

Long-term L-Arginine Supplementation Improves Small-Vessel Coronary Endothelial Function in Humans

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Background—Coronary endothelial dysfunction is characterized by an imbalance between endothelium-derived vasodilating and vasoconstricting factors and coronary vasoconstriction in response to the endothelium-dependent vasodilator acetylcholine. Thus, the present double-blind, randomized study was designed to test the hypothesis that long-term, 6-month supplementation of L-arginine, the precursor of the endothelium-derived vasodilator NO, reverses coronary endothelial dysfunction to acetylcholine in humans with nonobstructive coronary artery disease.

Methods and Results—Twenty-six patients without significant coronary artery disease on coronary angiography and intravascular ultrasound were blindly randomized to either oral L-arginine or placebo, 3 g TID. Endothelium-dependent coronary blood flow reserve to acetylcholine (10^{-6} to 10^{-4} mol/L) was assessed at baseline and after 6 months of therapy. There was no difference between the two study groups in clinical characteristics or in the coronary blood flow in the response to acetylcholine at baseline. After 6 months, the coronary blood flow in response to acetylcholine in the subjects who were taking L-arginine increased compared with the placebo group ($149 \pm 20\%$ versus $6 \pm 9\%$, $P < 0.05$). This was associated with a decrease in plasma endothelin concentrations and an improvement in patients' symptoms scores in the L-arginine treatment group compared with the placebo group.

Conclusions—Long-term oral L-arginine supplementation for 6 months in humans improves coronary small-vessel endothelial function in association with a significant improvement in symptoms and a decrease in plasma endothelin concentrations. This study proposes a role for L-arginine as a therapeutic option for patients with coronary endothelial dysfunction and nonobstructive coronary artery disease. (*Circulation*. 1998;97:2123-2128.)

Key Words: vessels ■ endothelium ■ arginine

The coronary vascular endothelium modulates vascular tone through release of vasodilating and vasoconstricting substances. The endothelium-derived relaxing factor NO is synthesized from the amino acid L-arginine by a family of enzymes, the NO synthases, through a metabolic route, namely the L-arginine-NO pathway.¹ NO mediates vascular smooth muscle cell relaxation and inhibits platelet aggregation and adhesion and smooth muscle cell proliferation.^{1,2} Endothelin-1, on the other hand, is an endothelium-derived peptide that produces coronary vasoconstriction at pathophysiological concentrations by binding to specific receptors on the vascular smooth muscle.² Repeated studies have established that coronary endothelial dysfunction is present in early atherosclerosis and is characterized by a coronary vasoconstriction in response to the endothelium-dependent vasodilator acetylcholine.³⁻⁵ Indeed, Zeiher and colleagues⁶ have suggested that coronary endothelial dysfunction may be a mechanism of exercise-induced myocardial ischemia in patients with effort angina and normal coronary arteriogram. We recently demonstrated that coronary endothelial dysfunction in humans is characterized by increased coronary and circulating endothelin and decreased production of the second

messenger of NO, cGMP.⁷ These studies underscore that endothelial dysfunction is characterized by an imbalance between endothelium-derived vasodilating and vasoconstricting factors.

Previous studies in experimental animals and humans that used either feeding or intravenous infusion of L-arginine have shown that L-arginine improves peripheral endothelium-dependent dilatation, inhibits platelet aggregation, and may reduce atherosclerosis.⁸⁻¹¹ Moreover, short-term intracoronary administration of L-arginine improves coronary endothelial response to acetylcholine in hypercholesterolemic subjects.¹² Supplemental oral L-arginine in patients with heart failure resulted in improvement in symptoms in association with a decrease in plasma endothelin concentrations.¹³ To date, the effect of long-term administration of L-arginine on coronary endothelial function is unknown. Thus, the present study was designed to test the hypothesis that long-term oral L-arginine supplementation for 6 months reverses coronary endothelial dysfunction in response to acetylcholine in humans with nonobstructive coronary artery disease. Moreover, the effect on plasma endothelin concentrations and symptoms was also assessed.

