



ORIGINAL ARTICLE

L-arginine improves endothelial function and reduces LDL oxidation in patients with stable coronary artery disease

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Summary

Background: We investigated the effects of oral L-arginine on endothelial function, intravascular oxidative stress, and circulating inflammatory markers in patients with stable coronary artery disease (CAD).

Methods: Thirty-one stable CAD patients were randomly assigned to oral L-arginine (10 g) or vitamin C (500 mg, an antioxidant, as active control) daily for 4 weeks, with crossover to the alternate therapy after 2 weeks off therapy, in this study. Brachial artery endothelial function studies were performed and serum concentrations of lipids and inflammatory markers were measured at baseline, at the end of each 4-week treatment period and at the 2-week wash-out period. Susceptibility of low-density lipoprotein (LDL) particles to oxidation, a marker of oxidative stress, was determined in 11 patients at random before and after 4-week treatment of oral L-arginine.

Results: We demonstrates that consumption of either L-arginine or vitamin C significantly increased brachial artery flow-mediated dilatation (mean diameter change from baseline of 4.87%, $P < 0.0001$ and of 3.17%, $P = 0.0003$, respectively). Neither oral L-arginine nor vitamin C affected lipid profiles and circulating levels of inflammatory markers. However, in the 11 patients whose LDL susceptibility to

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oxidation was determined, lag time significantly increased by 27.1% ($P = 0.045$) after consumption of L-arginine for 4 weeks.

Conclusions: Oral L-arginine supplement improved endothelial function and reduced LDL oxidation in stable CAD patients.

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Introduction

Current views regard atherosclerosis as a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation.¹⁻³ The monolayer endothelial cells perform a wide array of homeostatic functions within normal blood vessels. Endothelial dysfunction is an early manifestation of atherosclerosis, which may potentiate myocardial ischemia through paradoxical vasoconstriction and thrombosis.^{1,2} Endothelial dysfunction, as assessed in terms of vasomotor dysfunction, can occur well before the structural manifestation of atherosclerosis and thus serve as an independent predictor of future cardiovascular events.^{1,2,4-6} Modification or reversal of endothelial dysfunction may be of significant therapeutic benefit in the treatment of coronary artery disease (CAD).^{1,2,7,8}

L-arginine is the physiological substrate for nitric oxide (NO) synthesis by the vascular endothelium.⁸ The effect of L-arginine on endothelial function has been studied in hypercholesterolemic animals⁹ and humans.¹⁰⁻¹⁴ Intracoronary or intravenous infusion of L-arginine improved endothelium-dependent vasodilatation in hypercholesterolemic humans.¹⁰⁻¹² Oral L-arginine administration has been shown to improve coronary endothelial function in humans.^{13,14} It could also reduce human monocyte adhesion to endothelial cells and down-regulate the expression of cell adhesion molecules and proinflammatory cytokines.¹⁴⁻¹⁶ Recently, L-arginine was shown to improve the clinical symptoms in a selected group of patients with intractable angina pectoris.¹⁷ It could also attenuate the systemic rise in peripheral lymphocyte activation and anti-oxidized low-density lipoprotein cholesterol (LDL) antibodies induced by vessel wall injury following percutaneous coronary intervention in patients with unstable angina.¹⁸ Accordingly, it is possible that the mechanisms of vascular protection by L-arginine are multiple involving NO synthase substrate provision, reduction of the susceptibility of LDL-C to oxidative stress and anti-inflammation.

However, the multiple effects of oral L-arginine supplement on endothelial function, inflammatory

markers, and particularly LDL oxidation, a marker of oxidative stress, have not been completely studied in clinical atherosclerosis. This study was then sought to simultaneously evaluate the multiple potential vascular protective mechanisms of oral L-arginine treatment in a group of patients with stable CAD. Since the vascular protection of L-arginine might be also related to its antioxidant effect,¹⁸ all patients were scheduled to receive sequential treatment of L-arginine and vitamin C, an antioxidant, for the same duration for comparison.

