

Does Acute Improvement of Endothelial Dysfunction in Coronary Artery Disease Improve Myocardial Ischemia?

A Double-Blind Comparison of Parenteral D- and L-Arginine

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Objectives. Parenteral L-arginine will improve myocardial ischemia in patients with obstructive coronary artery disease.

Background. Endothelial dysfunction causes coronary arterial constriction during stress, and L-arginine improves endothelial dysfunction.

Methods. Twenty-two patients with stable coronary artery disease and exercise-induced ST-segment depression underwent assessment of forearm endothelial function with acetylcholine and symptom-limited treadmill exercise testing during dextrose 5% infusion and after double-blind intravenous administration of L- and D-arginine (5 mg/kg/min) for 20 min.

Results. Forearm blood flow increased with both L- and D-arginine ($33\% \pm 6\%$ and $38\% \pm 7\%$, respectively, $p < 0.001$). Acetylcholine-mediated forearm vasodilation also improved with both L- and D-arginine ($p < 0.0001$). The magnitude of improvement was similar with both enantiomers and was observed in patients throughout the range of acetylcholine responses and cholesterol levels.

Heart rate and blood pressure at rest and during each stage of exercise and exercise duration remained unchanged with L- and D-arginine compared to control. Ischemic threshold, measured either as the rate-pressure product or the duration of exercise at the onset of 1-mm ST-segment depression during exercise, also remained unchanged. Serum arginine, insulin and prolactin levels ($p < 0.01$) increased with both enantiomers.

Conclusions. Parenteral arginine produces non-stereo-specific peripheral vasodilation and improves endothelium-dependent vasodilation in patients with stable coronary artery disease by stimulation of insulin-dependent nitric oxide release or by non-enzymatic nitric oxide generation. Despite enhanced endothelial function, there was no improvement in myocardial ischemia during stress with either enantiomer. Whether parenteral arginine will be of therapeutic benefit in acute coronary syndromes and oral arginine in myocardial ischemia needs to be studied further.

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Blood vessel tone is modulated by balanced actions of vasoconstricting and dilating compounds that are either circulating or locally synthesized in the vascular wall. Predominance of vasoconstrictor influences, especially in the presence of atherosclerotic coronary arterial narrowing, leads to further restriction of myocardial blood flow and exacerbation of ischemia (1). Important vasoconstrictors include catecholamines, angiotensin II and endothelin, and their action appears to be counterbalanced by locally generated endothelium-derived vasodilators such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor. Nitric oxide, considered to be the predominant endothelium-derived relaxing factor, is constitutively synthesized by the action of a calcium-calmodulin-sensitive enzyme NO synthase on L-arginine (2). Nitric oxide diffuses lumenally into the blood stream and

inhibits platelet aggregation and adhesion, and penetrates abluminally into the vascular smooth muscle to cause vasodilation. Nitric oxide activity is impaired in the coronary and peripheral vasculature of patients with atherosclerosis and those with risk factors for atherosclerosis (3-8). This abnormality is associated with paradoxical vasoconstriction or failure of appropriate vasodilation of blood vessels in response to physiologic stress (9-12) that in turn may exacerbate myocardial ischemia in patients with obstructive coronary artery disease. Therapies designed to improve NO bioavailability would therefore be expected to increase vascular dilator tone at rest and during exercise and thereby ameliorate myocardial ischemia.

One strategy widely tested in recent years to improve vascular NO activity is the replenishment of L-arginine, the substrate for NO synthesis. L-arginine administration improved abnormal endothelium-dependent vasodilation in hypercholesterolemia (13-15). Intraarterial L-arginine also improved abnormal endothelium-dependent responses in the coronary vasculature of patients with atherosclerosis and inhibited the abnormal coronary constriction observed with pacing (16-18). A recent report demonstrated vasodilation of coronary stenoses with intracoronary L-arginine (19). On the

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basis of these observations, we hypothesized that parenteral L-arginine, by improving NO bioavailability, will improve myocardial ischemia in patients with obstructive atherosclerotic coronary artery disease by abrogating coronary constriction during stress.

Methods

Patients. We studied 22 patients, 19 men and 3 women (mean age 63.5 ± 2 years) with stable symptoms, angiographically documented coronary artery disease and exercise-induced ST-segment depression. Patients had undergone previous treadmill exercise testing on at least two or more occasions and had demonstrable ischemia on radionuclide testing. Mean serum cholesterol was 243 ± 12 mg/dl, seven patients were hypertensive, six diabetic and four were current smokers or had smoked in the past year. None had suffered from unstable angina or myocardial infarction in the 3-month period before the study, and all patients were free of symptoms of heart failure. Patients gave informed consent and the protocol was approved by the Investigational Review Board of the National Heart, Lung and Blood Institute.