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Selected Abbreviations and Acronyms

CBF = coronary blood flow
CFR = coronary flow reserve
IVUS = intravascular ultrasound
LAD = left anterior descending coronary artery

Methods

Subjects

The study protocol was approved by the Mayo Clinic Institutional Review Board. All patients gave written informed consent to participate in the study and were referred for coronary angiography because of recurrent chest pain. Exclusion criteria included $\geq 40\%$ diameter stenosis of any coronary artery, prior myocardial infarction, unstable angina pectoris, uncontrolled hypertension, peripheral vascular disease, ejection fraction $< 55\%$, left ventricular hypertrophy, active smoking, diabetes mellitus, and significant endocrine, hepatic, renal, or inflammatory disease. None of the patients had a left-dominant system.

Drug Infusion

Patients were brought to the cardiac catheterization laboratory between 8 AM and 10 AM in the fasting state after all cardiovascular medications had been discontinued for at least 72 hours. None of the patients were receiving lipid-lowering drugs. Diagnostic coronary angiography was performed via the percutaneous femoral approach without prior administration of nitrates or calcium channels blockers. The coronary angiogram was reviewed before the infusion of drugs; severity of stenosis was assessed by on-line quantitative coronary angiography. Patients with significantly obstructive coronary artery disease ($\geq 40\%$ diameter stenosis of any coronary artery) or diffuse disease were excluded from further studies.

The determination of endothelium-dependent and endothelium-independent CFR was performed as previously described.^{7,14} Additional intravenous heparin (5000 to 7500 U) was administered before instrumentation. Heart rate and mean arterial pressure were continuously monitored. A Doppler guidewire (FloWire, Cardiometrics Inc) 0.014 in. in diameter within a 2.2F coronary infusion catheter (Ultrafuse, SciMed Life System) was advanced and positioned in the middle portion of the LAD, 2 to 3 mm distal to the tip of the infusion catheter. Baseline average peak velocity as measured by Doppler echocardiography was recorded. Intracoronary bolus injections of incremental doses (18 to 36 μg) of adenosine (FujiSawa), an endothelium-independent vasodilator, were administered into the guiding catheter positioned in the ostium of the left main coronary artery until maximal hyperemia was achieved in all of the patients, and the maximal average peak velocity was recorded (in only 4 patients, or 13%, a dose $> 24 \mu\text{g}$ was required to achieve maximal hyperemia).^{14,15} To ensure that the increase in CBF did not merely reflect the forces of intracoronary bolus injection, CFR in response to normal saline (3 to 6 mL) was measured before adenosine injection and served as control.

The assessment of the endothelium-dependent CFR was performed by selective infusion of acetylcholine into the LAD. After a 5-minute equilibration period, baseline average peak velocity was recorded, followed by coronary angiography using nonionic contrast medium (Omnipaque, Winthrop Laboratories). Acetylcholine (Iolab Pharmaceuticals) at concentrations of 10^{-6} , 10^{-5} , and 10^{-4} mol/L (to achieve estimated final blood concentrations in the coronary bed of 10^{-8} , 10^{-7} , and 10^{-6} mol/L) was infused with a Harvard pump for 3 minutes at each concentration. Hemodynamic data (heart rate and mean arterial pressure), Doppler measurements, and coronary angiography were obtained after each infusion. The infusion was terminated when the largest dose of acetylcholine (10^{-4} mol/L) was reached. Nitroglycerin (Abbott Laboratories) was then injected as an intracoronary bolus (200 μg) through the guiding catheter.

At each time interval, average peak velocity was recorded, followed by coronary angiography. The angles, skew rotation, and

table height were kept constant during the procedure. In addition, the distances between the patient and the image intensifier and x-ray tube were kept constant. Coronary artery diameter was measured at three sites (proximal, middle, and distal) in three end-diastolic frames and averaged by an independent investigator unaware of the Doppler flow data using a computer-based image analysis system, as previously described.¹⁶ For the calculation of CBF, the measurements were performed in a 1-cm segment 5 mm distal to the tip of the Doppler wire. For each time interval, the diameter was measured in the same segment. CBF was calculated from the Doppler-derived time-velocity integral and vessel diameter^{17,18}: $\text{CBF} = \pi(\text{average peak velocity}/2)(\text{coronary artery diameter}/2)$.²

After the intracoronary infusions, IVUS of the LAD was performed with a Hewlett-Packard imaging system, as described previously,¹⁹ to confirm the absence of significant obstructive coronary artery disease. Continuous images were recorded throughout the LAD on a 0.5-in videotape for off-line analysis. A special effort was made to keep the IVUS catheter parallel to the long axis of the vessel lumen. Five or six segments of the LAD were identified in the videotape recording of the IVUS images by the digital counter, and the exact position of the IVUS catheter in relation to the artery was recorded on cine film at each position. The location of the catheter seen on the cine film of each segment was used to correlate the identified IVUS image with the angiographic segment.