Materials and methods

Study population

The study patients were recruited from a patient cohort followed up regularly at the outpatient cardiology clinic of a single local referral medical center between March 19, 2002 and March 27, 2003. Patients were included if they (1) had significant CAD with more than 50% luminal diameter stenosis of a major coronary artery documented by coronary angiography, (2) had stable angina pectoris that was less than or equal to functional class II and could be controlled by antiplatelet agents and β -adrenergic blockers, (3) had not actively smoked for at least 2 years and were taking no vasoactive medications. Patients were excluded if they had unstable angina, myocardial infarction within the previous 3 months, significant congestive heart failure (New York Heart Association more than or equal to class III), uncontrolled hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >100 mmHg), uncontrolled diabetes mellitus (fasting glucose level >160 mg/dl), impaired renal function (creatinine >3 mg/dl), systemic infection or inflammatory disease, malignancy, or other systemic illness. Those who had pre-scheduled coronary bypass surgery or coronary interventional therapy or who had undergone coronary intervention or bypass surgery during the 6 months prior to inclusion in the study were not recruited. As medications with vasodilator action may interfere with the efficacy assessment, those va-

soactive medications, including long-acting nitrates, alpha-blockers, calcium antagonists and angiotensin converting enzyme inhibitors and angiotensin II receptor blockers were prohibited throughout the study period. Those patients with antioxidant vitamin supplements or other antioxidant drugs such as probucol and allopurinol, lipid-lowering agents, and hormone replacement therapy were also excluded. Patients were instructed to stop such medications upon study entry and were asked not to alter their medical treatment throughout the duration of the study. Only aspirin, β -blockers, diuretics and sublingual nitroglycerin were allowed throughout the entire study period. In addition, subjects were prohibited from consuming grape juice, tea, or alcoholic beverages during the study. They were encouraged not to change their diet otherwise and daily dietary logs of all food and beverages consumed during the study were reviewed to assure dietary compliance. All subjects gave written informed consent, and the institutional review board approved the study protocol.

Study design

This study was conducted in a randomized, cross-over and open-label fashion because prior experience indicates that it is not possible to produce a placebo or active control substance that convincingly tastes like L-arginine. In this study, vitamin C was used as an active control substance to demonstrate the potential antioxidant effect on endothelial protection.

All patients underwent a full medical history, physical examination, basic laboratory screening, chest X-ray and electrocardiogram. After 14 days of run-in period, patients underwent initial endothelial function study and blood sampling for biochemical and inflammatory marker analyses. Thereafter they were randomly assigned to receive either 4-week treatment of oral L-arginine 5 g twice daily (10 g/d), followed by a 2-week wash-out period, and then another 4-week treatment of vitamin C 250 mg twice daily (sequence A), or the opposite sequence of treatments (sequence B). At the end of each 4-week treatment and the 2-week wash-out period, patients returned to the laboratory for testing of serum lipid profile and inflammatory markers, and underwent endothelial function study.

Eleven randomly selected patients (6 from sequence A and 5 from sequence B) had their susceptibility of LDL particles to oxidation deter-

mined before and after 4-week treatment of oral L-arginine.

Endothelial function study

Endothelium-dependent flow-mediated dilatation (FMD), endothelium-independent nitroglycerin-mediated vasodilatation, and hyperemic flow of the conduit brachial artery were determined by use of high-resolution vascular ultrasound and an upper-arm occlusive cuff as has been previously described.¹⁹ The brachial artery reactivity study was performed in the morning, with the patients having fasted overnight and having had no contact with tobacco smoke (including passive smoking) for 24 h. After a 10-min rest, the diameter of the brachial artery just above the antecubital fossa of the dominant arm of patients and baseline forearm blood flow were measured with a 6–15 MHz linear array vascular ultrasound transducer and a Angilent SONOS 5500 ultrasound system (Andover, MA, USA). Forearm blood flow was increased by inflation of a pneumatic blood pressure cuff placed around the proximal portion of the arm to a systolic pressure of 200 or 50 mmHg greater than the systolic blood pressure, whichever was lower, creating distal limb ischemia. This was followed by deflation after 5 min, when reactive hyperemia occurred. Repeated blood flow scans were obtained immediately thereafter, and repeat brachial artery diameter measurements were taken after 1 min. Fifteen minutes were allowed for vessel recovery, and repeat resting brachial artery diameter and blood flow scans were obtained. Sublingual NTG (0.4 mg) was administered, and final scans were performed after 3 min. A single lead electrocardiogram was monitored throughout the study. Blood pressure was measured in the opposite upper arm before the first scan, before administration of sublingual nitroglycerin, and every 5 min thereafter until it returned to baseline. Ultrasound images were digitized online and stored on magneto-optical disks for offline measurements. Vessel diameters were measured in triplicate and then averaged by a single observer who was unaware of subject information, drug ingestion and study date. Straight segments of the artery (10 mm in length) were chosen, and the brachial artery diameter was measured at end-diastole, using intima–media interface, or if they could not be visualized, media–adventitia interfaces, as landmarks. Previous studies have shown that maximal dilatation occurs 1 min after cuff deflation for reactive hyperemia and 3 min after sublingual NTG. The end point was the percent diameter change of brachial artery in response to reactive

