endothelium-dependent vasodi-

Table 2. Plasma L-Arginine and Dimethylarginine Concentrations

	Group	Baseline	1 Week	2 Weeks	3 Weeks	+6 Weeks
L-arginine (μmol/L)	L-Arginine	83.2 ± 11.6	161.1 ± 50.0*	138.1 ± 31.2*	163.2 ± 35.1*	118.5 ± 28.0
	PGE_1	93.0 ± 12.5	83.8 ± 11.3	95.0 ± 11.7	88.0 ± 11.2	83.9 ± 7.8
	Control	94.8 ± 8.3	83.5 ± 8.1	88.6 ± 9.4	94.5 ± 13.2	95.9 ± 12.2
ADMA (μmol/L)	L-Arginine	3.43 ± 0.37	3.61 ± 0.78	3.14 ± 0.40	3.51 ± 0.44	4.54 ± 0.94
	PGE_1	3.60 ± 0.66	3.49 ± 0.64	5.14 ± 1.07	4.32 ± 0.84	3.80 ± 0.74
	Control	3.43 ± 0.63	2.90 ± 0.43	3.03 ± 0.41	3.45 ± 0.69	3.42 ± 0.64
SDMA (μmol/L)	L-Arginine	2.74 ± 0.98	3.33 ± 1.22	3.20 ± 0.84	4.34 ± 1.98	4.40 ± 1.51
	PGE_1	2.97 ± 0.71	2.94 ± 0.91	3.81 ± 0.78	3.18 ± 0.73	2.84 ± 0.58
	Control	2.98 ± 0.60	2.55 ± 0.44	2.85 ± 0.56	3.06 ± 0.56	3.11 ± 0.53
L-arginine/ADMA ratio	L-Arginine	26.5 ± 4.4	65.0 ± 28.3*	45.4 ± 7.9*	51.7 ± 11.4*	28.9 ± 4.6
	PGE_1	34.5 ± 5.6	32.0 ± 5.7	30.9 ± 7.5	30.7 ± 6.9	33.1 ± 5.9
	Control	34.7 ± 4.6	35.8 ± 5.5	36.5 ± 6.9	37.7 ± 8.4	35.0 ± 7.2

Data are given as mean ± SEM. *$p < 0.05$ vs. baseline. SDMA = symmetric dimethylarginine; other abbreviations as in the text.

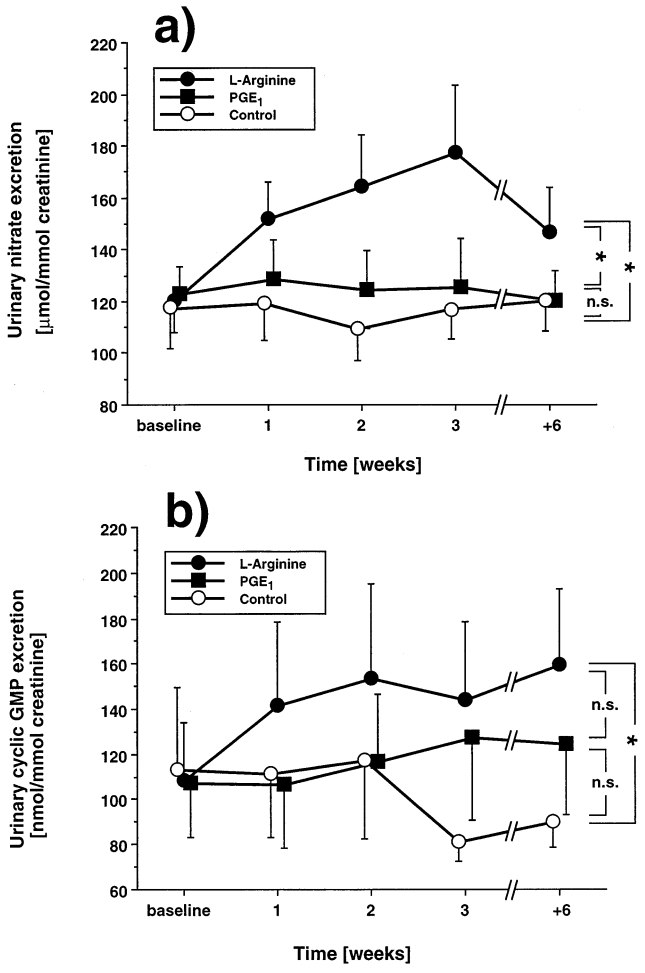

Figure 4. Urinary excretion rates of nitrate **(a)** and cGMP **(b)** in patients with chronic intermittent claudication before, during and after 3 weeks of infusion therapy with L-arginine or PGE_1, and in control patients. Data are mean ± SEM. *$p < 0.05$.