Protocol. On the first day, an intraarterial cannula was introduced under local anesthesia in the brachial artery of the nondominant arm. After 1-h rest, dextrose 5% (D5W) was given intravenously at 2 ml/min and patients were exercised on the treadmill, employing the National Institutes of Health Combined protocol (20). Blood pressure, heart rate and a 12-lead electrocardiogram were recorded every 30 s to accurately determine the ischemic threshold (heart rate and blood pressure at the onset of 1-mm ST-segment depression). Exercise was terminated for moderate intensity chest pain, limiting dyspnea, ≥ 3 mm ST-segment depression, hypertension or excessive fatigue.

After a 3-h recovery period on day 1, patients had determination of forearm endothelial function with intraarterial acetylcholine (ACH). This was followed by a double-blind randomized intravenous infusion of either L-arginine or D-arginine (Sigma Chemical) at 5 mg/kg/min for 20 min. In our preliminary experience, the infusion of 10 mg/kg/min arginine caused nausea in some patients. After 20 min of arginine infusion, determination of forearm endothelial function using intraarterial ACH was repeated. After this and approximately 30 min after commencing arginine infusion, treadmill exercise testing was performed and the infusion was terminated after the end of exercise.

The following day, repeat measurement of endothelial function was performed with intraarterial ACH and these measurements were repeated 20 min after either intravenous L-arginine or D-arginine. At the end of these measurements treadmill exercise testing was repeated while continuing infusion of intravenous arginine, as on the previous day.

Measurement of forearm endothelial function. All studies were performed in a quiet room with a temperature of approximately 22°C, as previously described (21,22). Patients were asked to refrain from drinking alcohol or beverages

containing caffeine and from smoking for ≥ 24 h before studies. A needle was inserted in the brachial artery of the nondominant arm that was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed on the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, D. E. Hokanson, Issaquah, Washington) calibrated to measure the percent change in volume; the plethysmograph in turn was connected to a chart recorder to record the forearm flow measurements. For each measurement, a cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, Hokanson) to occlude venous outflow from the extremity. A wrist cuff was inflated to suprasystolic pressures 1 min before each measurement to exclude hand circulation. Flow measurements were recorded for approximately 7 s every 15 s; 7 readings were obtained for each mean value.

Basal measurements were obtained during intraarterial infusion of 5% dextrose solution at 1 ml/min. Forearm flows were then measured after the infusion of intraarterial ACH at 30 and 60 $\mu\text{g}/\text{min}$. Each dose was infused for 5 min and forearm flow was measured during the last 2 min of the infusion. Measurements were repeated 20 min after intravenous L-arginine or D-arginine on both days.

Plasma arginine, insulin, prolactin and glucose measurements. Before the study and after 20-min infusions of L- and D-arginine, blood sampling was performed in 10 patients to measure serum glucose, prolactin, insulin and arginine levels.

Statistical analysis. Data are expressed as mean \pm SEM. The difference between means was tested with paired or unpaired Student's *t* test as appropriate. All tests were two-sided and a $p < 0.05$ was considered statistically significant. The hemodynamic changes during exercise and the dose response curves with ACH before and after L- and D-arginine were compared using a two-way repeated measures analysis of variance (ANOVA) (23) with appropriate interaction terms.

Results

Effects of intravenous D- and L-arginine on endothelial function. Figure 1 demonstrates the effect of both arginine enantiomers on forearm vascular endothelial function. Forearm vasodilation occurred with both intravenous L- and D-arginine; compared to baseline, forearm blood flow increased by $33\% \pm 6\%$ with L-arginine and $38\% \pm 7\%$ with D-arginine, and forearm vascular resistance decreased by $28\% \pm 5\%$ and $21\% \pm 5\%$, respectively (all $p < 0.001$). There was no difference between the magnitude of vasodilation with D- compared to L-arginine. Mean arterial pressure remained unchanged with both drugs; $-2\% \pm 1\%$ ($p = 0.1$) change with L-arginine and $2\% \pm 1\%$ ($p = 0.3$) change with D-arginine.

Acetylcholine produced progressive forearm microvascular dilation that was enhanced by both L-arginine ($p < 0.0001$, ANOVA) and D-arginine ($p < 0.0001$, ANOVA) (Fig. 1); thus, at the peak dose of ACH, forearm blood flow increased from 17.4 ± 2.2 ml/min/100 ml to 21.5 ± 2.3 ml/min/100 ml ($p = 0.01$) with L-arginine and from 14.4 ± 1.9 to 19.4 ± 2.4