hyperemia (endothelium-dependent vasodilatation) or NTG (endothelium-independent vasodilatation). The intra- and inter-observer variability for repeated measurements were 0.02 ± 0.07 and 0.06 ± 0.16 mm, respectively. When reactive hyperemia studies were performed on 2 separate days, the mean difference in brachial vasodilator response in absolute terms was $1.5 \pm 2.0\%$.

Measurement of lipid levels, isolation of LDL particles and determination of susceptibility to oxidation

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose were measured by an automated analyzer (Hitachi, Japan). LDL-C was calculated with the Friedewald formula.

Susceptibility of LDL particles to oxidation was determined from the rate of conjugated diene formation after exposure to copper sulfate (CuSO_4). The serum samples were fractionated by ultracentrifugation with the density adjusted by sodium bromide (NaBr). LDL particles were isolated from serum by sequential density ultracentrifugation between densities of 1.006 and 1.063 g/ml using an ultracentrifuge at 100,000 rpm ($>400,000g$). The formation of the conjugated dienes was stimulated by incubating $50 \mu\text{g}$ LDL with $5 \mu\text{mol/l}$ CuSO_4 in 1 ml PBS at 37°C . Oxidation of the LDL was determined by analyzing the production of conjugated dienes induced by copper ion and was measured by continuous monitoring of the changes in absorbance at 234 nm for 10 h. Absorbance measurements were obtained every 10 min using a Beckman DU 640 spectrophotometer (Beckman Instruments). From the kinetic profile of the LDL preparations, the lag time was defined as the time (in minutes) between initiation of conjugated diene production after addition of CuSO_4 and the intercept of the maximum slope of the absorbance curve at the time of maximum conjugated diene production. These studies were performed and interpreted by one investigator who was unaware of subject information, L-arginine consumption, and study date.

Inflammatory marker analyses

Serum concentrations of vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and P-selectin were measured with commercial enzyme immunoassays (R&D Systems, Inc., Minneapolis, MN, USA). Immunoassays were

also used to measure serum concentrations of high-sensitivity C-reactive protein (hsCRP) (IMMAGE Immunochemistry Systems, Beckman Coulter, Inc., CA, USA) and von Willebrand factor (vWF) (American Diagnostica Inc., CT, USA). Assays for all these factors were done concurrently to minimize any effects of repeated freeze-thaw cycles. The intra- and inter-assay coefficients for each factor in our laboratory were about 5% and about 10%, respectively.

Safety

Safety assessments consisted of physical examinations, ECG recordings, vital signs, standard laboratory safety evaluations and adverse-event monitoring. For each adverse event, the duration, frequency, severity, and relation to study treatment were assessed.

Statistical analysis

Data are given as mean \pm SE. The analysis based on the intention-to-treat population was considered as the primary analysis for this trial. The intention-to-treat population is defined as all randomized subjects who had at least one post-treatment efficacy evaluation.

All variables concerning baseline characteristics were summarized by treatment sequences and all sequences combined. For continuous variables, an analysis of variance (ANOVA) was performed. For categorical variables, the Fisher exact test was performed. For the susceptibility of LDL particles to oxidation study, changes from pre- to post-treatment were assessed using paired *t*-test.

For all efficacy variables, results were summarized for all study visits and for changes between pre-treatment and the end of each study period. The generalized linear model was used to compare changes from baseline within a sequence to indicate whether there was a significant carry-over effect within a sequence. The generalized linear model considered sequence variation, variation between patients within group, period variation and treatment variation. Mean \pm SE of efficacy variables were graphed over time by treatment groups. An analysis of covariance model was used to assess sequence, treatment and sequence-by-treatment interactions using pre-treatment values as covariate.