lation in response to ischemia in the diseased leg and with increased urinary NO metabolite excretion rates.

Improvement in walking distance. While recent placebo-controlled studies have shown that intravenous PGE_1 improves claudication distances in PAOD patients (4), the finding that infusion therapy with L-arginine improves the pain-free and absolute walking distances is novel. Treadmill testing is the method of choice to assess the patients' walking distances. A treadmill velocity of ~3 km/h (~2 mph) and a slope of ~12% have been recommended in national and international guidelines to ensure reproducibility and to limit variability of the results obtained (14–16). Treatment with L-arginine and with PGE_1 resulted in a significant increase of both the pain-free and the absolute walking distances. The percent increase over baseline was similar for both drugs during 3 weeks of active treatment (pain-free walking distance: L-arginine, +230 ± 63%; PGE_1, +209 ± 63%; absolute walking distance: L-arginine, +155 ± 48%; PGE_1, +144 ± 28%). It is important to note that the walking distances assessed on a treadmill under the conditions we used are to be multiplied by about three to four to reflect the change of the patients' community-based walking ability (22).

Hemodynamic effects of L-arginine. The mechanism(s) behind this therapeutic effect of L-arginine may be multifactorial. In a previous study we found that a single intravenous infusion of L-arginine increases femoral arterial blood flow in patients with critical limb ischemia (13), suggesting peripheral arteriolar vasodilation. This was paralleled by increased urinary excretion rates of nitrate and cGMP. We could recently corroborate this finding by demonstrating improved nutritive capillary muscular perfusion of the calves in PAOD patients after a single intravenous infusion of 30 g of L-arginine, as measured using ^{15}O-water positron emission tomography (23). Peripheral vasodilation is also achieved with PGE_1 (13), although a beneficial effect of intravenous PGE_1 on nutritive tissue perfusion has not yet been conclusively shown (24). The major difference between both treatments is that L-arginine restores endogenous NO formation rates, which are impaired in atherosclerotic subjects (10), whereas PGE_1 mimicks the effects of prostacyclin.

Nitric oxide, but not prostacyclin, is the principal mediator of flow-induced vasodilation of human peripheral conductance arteries (25). Joannides et al. (25) reported that infusion of the NO synthase inhibitor N^G-monomethyl-L-arginine reduced flow-induced brachial artery vasodilation during hyperemia as well as the duration of the hyperemic response, but not the peak increase in hyperemic blood flow in healthy humans. In the present study we observed the opposite effects during chronic intermittent intravenous L-arginine therapy. At baseline, flow-induced femoral vasodilation was reduced in the femoral artery of PAOD patients, as previously observed by Cox et al. (26) and Nabel et al. (27) in atherosclerotic human coronary arteries. Despite no significant change in peak hyperemic blood flow, the duration of hyperemia (AUC and $t_{1/2}$ of the hyperemic reaction) was markedly increased after 3 weeks of L-arginine therapy, as was the flow-induced vasodilator response. Neither PGE_1 infusion nor exercise alone had a significant effect on flow-induced vasodilation.

The increased flow-induced vasodilator response after L-arginine therapy may indicate an improved ability of the peripheral circulation to adapt to changes in nutritive demand in skeletal muscle, which was also reflected by the higher increase in postexercise ankle-arm blood pressure ratios in this group as compared with PGE_1 and controls. These results suggest that the known beneficial effects of short-term L-arginine infusion on endothelium-mediated vasodilation (9,12) persist during longer-term intermittent infusion therapy and result in improved peripheral blood flow at rest and during exercise in patients with generalized atherosclerosis. A recent study Rector et al. (28) reported that dietary supplementation of patients with heart failure with L-arginine during 6 weeks not only improved endothelium-mediated vasodilation, but also increased exercise capacity and functional status of these patients.