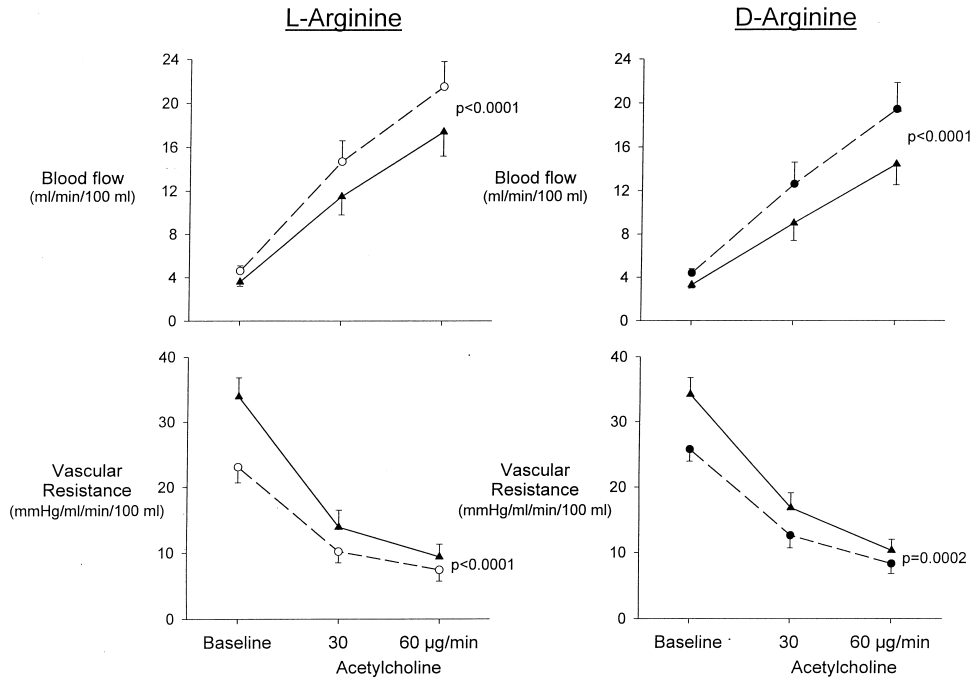


Figure 1. Forearm blood flow and vascular resistance changes with intraarterial ACH before and after L- and D-arginine. p values represent two-way repeated measures ANOVA.

ml/min/100 ml ($p < 0.001$) with D-arginine. Because of the change in resting blood flow with L- and D-arginine, we also calculated the flow increment, compared to baseline, with ACH before and after arginine (Fig. 2). This analysis demonstrated a significant further enhancement (above resting levels) in ACH-induced vasodilation with both L- and D-arginine. There was no change in arterial pressure during administration of ACH either before or after L-arginine or D-arginine.

Effects of intravenous D- and L-arginine on exercise hemodynamics. There was no alteration in heart rate, systolic, diastolic or mean arterial pressures with either L-arginine or D-arginine at rest (Fig. 3). During treadmill exercise there was also no significant change in heart rate, systolic blood pressure or rate-pressure product achieved at each stage during D5W, L-arginine or D-arginine administration (Fig. 3). Peak exercise duration remained unchanged, and the heart rate, blood pressure and rate-pressure product achieved at peak exercise were unchanged during D- and L-arginine therapy and D5W administration (Fig. 4). Finally, there was no significant change in the duration of exercise or heart rate and blood pressure at

1-mm ST-segment depression during L-arginine or D-arginine therapy compared to control exercise with D5W.

Subgroup analysis. Previous studies have demonstrated improvement in peripheral vascular endothelial function with L-arginine in subjects with hypercholesterolemia and in those with more severe coronary vascular endothelial dysfunction (21,23). We therefore analyzed two subgroups, those with more severe vascular endothelial dysfunction and patients with hypercholesterolemia, to investigate whether arginine was of greater benefit in these subsets.

Patients were divided into two equal-sized groups with either “depressed” or “normal” endothelial function based on that peak response to ACH. Both groups of patients had improvement of ACH-mediated vasodilation with L- and D-arginine (Fig. 5). During treadmill exercise testing, there was no change in the ischemic threshold or hemodynamics at peak exercise with L-arginine or D-arginine compared to D5W in either patients with depressed or normal endothelial function (Fig. 5).