All statistical analyses are based on two side hypothesis tests with a significance level of $P < 0.05$.

Results

Baseline data of the study population (Table 1)

Of the 36 consecutive patients initially screened, 33 (21 men and 12 women; mean age 58 ± 7 years, range 51–65 years) fulfilled the inclusion criteria and were eligible for the study. Among these 33 patients randomized to the treatment, two were excluded (one withdrew and one dropped out for uncontrolled angina) from the intention-to-treat population of the efficacy analysis owing to a lack of available post-baseline efficacy data. Thirty-one patients therefore remained in the intention-to-treat population. Of these, 16 patients were randomized to sequence A and 15 patients to sequence B. The baseline characteristics are displayed in Table 1. There were no differences in baseline characteristics between the two treatment groups (sequences A and B). Concurrent cardiac medications, including aspirin, β -blockers, diuretics and sublingual NTG, were also comparable between the two groups. In the intention-to-treat population, the mean

values of total cholesterol, LDL-C, HDL-C, triglycerides, and the inflammatory markers were all comparable between the two treatment sequences.

Hemodynamic parameters and brachial artery ultrasound study (Table 2)

Hemodynamic parameters

Baseline systolic blood pressure, diastolic blood pressure and heart rate were comparable in the L-arginine- and vitamin C-treated groups. Both L-arginine and vitamin C had no effect on these hemodynamic parameters.

Endothelium-dependent FMD

Baseline brachial artery FMD was comparable in the L-arginine- and vitamin-C-treated groups ($4.19 \pm 0.46\%$ versus $4.72 \pm 0.39\%$, $P = 0.387$) and there was no significant carryover effect (carryover effect $P = 0.9776$) (Fig. 1). Both L-arginine and vitamin C consumption significantly improved brachial artery FMD (mean diameter change from baseline of 4.87% , $P < 0.0001$ and mean diameter

Table 1 Baseline characteristics, lipid profile and inflammatory markers of the intention-to-treat population.

	All groups (N = 31)	Sequence A (N = 16)	Sequence B (N = 15)	P-value
Male gender (%)	20 (65)	11 (69)	9 (60)	NS
Age (years)	59 ± 1	58 ± 2	60 ± 2	NS
Body mass index (kg/m^2)	27 ± 0.6	28 ± 0.6	26 ± 0.6	NS
Diabetes mellitus, N (%)	6 (19)	3 (19)	3 (20)	NS
Hypertension, N (%)	22 (71)	11 (69)	11 (73)	NS
Hyperlipidemia, N (%)	21 (68)	10 (63)	11 (73)	NS
<i>Concurrent medications</i>				
Aspirin, N (%)	18 (58)	11 (69)	7 (47)	NS
β -Blockers, N (%)	28 (90)	15 (94)	13 (87)	NS
Diuretics, N (%)	5 (16)	4 (25)	1 (7)	NS
Sublingual nitroglycerin, N (%)	9 (29)	5 (31)	4 (27)	NS
<i>Lipid profile</i>				
Total cholesterol (mg/dl)	198 ± 6	196 ± 9	200 ± 7	NS
LDL-cholesterol (mg/dl)	131 ± 6	128 ± 8	134 ± 7	NS
HDL-cholesterol (mg/dl)	50 ± 2	49 ± 3	52 ± 3	NS
Triglycerides (mg/dl)	140 ± 13	150 ± 16	130 ± 22	NS
Total cholesterol/HDL-cholesterol	4.2 ± 0.2	4.3 ± 0.3	4.1 ± 0.3	NS
<i>Inflammatory markers</i>				
vWF (mU/ml)	624 ± 43	667 ± 60	579 ± 61	NS
VACM-1 (ng/ml)	475 ± 17	490 ± 27	459 ± 21	NS
ICAM-1 (ng/ml)	162 ± 8	171 ± 12	152 ± 11	NS
P-Selectin (ng/ml)	33 ± 3	31 ± 4	35 ± 5	NS
High-sensitivity CRP (mg/l)	3.4 ± 1.4	4.4 ± 2.5	2.4 ± 1.4	NS

CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cellular adhesion molecule-1; vWF = von Willebrand factor.