An off-line computer-interactive analysis system was used to digitize the intracoronary IVUS video images onto a 256×256 -bit matrix. Standard calibration markers obtained directly from the IVUS images were used for calibration of absolute measurements. All measurements were made in end diastole and measured at the media-adventitia interface. Measurements of coronary artery diameters and areas were averaged from two orthogonal planes. All measurements were made by an observer blinded to the Doppler or angiographic findings.

After the procedure, patients with coronary endothelial dysfunction, defined as an attenuated increase ($< 50\%$) or a decrease in CBF in response to graded infusion of acetylcholine¹² and normal CFR in response to adenosine ($\text{CFR} > 2.5$), were recruited into the study and randomized. Oral L-arginine was given at a dose of 1 g TID for week 1, 2 g/d for week 2, and at the target dose of 3 g TID beginning in week 3. Both the active drug and the placebo were prepared as identical capsules and supplied by Triage Pharmaceuticals Inc. This dose has previously been shown to improve endothelial function in hypercholesterolemic subjects.^{8,10,11} The patients were instructed to avoid taking other medication, over-the-counter vitamins, or amino acids. The patients were thereafter contacted, in a blinded fashion, weekly for month 1 and monthly for the remainder of the 6-month study duration for monitoring of any side effects and to ensure compliance with treatment instructions. During these interviews, the patients were also questioned about their symptoms of chest pain. Their symptoms were scored as follows: 0, no symptoms; 0.5, improvement in their symptoms; and 1.0, no change or worsening of their symptoms.

One week before the repeat angiogram, all medications except for the study drug were discontinued. The study drug was continued until the evening before the repeat study. During the repeat angiogram at 6 months, the entire intracoronary infusion protocol was repeated by the investigator, who was blinded to the randomization. The same angles, skew rotation, and table height as in the baseline study were used. There were no complications at the baseline or follow-up procedures, and all patients tolerated the entire procedures without side effects.

Biochemical Studies

Venous blood samples were collected before the baseline procedure and after the 6 months of treatment. Fasting lipid analyses were performed for total cholesterol, HDL cholesterol, and triglycerides; LDL was measured in the Mayo Clinic Immuno Chemistry Laboratory by use of the Roch reagent, a colorimetric assay on the COBAS Mira System. The concentration of free L-arginine was analyzed with an automated amino acid analyzer by the Mayo Clinic laboratory, the normal range being from 20 to 180 $\mu\text{mol/L}$. Plasma endothelin was

TABLE 1. Demographic Characteristics of the Study Population

	Group 1: L-Arginine (n=13)	Group 2: Placebo (n=13)
Age, y (range)	48 (32–63)	50 (33–68)
Sex, M/F	7/6	6/7
Hypertension, %	23	31
History of smoking, %	46	38
Family history of CAD, n	31	31
Hypercholesterolemia, n	38	38

CAD indicates coronary artery disease.

determined by the ET-1,¹²⁵I assay system (Amersham), as previously described from our laboratory.⁷ The recovery of the extraction procedure is 81%, as determined by addition of synthetic endothelin to plasma, and interassay and intra-assay variations are 9% and 5%, respectively. The minimal level of detection is 0.5 pg per tube. The cross-reactivities of endothelin-2, endothelin-3, and proendothelin in this assay are <5%, <3%, and <37%, respectively.

Statistics

Continuous variables are presented as mean±SEM. The effect of acetylcholine infusion is expressed as percent change (mean±SEM) in CBF during the infusion of 10⁻⁴ mol/L relative to baseline. The effect of adenosine is expressed as the maximal ratio between the coronary flow velocities after and immediately before the bolus injection. The differences within the groups were analyzed by repeated-measures ANOVA, and the differences between groups were analyzed by ANOVA and Student's unpaired *t* test and the χ^2 test. A value of *P*<0.05 was accepted as significant.

Results

Of the 52 patients screened, 30 subjects fulfilled the selection criteria and were randomized. However, during the 6-month study, 4 patients discontinued the study. One patient from each group discontinued the study secondary to minor gastrointestinal side effects and 1 from each group secondary to personal preference. The remaining 26 participants completed the study (L-arginine, group 1, n=13, and placebo, group 2, n=13).