Finally, responses in patients with hypercholesterolemia

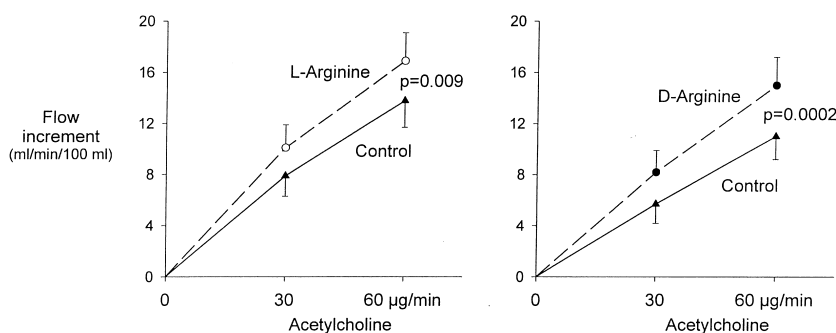
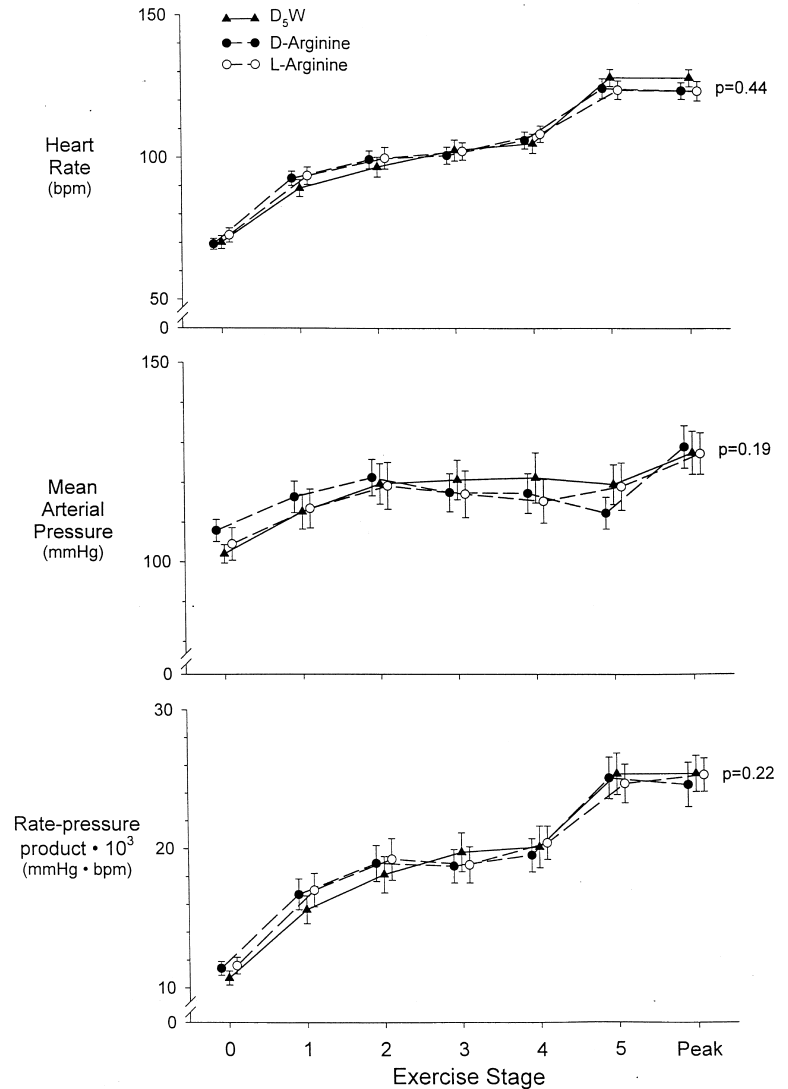


Figure 2. Increase in forearm blood flow with ACH during dextrose infusion (control) and after L-arginine and D-arginine. The flow increment beyond baseline changes observed with both arginine enantiomers is shown. p values represent two-way repeated measures ANOVA.

Figure 3. Changes in heart rate, mean arterial blood pressure and rate-pressure product during exercise with intravenous dextrose 5% (D5W), L-arginine or D-arginine. p values represent two way repeated measures ANOVA.



(total cholesterol >250 mg/dl, mean 293 ± 10 , $n = 11$) were compared to those with lower serum cholesterol levels (<250 mg/dl, mean 193 ± 7 , $n = 11$). Both hypercholesterolemic and normocholesterolemic patients had improvement of ACH-mediated forearm vasodilation with L- and D-arginine, with no difference in the magnitude of improvement between the two arginine enantiomers in either subgroup ($p = 0.38$ in normocholesterolemic and $p = 0.34$ in hypercholesterolemic patients, D- vs. L-arginine). There was no change in the ischemic threshold or hemodynamics at peak exercise with either L-arginine or D-arginine compared to D5W either in patients with or in patients without hypercholesterolemia.

Serum arginine, prolactin, glucose and insulin levels (Table 1). Serum arginine level increased from 17.9 ± 2 to $406 \pm 64 \mu\text{mol/dl}$ ($p < 0.001$) with L-arginine administration for 30 min and from 16.5 ± 4 to $519 \pm 64 \mu\text{mol/dl}$ ($p < 0.001$) with intravenous D-arginine. This was associated with a simultaneous increase in serum prolactin and insulin levels with both L- and D-arginine. There was no significant difference between

the magnitude of increase in serum insulin ($85\% \pm 22\%$ vs. $108\% \pm 35\%$) or prolactin ($207\% \pm 29\%$ vs. $191\% \pm 49\%$) with L-arginine or D-arginine, respectively. Glucose levels remained unchanged during both infusions (Table 1).

Discussion

Our study demonstrates that both enantiomers of arginine, when administered intravenously to patients with atherosclerotic coronary artery disease, produce forearm vasodilation at rest and enhance endothelium-dependent dilation in response to ACH. Despite improvement of vascular function at rest and with pharmacologic stimulation, there is no significant amelioration of myocardial ischemia during exercise.