Improved peripheral blood flow also may be mirrored by increased systolic ankle blood pressure. Ankle-arm blood

pressure indices have been found to be an independent measure of the mortality risk of PAOD patients (29). In the present study, both L-arginine and PGE_1 increased resting and postexercise ankle-arm blood pressure indices. This effect was greater in the L-arginine group; however, due to the small group size the difference between both treatments did not achieve statistical significance. Experimental data suggest that improved collateral perfusion may be involved in the increased peripheral blood supply after the infusion of L-arginine (30) as well as PGE_1 (31). Six weeks after the end of active treatment, a slight tendency of walking distances and ankle-arm blood pressure indices was noted to further increase in the L-arginine group. No such tendency was observed in the PGE_1 group. These differences were not statistically significant and variability was relatively high. However, it may be speculated that other than direct hemodynamic effects of L-arginine may have contributed to improved clinical outcome. Induction of collateral growth by L-arginine has been demonstrated in rabbits (32); it may also take place in atherosclerotic humans treated with L-arginine. Collateral perfusion would still be present after the end of intermittent intravenous L-arginine administration. Whether such effects contribute to L-arginine's effects in human vascular disease remains to be established.

Limitations of the study. One limitation of our study is the lack of a placebo group. We compared the effects of L-arginine with those of PGE_1, which is the current approved standard therapy for patients with limb ischemia in our country, where many patients with severe claudication are treated as inpatients to receive a twice-daily infusion regimen of intravenous PGE_1. To eliminate any potential differences between both groups due to incomplete absorption, L-arginine was also administered as a twice-daily intravenous infusion regimen. Prostaglandin E_1 has been shown previously to induce superior clinical benefit in patients with intermittent claudication as compared with placebo. In a recently published study, intravenous PGE_1 improved the claudication distance of PAOD patients significantly more than placebo (4). Moreover, PGE_1 has been shown to exert a significant additive beneficial effect during vascular training in patients with PAOD Fontaine stage IIb (5). In the present study a third group of patients without any vasoactive medication was studied as a control group. Both hemodynamically active treatments had superior effects as compared with vascular training alone, which caused only insignificant increases in the pain-free and absolute walking distances by 34 ± 7% and 49 ± 16%, respectively. These rates of improvement are within the range of changes induced by exercise programs reported previously by others (for reviews, see references 16 and 33). Although all patients in the three groups were instructed about vascular training with the help of a formalized instruction sheet and training was supervised once a week in each group, we cannot completely exclude the possibility that patients in the L-arginine and PGE_1 groups may have adhered more closely to the training program, and that better compliance may have contributed to better clinical outcome in these groups.

L-Arginine administration and vascular NO production. We have recently reported that NO formation rate is significantly reduced in PAOD patients in a manner related to the severity of the atherosclerotic disease (10). These data, which were obtained using the urinary excretion rates of nitrate and cGMP as noninvasive indices of systemic NO production, corresponded well to the impaired endothelium-dependent vasodilator response in patients with cardiovascular disease previously reported by others (34,35). Moreover, we found that accumulation of ADMA, an endogenous inhibitor of NO synthesis (36), might be one causal factor for reduced NO synthase activity (10). Increased ADMA concentrations result in a reduced L-arginine/ADMA ratio, which may point to reduced NO synthase substrate availability in atherosclerosis and explain at least in part impaired NO elaboration in this disease (37). The present study confirms the presence of increased ADMA plasma concentrations in PAOD patients, concomitantly with reduced L-arginine/ADMA ratio and low urinary nitrate and cGMP excretion rates. L-Arginine infusion restored this ratio to normal and increased the urinary excretion rates of nitrate and cGMP.

Under conditions of impaired substrate availability, the endothelial NO synthase may undergo structural disarrangement, resulting in the conversion of this enzyme from an NO synthase into a generator of superoxide anion in ischemic rabbit hindlimb skeletal muscle (38,39). This effect is antagonized by excess L-arginine (38). Indeed, we have previously demonstrated that chronic treatment with L-arginine prevents vascular superoxide radical generation in cholesterol-fed rabbit aorta (8), an effect in which elevated levels of ADMA may be involved (37). Decreased vascular oxidative stress may have contributed to improved endothelial function in the L-arginine group of the present study.