Table 2 Hemodynamic parameters and brachial artery ultrasound study.

	L-arginine			Vitamin C		
	Baseline	End	P-value	Baseline	End	P-value
Systolic blood pressure (mmHg)	128 ± 3	123 ± 3	NS	126 ± 3	127 ± 3	NS
Diastolic blood pressure (mmHg)	80 ± 2	78 ± 2	NS	78 ± 2	80 ± 2	NS
Heart rate (bpm)	60 ± 2	60 ± 2	NS	61 ± 2	61 ± 2	NS
<i>Flow-mediated dilatation</i>						
% Diameter changes	4.19 ± 0.46	9.06 ± 0.69	<0.0001	4.72 ± 0.39	7.90 ± 0.72	0.0003
Mean diameter changes (%)		4.87 ± 0.68			3.17 ± 0.67	0.080
<i>Nitroglycerin-mediated dilatation</i>						
% Diameter changes	12.0 ± 1.22	13.6 ± 0.99	0.310	11.8 ± 1.08	12.1 ± 1.05	0.802
Mean diameter changes (%)		1.61 ± 1.23			0.38 ± 1.08	0.458

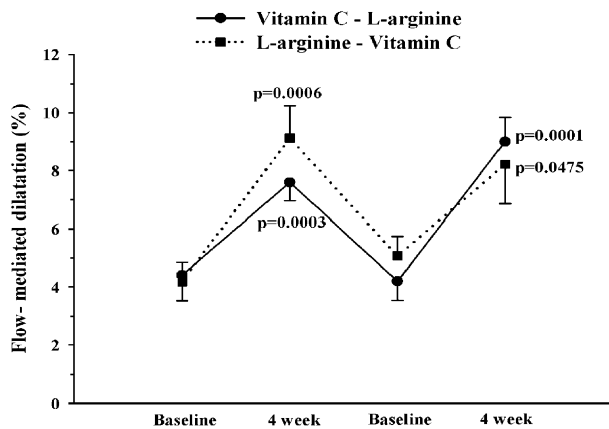


Figure 1 Mean ± SE of FMD were graphed over time by treatment groups. An analysis of covariance model was used to assess sequence, treatment and sequence-by-treatment interactions using pre-treatment values as covariate. At baseline, FMD was impaired. After treatment with L-arginine and vitamin C, FMD increased significantly. There was no carryover effect.

change from baseline of 3.17%, $P = 0.0003$, respectively). Although the mean diameter change from baseline in patients with L-arginine was higher than that of those who were treated with vitamin C, the difference was not statistically significant ($P = 0.080$).

Endothelium-independent nitroglycerin-mediated dilatation

Baseline brachial artery nitroglycerin-mediated dilatation was also comparable in the L-arginine- and vitamin-C-treated groups ($12.0 \pm 1.22\%$ versus $11.8 \pm 1.08\%$, $P = 0.855$) and there was no significant carryover effect (carryover effect $P = 0.406$). Neither L-arginine nor vitamin C consumption significantly affected nitroglycerin-induced endothelium-independent vasodilation (mean diameter change from baseline of 1.61%, $P = 0.310$

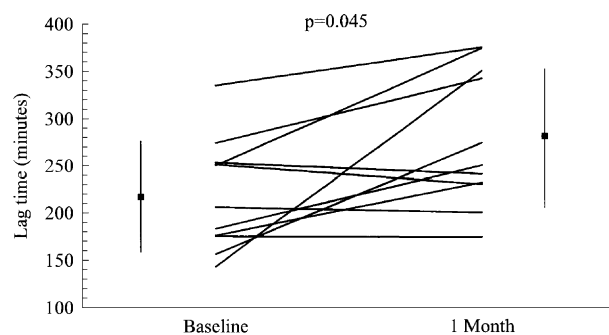


Figure 2 Effect of L-arginine on LDL susceptibility to oxidation. Susceptibility of LDL particles to oxidation was determined from the rate of conjugated diene formation after exposure to CuSO_4 . At baseline, the lag time to conjugated diene formation was 218 ± 59 min. After 4 weeks of ingesting L-arginine, the lag time increased to 277 ± 72 min (+27.1%, $P = 0.045$). Changes from pre- to post-treatment were assessed using paired t -test.

and mean diameter change from baseline of 0.38%, $P = 0.802$, respectively). The difference between the mean diameter change from baseline in patients with L-arginine and that of those who were treated with vitamin C was insignificant ($P = 0.458$).