Arginine and endothelial function. Demonstration of enhanced NO production from the vasculature of hypercholesterolemic and atherosclerotic animals with endothelial dysfunction has been a stimulus for using L-arginine in human studies (13,14). Peripheral arteriolar dilation in response to

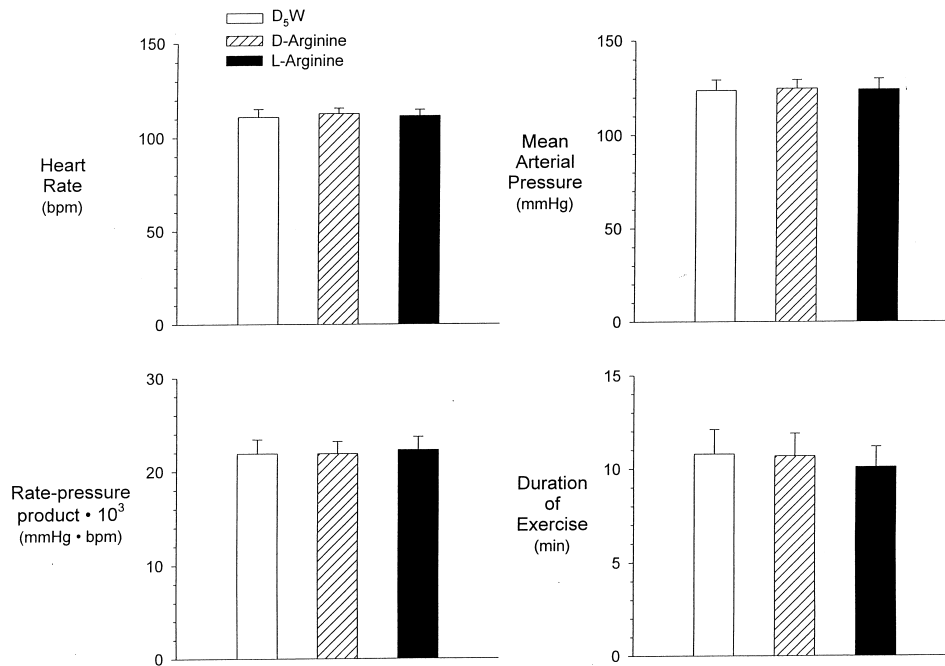


Figure 4. The heart rate, mean arterial blood pressure, rate-pressure product and the duration of exercise at 1-mm ST-segment depression during treadmill exercise testing with dextrose 5% (D₅W), L-arginine and D-arginine. There were no significant differences between the treatments by two-way repeated measures ANOVA.

ACH improved with intraarterial L-arginine but not D-arginine in normal subjects, but not in those with hypertension, hypercholesterolemia or atherosclerosis (21,24–27). In contrast, intravenous L-arginine but not D-arginine improved forearm dilation in hypercholesterolemic subjects in response to methacholine (14). In the coronary vasculature, improvement in ACH responses in hypercholesterolemic and atherosclerotic patients with intraarterial L-arginine could not be reproduced with intraarterial D-arginine (16–18). Finally, oral arginine also improved brachial artery flow-mediated dilation, a surrogate measure of endothelial cell integrity in hypercholesterolemic subjects, but not in normal individuals (28). Taken together, these studies suggest that the route of administration of arginine, the vascular bed and the specific causes leading to endothelial dysfunction may, to some extent, influence the response of arginine *in vivo*.

It is noteworthy that non-stereo-specific arteriolar and venous dilation accompanied by hypotension occurs in normal subjects at high parenteral concentrations of both L- and D-arginine (29–31). We observed peripheral vasodilation at somewhat lower concentration in patients with atherosclerosis in whom blood pressure remained unchanged, suggesting that either reflex constriction occurred in other vascular beds or there was a simultaneous increase in cardiac output (30). Of interest, heart rate also remained unchanged, indicating that there was no systemic compensation for the mild peripheral vasodilation observed with arginine. Surprisingly, both L- and D-arginine produced enhancement of ACH-mediated forearm vasodilation to a similar extent, a finding present in patients throughout the range of responses to ACH. This contrasts with previous observations where intravenous L-arginine but not D-arginine improved methacholine responses in hypercholes-

terolemic patients (14). These differences might be explained either because the serum arginine levels were lower than those achieved in our study, or fewer patients were studied with D-arginine or methacholine and not ACH was used as a probe for testing endothelial function in that study.

Mechanisms underlying arginine-mediated improvement in endothelial function. L-arginine but not D-arginine is a substrate for NO synthesis. L-arginine is catalyzed by the action of NO synthase to yield NO and L-citrulline (32). The K_m for L-arginine in normal endothelial cells (2.9 $\mu\text{mol/liter}$) is several magnitudes lower than the intracellular concentrations of L-arginine (0.8 to 2 mmol/liter), which makes it unlikely that substrate deficiency is limiting for NO synthesis (33). Indeed, *in vitro*, improvement with L-arginine can only be seen when its levels are depleted (34). In contrast, parenteral arginine *in vivo* increases vascular NO activity (29,30). Although it is believed that endothelial dysfunction in hypercholesterolemia, diabetes and atherosclerosis is due to inactivation of NO by oxygen-free radicals, it is unlikely that substrate limitation occurs even in these circumstances of increased NO turnover (35). Furthermore, were this to be the mechanism, only L-arginine but not D-arginine would be expected to produce improvement of the ACH response.