Of the remaining patients, 23% from group 1 (L-arginine) and 31% from group 2 (placebo) received calcium channel blockers for treatment of hypertension. None of the patients

received nitrates, β -blockers, ACE inhibitors, or cholesterol-lowering drugs. All the patients were referred for the evaluation of stable exertional chest pain suspected to be of cardiac origin. Before the coronary angiogram, 10 patients (77%) from each group underwent a noninvasive functional test, which was positive and consistent with myocardial ischemia in 6 patients from group 1 and 5 patients from group 2.

The demographic and clinical characteristics of the two groups at baseline are outlined in Table 1. There was no difference between the study groups in age, sex, or coronary artery disease risk factors. Nor was there any difference between groups 1 and 2 in the extent of coronary artery atherosclerosis as determined by IVUS (maximal area stenosis, 35±11% and 37±9%, respectively) or in the left ventricular ejection fraction (68±3% and 67±5%, respectively).

At baseline, the two groups also had similar resting CBF, CFR in response to adenosine, and CBF response to graded infusion of acetylcholine (peak response to 10⁻⁴ mol/L: group 1, 10±8% versus group 2, 5±6%) (Tables 2 and 3).

In the subjects who were taking L-arginine for 6 months, there was a significant improvement in CBF (149±20% versus 6±9%, *P*<0.05) and epicardial coronary artery diameter (16±1.4% versus -25.9±1.8%, *P*<0.05) in response to acetylcholine compared with the placebo group (Table 3, Figure 1). This change was associated in group 1 with a significant decrease in plasma endothelin concentrations (35±5%) and a mild but significant increase in plasma L-arginine levels (33±8%), which remained unaltered in group 2 (Table 2). There was no correlation between the decrease in plasma endothelin concentrations and the CBF response to acetylcholine. There was no difference in the response to L-arginine between the hypertensive and normotensive patients. There was no significant difference between the subjects on L-arginine or placebo in respect to mean arterial pressure, plasma cholesterol, CFR in response to adenosine, and CBF in response to intracoronary nitroglycerin (Table 2). Also, there was no difference between the groups in the extent of coronary artery atherosclerosis at follow-up angiogram.

A significant improvement of the symptoms score was observed in the group that was treated with L-arginine

TABLE 2. Coronary Hemodynamics and Biochemical Results of Study Groups at Baseline and at 6 Months

	Baseline		6-mo Follow-up	
	Group 1	Group 2	Group 1	Group 2
Mean arterial pressure, mm Hg	85±9	86±8	82±7	87±3
CFR to adenosine	2.8±1.2	2.7±0.9	2.9±0.9	2.7±1.0
CBF, mL/min	50±8	51±5	52±5	53±6
% Δ CBF to nitroglycerin	121±15	129±19	127±21	129±16
Total cholesterol, mg/dL	196±20	190±15	189±12	187±11
HDL cholesterol, mg/dL	44±4	43±7	46±6	43±8
Endothelin, pg/mL	15.1±0.9	14.9±1.2	9.5±0.9*	13.4±1.1
L-Arginine, μ mol/L	83±19	87±21	112±11*	89±17

Group 1 received L-arginine; group 2, placebo.

**P*<0.05 vs group 1.

TABLE 3. Dose Response to Acetylcholine

	Baseline		6-mo Follow-up	
	Group 1	Group 2	Group 1	Group 2
% Δ CAD to acetylcholine, 10^{-6} mol/L	-4.2 \pm 0.8	-3.2 \pm 0.8	-1.2 \pm 1.1	-4.2 \pm 0.8
% Δ CAD to acetylcholine, 10^{-5} mol/L	-17.2 \pm 1.2	-15.2 \pm 1.1	8.8 \pm 1.6*	-16.2 \pm 1.1
% Δ CAD to acetylcholine, 10^{-4} mol/L	-26.5 \pm 1.6	-24.9 \pm 1.7	16 \pm 1.4*	-25.9 \pm 1.8
% Δ CBF to acetylcholine, 10^{-6} mol/L	-5.2 \pm 1.2	-5.0 \pm 1.6	25 \pm 9*	-7.2 \pm 1.2
% Δ CBF to acetylcholine, 10^{-5} mol/L	5.2 \pm 1.3	2.7 \pm 0.9	72 \pm 12*	4.7 \pm 1.9
% Δ CBF to acetylcholine, 10^{-4} mol/L	10 \pm 8	5 \pm 6	149 \pm 20*	6 \pm 9

CAD indicates coronary artery disease. Group 1 received L-arginine; group 2, placebo.