Other potential mechanisms of action of L-arginine. Other mechanisms than hemodynamic mechanisms related to the biological activity of NO may also have contributed to the beneficial therapeutic effects of L-arginine in this study. Nitric oxide is a known inhibitor of platelet activity (40). L-Arginine also inhibits monocyte adhesion to the vascular endothelium (41) and reduces vascular oxidative stress (8,42). Moreover, L-arginine has been reported to exert analgesic effects (43), whereas PGE_1 itself induced pain and an inflammatory reaction at the site of the infusion in one-third of our patients. No such effects were observed for L-arginine, except for one case of a mild allergic skin reaction. L-Arginine has previously been reported to cause allergic reactions (44), but in general the tolerability of L-arginine has been reported to be good (45).

In conclusion, our present study provides evidence that chronic intermittent infusion therapy with L-arginine restores systemic endogenous NO formation and concomitantly improves the clinical symptoms of patients suffering from severe intermittent claudication due to atherosclerotic peripheral arterial disease. Restoration of endogenous NO production by L-arginine may therefore be a novel approach in the therapy of atherosclerotic vascular disease.

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References

1. Coffman JD. Intermittent claudication: not so benign. Am Heart J 1986;112: 1127–8.
2. Förstermann U, Mügge A, Alheid U, Haverich A, Frölich JC. Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. Circ Res 1988;62:185–90.
3. Cohen RA. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. Prog Cardiovasc Dis 1995;38:105–28.
4. Diehm C, Balzer K, Bisler H, et al. Efficacy of a new prostaglandin E_1 regimen in outpatients with severe intermittent claudication: results of a multicenter placebo-controlled double-blind trial. J Vasc Surg 1997;25:537–44.
5. Scheffler P, de la Hamette D, Leipnitz G. Therapeutic efficacy of intravenously applied prostaglandin E_1. Vasa 1989;28 Suppl:19–25.
6. Moncada S, Higgs EA. The L-arginine—nitric oxide pathway. N Engl J Med 1993;329:2002–12.
7. Cooke JP, Andon NA, Girerd XJ, Hirsch AT, Creager MA. Arginine restores cholinergic relaxation of hypercholesterolemic rabbit thoracic aorta. Circulation 1991;83:1057–62.
8. Böger RH, Bode-Böger SM, Mügge A, et al. Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis 1995;117:273–84.
9. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 1991;338:1546–50.
10. Böger RH, Bode-Böger SM, Thiele W, Junker W, Alexander K, Frölich JC. Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. Circulation 1997;95:2068–74.
11. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherosclerotic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 1992;90:1168–72.
12. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP. L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. J Clin Invest 1992;90:1248–53.
13. Bode-Böger SM, Böger RH, Alfke H, et al. L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. Circulation 1996;93:85–90.
14. European Working Group on Critical Limb Ischemia. Second European Consensus Document on Chronic Critical Limb Ischemia. Circulation 1991;84 Suppl IV:1–26.
15. Heidrich H, Allenberg J, Cachovan M, et al. Guidelines for therapeutic studies on peripheral arterial occlusive disease in Fontaine stages II–IV. Vasa 1992;21:339–43.
16. Hiatt WR, Hirsch AT, Regensteiner JG, Brass EP. Clinical trials for claudication. Assessment of exercise performance, functional status, and clinical end points. Circulation 1995;92:614–21.
17. Sorensen KE, Celermajer DS, Spiegelthaler DJ, et al. Non-invasive measurement of human endothelium-dependent arterial responses: accuracy and reproducibility. Br Heart J 1995;74:247–53.
18. Ranke C, Hendrickx P, Roth U, Brassel F, Creutzig A, Alexander K. Color and conventional image-directed Doppler ultrasonography: accuracy and sources of error in quantitative blood flow measurements. J Clin Ultrasound 1992;20:187–93.