There are several other proposed mechanisms whereby L-arginine may improve endothelial function. These include: 1) reversal of inhibitory effects of L-glutamine on L-arginine bioavailability (34), 2) counteracting the inhibitory effects of asymmetric dimethyl arginine (ADMA), a naturally occurring inhibitor of L-arginine (36–38), 3) its anti-oxidant actions (38,39), 4) stimulation of insulin release (40–44) and 5) nonenzymatic and non-stereo-specific generation of NO by arginine (45).

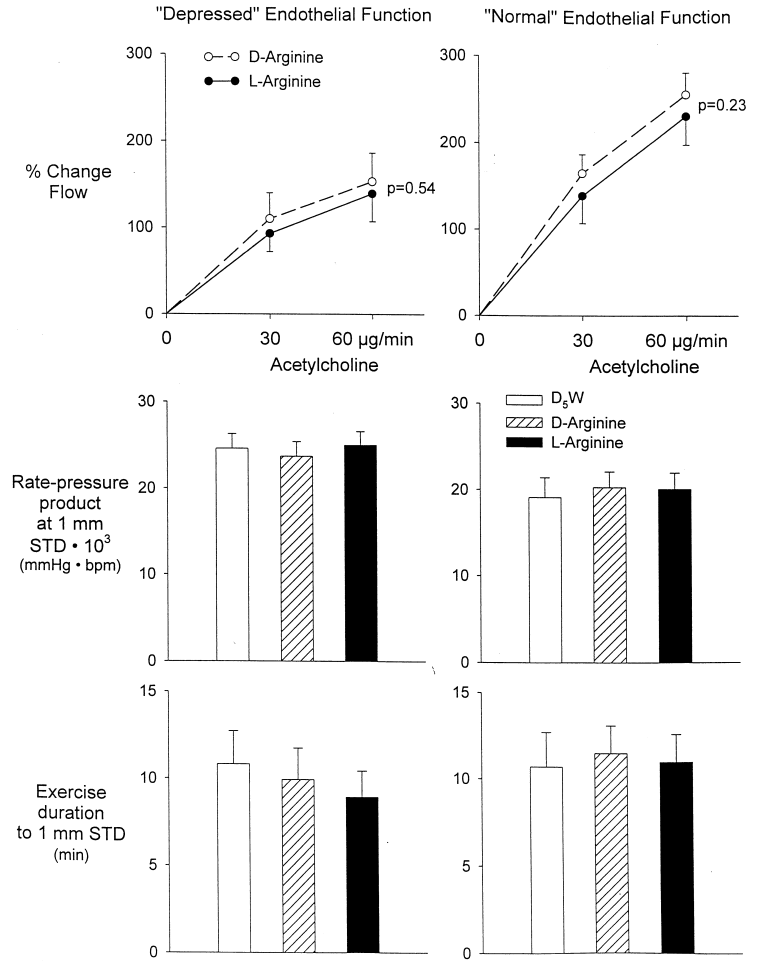


Figure 5. The increase in forearm blood flow with L-arginine and D-arginine compared to control in patients with initially "depressed" endothelial function (n = 11) is compared to responses in patients with better ("normal") endothelial function (n = 11). The rate-pressure product and duration of exercise at 1-mm ST-segment depression during exercise is also demonstrated. There was no significant difference between the groups by two-way repeated measures ANOVA. (D5W = dextrose 5%.)

Because it is clear that the actions of arginine in reversing the inhibitory effects of L-glutamine, superoxide anions and ADMA are all specific for the L-enantiomer, it is likely that our observations of non-stereo-specific improvement in ACH responses are either a result of hormonal stimulation, in particular of insulin, or due to NO generation by the nonenzymatic pathway.

Myocardial ischemia and coronary artery disease. Myocardial ischemia in patients with obstructive coronary artery disease is believed to be primarily due to the myocardial oxygen demand during exercise outstripping coronary blood flow delivery across the atherosclerotic narrowing. However, overwhelming evidence indicates that coronary vasomotion

importantly modifies the ease with which myocardial ischemia occurs (1). Thus, increased coronary vascular tone can sometimes dramatically reduce coronary blood flow delivery, exacerbating myocardial ischemia. Vasoconstriction or failure of normal vasodilation during physiologic stress of the coronary vasculature in the presence of atherosclerosis and risk factors for atherosclerosis, is believed to be secondary to the development of endothelial dysfunction and reduced NO bioavailability during stress (3-12).

Using L-N^Gmonomethyl arginine (L-NMMA), a specific antagonist of NO synthesis, it was shown that the deficiency in NO activity during stress accounted for the abnormal vasodilation during atrial pacing of the coronary circulation and during hyperemia in the peripheral circulation (24,46). Based on these findings, we hypothesized that improvement of endothelial NO activity with parenteral L-arginine, by abrogating vasoconstriction during stress, will ameliorate myocardial ischemia. Other effects of arginine, including reduction in viscosity and inhibition of platelet activation, may theoretically have contributed to the beneficial effects that were anticipated (29,47).