* P <0.05 vs group 2.

compared with the placebo group (Figure 2) after 1 week and persisted for the 6-month study.

Discussion

The present study demonstrates that long-term oral L-arginine supplementation for 6 months in humans with nonobstructive coronary artery disease improves coronary small-vessel endothelial function in response to acetylcholine in association with a significant improvement in their symptoms. The group that received L-arginine demonstrated an improvement in endothelial function at the level of both the epicardial and small coronary vessels. This improvement in coronary endothelial function was associated with a decrease in plasma endothelin concentrations. This study proposes a role for L-arginine as a therapeutic option for patients with coronary endothelial dysfunction and nonobstructive coronary artery disease.

The endothelium is an important modulator of coronary vascular tone through the release of endothelium-derived relaxing factors such as NO and endothelium-derived vasoconstrictors such as endothelin.² Early coronary atherosclerosis is associated with coronary endothelial dysfunction, which is characterized by an attenuated or absent endothelium-dependent vasodilatation, such as the response to intracoronary administration of acetylcholine.³⁻⁵ The significance of coronary endothelial dysfunction is underscored by its implications as a mechanism of exercise-induced myocardial

ischemia⁶ and by its association with myocardial perfusion defects, as recently observed in our laboratory.²⁰

A growing body of evidence suggests that dietary supplementation of arginine improves endothelial function in experimental animals and in humans. Short-term administration of intracoronary L-arginine restored the impaired endothelium-dependent dilatation in hypercholesterolemic patients.¹² Recently, Clarkson and colleagues⁸ reported that a 4-week regimen of oral L-arginine improved peripheral endothelium-dependent dilation in hypercholesterolemic subjects, and Rector and colleagues¹³ demonstrated beneficial effects of oral L-arginine in patients with heart failure. The present study extends these previous observations and demonstrates that long-term L-arginine improves coronary endothelial function in humans.

The patients treated with L-arginine demonstrated a significant improvement in symptoms compared with the placebo group. This observation may support the hypothesis that their symptoms were related to their abnormalities in coronary endothelial function. This observation is also in accord with previous studies that demonstrated an improvement in symptoms in patients with heart failure subsequent to L-arginine treatment¹³ and with a recent report that oral L-arginine supplementation has a beneficial effect on exercise capacity in patients with stable angina.²¹

The mechanism by which L-arginine improves endothelial function may be multifactorial. L-Arginine is the substrate for NO synthase, the enzyme that catalyzes the production of NO

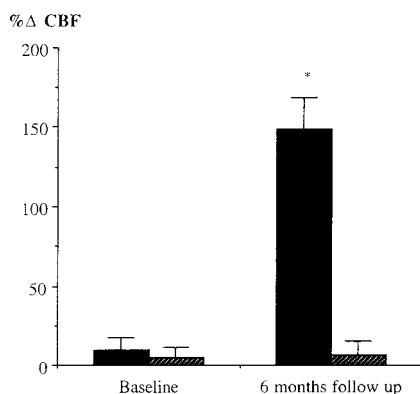


Figure 1. Percent changes in CBF in response to 10^{-4} mol/L of acetylcholine at baseline and at 6-month follow-up in two study groups (solid bars, group 1, L-arginine; hatched bars, group 2, placebo; * P <0.05 between groups and compared with baseline).

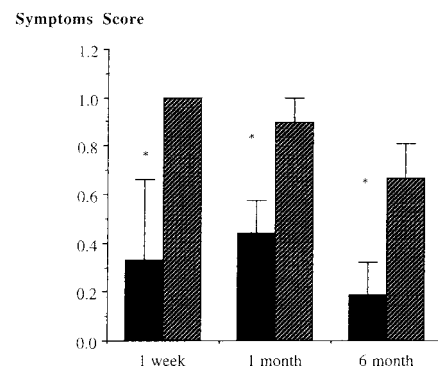


Figure 2. Symptoms scores at 1-week, 1-month, and 6-month follow-up in two study groups (solid bars, group 1, L-arginine; hatched bars, group 2, placebo; * P <0.05 between groups and compared with baseline).