Lipid levels and LDL susceptibility to oxidation

Long-term L-arginine or vitamin C consumption had no effect on fasting glucose, total cholesterol, LDL-C, HDL-C, the total cholesterol/HDL-C ratio or triglyceride levels (data not shown).

At baseline, the lag time to conjugated diene formation was 218 ± 59 min. After 4 weeks of ingesting L-arginine, the lag time increased to 277 ± 72 min (+27.1%, $P = 0.045$) (Fig. 2).

Inflammatory markers

Neither L-arginine nor vitamin C consumption affected circulating levels of inflammatory markers, including vWF, VCAM-1, ICAM-1, P-selectin and hsCRP (data not shown).

Treatment-related adverse events

The adverse events diagnosed as possibly, probably or related were considered as treatment-related adverse events. No deaths were reported and there were no treatment discontinuations or interruptions due to adverse events. One serious adverse event was reported which occurred in a patient assigned to sequence B. This patient experienced muscle sprain approximately 2 weeks after vitamin C treatment and was briefly hospitalized and underwent physiotherapy. The investigator deemed the event to be unrelated to study medication and attributed it to the patient's co-existing disease state. During the study period, there were 13 treatment-related adverse events occurring in 10 patients. Eight events in 5 patients during L-arginine treatment and 5 events in 5 patients during vitamin C treatment were reported. The treatment-related adverse events after L-arginine treatment were frequent bowel movements (5 patients, 15.2%), epigastralgia (1 patient, 3%), atypical chest pain (1 patient, 3%) and increased glucose levels (1 patient, 3%). The treatment-related adverse events after vitamin C treatment were retching (2 patients, 6.1%), palpitations (1 patient, 3%), diarrhea (1 patient, 3%) and frequent bowel movements (1 patient, 3%). There were no statistically significant differences regarding the incidence of treatment-related adverse events between the two treatment groups.

Discussion

It was demonstrated in this study that consumption of either L-arginine or vitamin C significantly increased brachial artery FMD, indicating that both of them could improve endothelial function in CAD patients. These findings are comparable to that reported previously.^{13,14,17,20} However, in the same group of patients, consumption of vitamin C increased FMD to a lesser, though borderline significant ($P = 0.080$), extent than L-arginine treatment did, suggesting the potential advantage of L-arginine rather than vitamin C supplement in endothelial protection. On the other hand, neither L-arginine nor vitamin C consumption affected lipid

profile or circulating levels of inflammatory markers, including vWF, VCAM-1, ICAM-1, P-selectin, and hsCRP. However, in the 11 randomly selected patients whose susceptibility of LDL particles to oxidation was determined from the rate of conjugated diene formation after exposure to CuCl_2 , lag time significantly increased by 27.1%, suggesting the reduction of oxidative stress after L-arginine supplement for 4 weeks.

L-arginine is the precursor of NO, the most important vasodilator substance produced by endothelial cells. Endothelial dysfunction, which is characterized by decreased bioavailability of NO and increased release of oxygen-derived free radicals, occurs early in atherosclerosis.^{1,2,7,8} Recent study has shown that the beneficial effect of L-arginine on the improvement of endothelial function may involve NO synthase substrate provision, especially in patients with elevated levels of asymmetric dimethylarginine (ADMA), the endogenous NO synthase inhibitor.⁸ Although we did not measure NO, arginine or ADMA levels in this study, the LDL levels of our study patients were normal or slightly increased. Therefore it is difficult to hypothesize an increase in ADMA levels in these patients. Nevertheless, our results did support the notion that L-arginine supplementation could improve vasodilatation by a NO-mediated endothelial-dependent mechanism.