Despite producing baseline dilation and improving endothelium-dependent responses, there was no improvement

Table 1. Plasma Levels of Arginine, Prolactin, Insulin and Glucose

	L-Arginine		D-Arginine	
	Baseline	Peak	Baseline	Peak
Arginine (μmol/dl)	17.9 ± 2.4	406 ± 64**	16.5 ± 4	519 ± 64**
Prolactin (mg/ml)	5.5 ± 0.8	16 ± 1.2**	6.0 ± 1.2	16.2 ± 2.9**
Insulin (μU/ml)	40 ± 9	85 ± 15.4**	27.1 ± 6.9	51.6 ± 12.3*
Glucose (mg/dl)	140 ± 15	131 ± 14	149 ± 27	166 ± 26

*p < 0.02; **p < 0.01.

in the ischemic threshold or the peak exercise performance with L-arginine or D-arginine. Furthermore, improved vascular tone at rest and with ACH did not affect blood pressure changes with exercise during arginine compared to control periods. Failure of improvement in myocardial ischemic threshold when NO activity was likely enhanced (29,30) may mean that vasomotor tone changes dependent on the endothelial NO pathway are not playing an important role in precipitation of exercise-induced myocardial ischemia. Alternatively, it is possible that the sympathetic nervous system activation associated with parenteral arginine infusion and precipitated by increased insulin release (44,47) may counteract the positive effects of increased NO availability on vasomotor tone. Also, it is possible that arginine-mediated improvement of ACH responses does not translate into improved NO availability during physical stress in the coronary vasculature. However, this is unlikely because we have demonstrated improved ACH-mediated and pacing-induced coronary vasodilation in patients with atherosclerosis during local infusions of L-arginine in the coronary vasculature (18).

Limitations. We have not measured the forearm vascular responses to an endothelium-independent vasodilator to confirm whether the observed improvement with arginine was specific for endothelial function and did not extend to the vascular smooth muscle. However, many previous studies have shown that neither L-arginine nor D-arginine improve sodium nitroprusside responses (14,24). Because of the double-blind nature of the study design, we were unable to test a range of arginine dosages. We selected our infusion rate based on previous studies demonstrating improvement of vascular endothelial function with doses of L-arginine between 0.5 and 1.0 mg/kg/min.

Our findings cannot be extended to other subgroups of patients with myocardial ischemia, such as variant angina, Syndrome X or unstable angina, where both vasoconstriction and platelet activation are major pathophysiologic etiologic factors, and may not be applicable to spontaneous ischemic episodes. Inhibition of platelet aggregation and coronary vasodilation with L-arginine could be of therapeutic benefit in these conditions.

Conclusions. Our study demonstrates non-enantiomer-specific improvement of peripheral vascular endothelial function with arginine, an effect that is likely to be due either to stimulation of insulin-dependent NO release or by nonenzymatic NO generation by parenteral D- and L-arginine. Acute administration of arginine did not improve myocardial ischemia during stress in patients with stable coronary artery disease. Whether long-term oral therapy will achieve this goal and whether parenteral arginine will be beneficial in other acute ischemic syndromes needs to be explored further.

References

- Mulcahy D, Dakak N, Zalos G, et al. Patterns and underlying mechanisms of transient myocardial ischemia in stable coronary artery disease are the same in both sexes: a comparative study. *J Am Coll Cardiol* 1996;27:1629-36.
- Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43:109-41.
- Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986;315:1046-51.
- Zeiger AM, Drexler H, Wollschläger H, Just H. Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991;83:391-401.
- 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.
- Quyyumi AA, Dakak N, Andrews NP, et al. Nitric oxide activity in the human coronary circulation: impact of risk factors for coronary atherosclerosis. *J Clin Invest* 1995;95:1747-55.
- Quyyumi AA, Dakak N, Mulcahy D, et al. Impaired release of nitric oxide from atherosclerotic human coronary vasculature. *J Am Coll Cardiol* 1997;29:308-17.
- Quyyumi AA, Mulcahy D, Andrews NP, Husain S, Panza JA, Cannon RO III. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia: comparison of acetylcholine and substance P. *Circulation* 1997;95:104-10.
- Quyyumi AA, Cannon RO, Panza JA, Diodati JG, Epstein SE. Endothelial dysfunction in patients with chest pain and normal coronary arteries. *Circulation* 1992;86:1864-71.
- Nabel EG, Selwyn AP, Ganz P. Paradoxical narrowing of atherosclerotic arteries induced by increases in heart rate. *Circulation* 1990;81:850-9.
- Gordon JB, Ganz P, Nabel EG, et al. Atherosclerosis influences the vasomotor response of epicardial coronary arteries to exercise. *J Clin Invest* 1989;83:1946-52.
- Yeung AC, Vekshtein VI, et al. The effect of atherosclerosis on the vasomotor response of coronary arteries to mental stress. *N Engl J Med* 1991;325:1551-6.
- Girerd XJ, Hirsch AT, Cooke JP, Creager MA. L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits. *Circ Res* 1990;67:1301-8.
- 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.
- Rossitch E Jr, Alexander E III, Black PM, Cooke JP. L-arginine normalizes endothelial function in cerebral vessels from hypercholesterolemic rabbits. *J Clin Invest* 1991;87:1295-9.
- Drexler H, Zeiger AM, Meinertz T, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolemic patients by L-arginine. *Lancet* 1991;338:1546-50.
- Dubois-Rande JL, Zelinsky R, Rovoot F, et al. Effects of infusion of L-arginine into the left anterior descending coronary artery on acetylcholine-induced vasoconstriction of human atheromatous coronary arteries. *Am J Cardiol* 1992;70:1269-75.
- Quyyumi AA, Dakak N, Diodati J, Gilligan DM, Panza JA, Cannon RO III. Effect of L-arginine on human coronary endothelium-dependent and physiologic vasodilation. *J Am Coll Cardiol* 1997;30:1220-7.
- Tousoulis D, Davies G, Tentolouris C, Crake T, Toutouzas P. Coronary stenosis dilation induced by L-arginine. *Lancet* 1997;349:1812-3.
- Panza JA, Quyyumi AA, Diodati JG, Callahan TS, Bonow RO, Epstein SE. Long-term variation in myocardial ischemia during daily life in patients with stable coronary artery disease: its relation to changes in the ischemic threshold. *J Am Coll Cardiol* 1992;19:500-6.
- Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. *Circulation* 1993;87:1475-81.
- Whitney RJ. Measurements of changes in human limb volume by means of a mercury-in-rubber strain gauge. *J Physiol (Lond)* 1948;108:5P-6P.
- Glantz SA, Slinker BK. Repeated measures. In: Glantz SA, Slinker BK, editors. *Primer of Applied Regression and Analysis of Variance*. New York: McGraw-Hill, 1990:381-463.