19. Tsikas D, Böger RH, Bode-Böger SM, Gutzki FM, Frölich JC. Quantification of nitrite and nitrate in human urine and plasma as pentafluorobenzyl derivatives by gas chromatography-mass spectrometry using their ^{15}N-labelled analogs. J Chromatogr B 1994;661:185–91.
20. Böger RH, Bode-Böger SM, Gerecke U, Gutzki FM, Tsikas D, Frölich JC. Urinary NO_3^- excretion as an indicator of nitric oxide formation in vivo during oral administration of L-arginine or L-NAME in rats. Clin Exp Pharmacol Physiol 1996;23:11–5.
21. Bode-Böger SM, Böger RH, Kienke S, Junker W, Frölich JC. Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Commun 1996;219:598–603.
22. Lindgärde F, Labs KH, Rößner M. The pentoxifylline experience: exercise testing reconsidered. Vasc Med 1996;1:145–54.
23. Schellong SM, Böger RH, Burchert W, et al. Dose-related effect of intravenous L-arginine on muscular blood flow of the calf in patients with peripheral vascular disease. A ^{15}O-water positron emission tomography study. Clin Sci 1997;93:159–65.
24. Schellong SM, Burchert W, Böger RH, Creutzig A, Hundeshagen H, Alexander K. Prostaglandin E_1 in peripheral vascular disease. A PET-study of muscular blood flow. Scand J Clin Lab Invest 1998;58:109–17.
25. Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314–9.
26. Cox DA, Vita JA, Treasure CB, et al. Atherosclerosis impairs flow-mediated dilation of coronary arteries in humans. Circulation 1989;80:458–65.
27. Nabel EG, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. J Am Coll Cardiol 1990;16:349–56.
28. Rector TS, Bank AJ, Mullen KA, et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. Circulation 1996;93:2135–41.
29. McKenna M, Wolfson S, Kuller L. The ratio of ankle and arm blood pressure as an independent predictor of mortality. Atherosclerosis 1991;87:119–28.
30. Randall MD, Ujiie H, Griffith TM. L-arginine reverses the impairment of nitric oxide-dependent collateral perfusion in dietary-induced hypercholesterolemia in the rabbit. Clin Sci 1994;87:53–9.
31. Rudofsky G, Gerngroß U. Effect of prostaglandin E_1 on collateral vessels in peripheral occlusive vascular disease. Progr Clin Biol Res 1989;301:407–9.
32. Randall MD, Griffith TM. EDRF plays a central role in collateral flow after arterial occlusion in rabbit ear. Am J Physiol 1992;263:H752–60.
33. Gardner AW, Poehlman ET. Exercise rehabilitation programs for the treatment of claudication pain. JAMA 1995;274:975–80.
34. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–7.
35. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol 1994;24:1468–74.
36. Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992;20 Suppl 12:60–2.
37. Böger RH, Bode-Böger SM, Brandes RP, et al. Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation 1997;96:1282–90.
38. Huk I, Nanobashvili J, Neumayer J, et al. L-arginine treatment alters the kinetics of nitric oxide and superoxide release and reduces ischemia/reperfusion injury in skeletal muscle. Circulation 1997;96:667–75.
39. Cooke JP, Dzau VJ. Derangements of the nitric oxide synthase pathway, L-arginine, and cardiovascular diseases. Circulation 1997;96:379–82.
40. Radomski MW, Palmer RMJ, Moncada S. Characterization of the L-arginine: nitric oxide pathway in human platelets. Br J Pharmacol 1987;101: 325–8.
41. Theilmeier G, Chan JR, Zalpour C, et al. Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine. Arterioscler Thromb Vasc Biol 1997;17:3557–64.
42. Candipan RC, Wang B, Buitrago P, Tsao PS, Cooke JP. Regression or progression. Dependency on nitric oxide. Arterioscler Thromb Vasc Biol 1996;16:44–50.
43. Harima A, Shimizu H, Takagi H. Analgesic effect of L-arginine in patients with persistent pain. Eur Neuropsychopharmacol 1991;1:529–33.
44. Tiwary CM, Rosenbloom AL, Julius RL. Anaphylactic reaction to arginine infusion (letter). N Engl J Med 1973;288:218.
45. Walter JH, Leonhard V. Inborn errors of the urea cycle. Br J Hosp Med 1987;38:136–83.