in vascular endothelial cells.¹ NO contributes to the resting epicardial and the coronary microvascular tone. Moreover, the acetylcholine-induced increase in CBF is largely due to release of NO from the coronary epicardial and microvascular bed.²² Thus, one possible mechanism for the beneficial effect of L-arginine is the enhanced production of NO from the coronary endothelium. This mechanism was recently challenged because the intracellular concentration of L-arginine far exceeds the K_m of the NO synthases,^{22,23} and L-arginine did not improve relaxation to acetylcholine in aortic rings from hypercholesterolemic rabbits in vitro.²⁴ However, the composition of the buffer solution in the in vitro studies may have contributed to this discrepancy, because when L-glutamine was added to the solution, L-arginine did enhance endothelium-dependent vascular relaxation.^{22,23} Another hypothetical mechanism whereby L-arginine administration could directly affect the activity of the endothelial NO synthase may be uncovered by a recent observation that an endogenous inhibitor of NO synthase, asymmetric dimethylarginine, accumulates in the serum of cholesterol-fed rabbits and might antagonize the normal intracellular concentrations of L-arginine.²⁵ Although we did not measure the levels of asymmetric dimethylarginine in the present study, it may be speculated that large doses of L-arginine might overcome this effect.

An additional, indirect mechanism by which L-arginine may improve endothelial function is through other vasoactive factors, such as endothelin-1. Indeed, in the present study, plasma endothelin concentration decreased after 6 months of L-arginine supplementation, and this observation is supported by a previous study that demonstrated a beneficial effect of oral L-arginine in patients with heart failure in association with a decrease in plasma endothelin-1 concentrations.¹³ The increase in NO activity by L-arginine may decrease endothelin production, because endothelium-derived NO inhibits the production of endothelin via a cGMP-dependent pathway.²⁶ Moreover, we recently reported that experimental hypercholesterolemia, which is associated with coronary endothelial dysfunction and a decrease in endogenous coronary NO activity, is characterized by an enhanced coronary vasoconstrictive response to pathophysiological doses of endothelin-1.²⁷ Thus, a decrease in plasma endothelin concentration may both directly and indirectly contribute to the improvement in coronary endothelial function by decreasing the sensitivity of the coronary circulation to other vasoconstrictor factors, such as angiotensin II and norepinephrine.^{28,29} Indeed, recent studies have demonstrated a clear benefit of pharmacological therapy, such as ACE inhibitors, on coronary endothelial dysfunction.³⁰

We previously reported that plasma and circulating endothelin concentrations are increased in patients with coronary endothelial dysfunction.⁷ The decrease in circulating endothelin-1 in the present study in association with an improvement in coronary endothelial function further supports the hypothesis that in early coronary atherosclerosis, an imbalance between production and release of NO and endothelin occurs that leads to augmented coronary vasoconstriction. Because endothelin and NO act primarily as local, paracrine/autocrine factors, it seems reasonable to assume that the

circulating levels of endothelin underrepresent the local and tissue concentrations in the coronary circulation.

An increasing body of evidence suggests that dietary supplementation of arginine inhibits atherogenesis.³¹ Aji and colleagues⁹ recently reported that oral L-arginine for 6 months prevented xanthoma development and inhibited atherosclerosis in LDL cholesterol receptor knockout mice, suggesting that this response may be mediated by NO. Moreover, long-term administration of supplementary dietary arginine markedly inhibits intimal lesion formation in hypercholesterolemic rabbits³² and preserves endothelium-dependent vasodilatation.³³ NO can inhibit monocyte adhesion to the endothelium^{1,31} and may also inhibit the production of superoxide anion by activated neutrophils.³⁴ Thus, it may be speculated that long-term L-arginine administration improves coronary endothelial function by attenuating the progression of coronary atherosclerosis.³⁵ In the present study, a small number of selected patients were studied, and the results may be influenced by altered hemodynamics or other mechanisms. Moreover, we did not measure biochemical markers to evaluate the NO pathways, such as urinary nitrogen oxides or cGMP.³⁶ Thus, a larger, comprehensive study may be required before the therapeutic role for L-arginine and the mechanism of its beneficial effect can be elucidated.

In summary, this study demonstrated that oral L-arginine supplementation for 6 months improves coronary endothelial function in association with improvement in symptoms. This study proposes a therapeutic role for L-arginine in patients with chest pain and coronary endothelial dysfunction.

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