24. Casino PR, Kilcoyne CM, Quyyumi AA, Hoeg JM, Panza JA. Investigation of decreased availability of nitric oxide precursor as the mechanism responsible for impaired endothelium-dependent vasodilation in hypercholesterolemic patients. *J Am Coll Cardiol* 1994;23:844-50.
25. Dakak N, Hussain S, Mulcahy D, et al. Contribution of nitric oxide to reactive hyperemia: importance of endothelial dysfunction and effect of L-arginine. *Hypertension* 1998 (in press).
26. MacAllister RJ, Calver AL, Collier J, et al. Vascular and hormonal responses to arginine: provision of substrate for nitric oxide or nonspecific effect? *Clin Sci* 1995;89:183-90.
27. Imaizumi T, Hirooka Y, Masaki H, et al. Effects of L-arginine on forearm vessels and responses to acetylcholine. *Hypertension* 1992;20:511-7.
28. Clarkson P, Adams MR, Powe AJ, et al. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. *J Clin Invest* 1996;97:1989-94.
29. Bode-Boger SM, Boger RH, Creutzig A, et al. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. *Clin Sci* 1994;87:303-10.
30. Hishikawa K, Nakaki T, Tsuda M, et al. Effect of systemic L-arginine administration on hemodynamics and nitric oxide release in man. *Jpn Heart J* 1992;33:41-8.
31. Calver A, Collier J, Vallance P. Dilator actions of arginine in human peripheral vasculature. *Clin Sci* 1991;81:695-700.
32. Palmer RMJ, Moncada S. A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun* 1989;158:348-52.
33. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 1990;685:682-5.
34. Arnal J-F, Münzel T, Venema RC, et al. Interactions between L-arginine and L-glutamine change endothelial nitric oxide production. An effect independent of nitric oxide synthase substrate availability. *J Clin Invest* 1995;95:2565-72.
35. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 1993;91:2546-51.
36. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572-5.
37. Bode-Boger SM, Boger RH, Kienke S, Juner W, Frohlich 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.
38. Boger RH, Bode-Boger 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.
39. Boger RH, Bode-Boger SM, Mugge 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.
40. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 1994;94:2511-5.
41. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. *J Clin Invest* 1994;94:1172-9.
42. Schmidt HH, Warner TD, Ishi K, Sheng H, Murad F. Insulin secretion from pancreatic β -cells caused by L-arginine-derived nitrogen oxides. *Science* 1992;255:721-3.
43. Anderson EA, Mark AL. The vasodilator action of insulin. Implications for the insulin hypothesis of hypertension. *Hypertension* 1993;21:136-41.
44. Giugliano D, Marfella R, Verrazzo G, et al. The vascular effects of L-arginine in humans. *J Clin Invest* 1997;99:433-8.
45. Nagase S, Takemura K, Ueda A, et al. A novel nonenzymatic pathway for the generation of nitric oxide by the reaction of hydrogen peroxide and D- or L-arginine. *Biochem Biophys Res Commun* 1997;233:150-3.
46. Quyyumi AA, Dakak N, Andrews NP, Gilligan DM, Panza JA, Cannon RO III. Contribution of nitric oxide to metabolic vasodilation in the human heart. *Circulation* 1995;92:320-6.
47. Adams MR, Forsyth CJ, Jessup W, Robinson J, Celermajer DS. Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. *J Am Coll Cardiol* 1995;26:1054-61.