Another interesting finding of the present study is that oral L-arginine supplementation decreased the susceptibility of LDL to copper-catalyzed oxidation, despite the fact that the lipid levels were not significantly changed after treatment. Because in vitro lag phase values most depend on the ratio between polyunsaturated fatty acid and lipophilic antioxidants in LDL (mainly vitamin E), we tried our best to control this factor. As mentioned before, we carefully excluded those patients with antioxidant vitamin supplements or other antioxidant drugs. Furthermore, patients were asked not to alter their medical treatment and diet otherwise throughout the duration of the study. Even though the mechanism behind this finding has not been fully elucidated, it has been shown that in the presence of LDL, NO production is reduced with increased superoxide generation by endothelial cell NO synthase. Such effect could be overcome by excess L-arginine.²¹ On the other hand, it was also shown that in vivo supplement of L-arginine could reduce oxidative stress by restoring glutathione concentration as well as superoxide dismutase and catalase activities.²² Thus, theoretically, L-arginine supplement may reduce oxidative stress either by preventing LDL-induced superoxide generation of endothelial cell NO synthase or by upregulating

antioxidant enzymes or both, which could then protect circulating LDL from oxidation as that shown in the present study.

In the present study, vitamin C was chosen as positive control. A significant improvement in endothelial function in patients with CAD after short- and long-term oral vitamin C administration had been reported.^{20,23} Ascorbic acid, which is the main water-soluble antioxidant, has been suggested to improve endothelial function by quenching superoxide and other reactive oxygen species,²⁴ thereby preventing NO inactivation. However, this is unlikely, as very high concentrations of vitamin C (>10 mmol/l) are needed to prevent the interaction of superoxide anion and NO: such high concentrations might be achievable by intraarterial infusions of high dose vitamin C, but not when given orally.²⁵ Several mechanisms that might be responsible for the improvement in endothelial function seen after oral administration of vitamin C other than superoxide scavenging have been proposed. Firstly, vitamin C is actively transported into cells and may play a role in the regulation of intracellular redox state and antioxidant defenses,²⁶ possibly via regulation of intracellular thiol species such as glutathione. This may improve NO action.^{27,28} Secondly, physiologic concentrations of intracellular ascorbate, reported to be in the range of 1–2.5 mmol/l,²⁹ might be sufficient to compete in the reaction between superoxide and NO.²⁵ Thus, intracellular sources of superoxide that impair NO might be scavenged by concentrations of vitamin C that may be achievable by oral supplementation of 500 mg daily. Thirdly, vitamin C supplement may enhance endothelial NO synthase activity by increasing intracellular tetrahydrobiopterin, an important co-enzyme of NO synthesis.³⁰ Previously used low L-arginine doses (dietary supplement of 0.5–1 g daily) have been essentially ineffective in providing any beneficial results on endothelial function. The lowest effective dose of L-arginine for endothelial-dysfunction-related condition is 6–8 g daily for adults, whereas a daily dose of 18–20 g provides maximal benefit without significant side effects.³¹ However, patient compliance for such a high dose of this amino acid may be poor and may require continuous reinforcement,³¹ hence our choice of a 10 g/d dose of L-arginine in this study.

Since both of the L-arginine and vitamin C supplement could exert endothelial protection through multiple but differential mechanisms, our results provide a rationale to combine use of the both agents in clinical vascular protection. It was recently shown the combination treatment of antioxidative vitamins and L-arginine, better than

L-arginine or antioxidative vitamins alone, could increase plasma NO concentration, reduce free radical generation, and improve microvascular circulation during in vivo ischemia-perfusion injury.³²

Finally, although previous study suggested that long-term administration of oral L-arginine improves endothelial function, downregulates cell adhesion molecules and proinflammatory cytokines, and reduces monocyte adherence to cultured human umbilical vein endothelial cells both in vitro and in vivo,^{13–17} there were no significant changes in serum inflammatory markers in the studied patients. However, brachial artery FMD was significantly improved after chronic administration of L-arginine. Our findings indicated that even in such a group of well-controlled CAD patients with minimal vascular inflammation, L-arginine could still improve endothelial function by its multiple vascular protection mechanisms.

Study limitations

There were some issues bearing on potential limitations of this study. One of them is the relevance of peripheral artery endothelial function for coronary events though prior studies have shown a close relationship between vasomotor responses in these two vascular beds.³³ In addition, serum concentration of L-arginine, NO, and ADMA were not checked in the present study. Instead, we showed that NO-induced FMD was improved by L-arginine supplement. Finally, the L-arginine was administered for only 4 weeks in a limited number of patients, longer-term effects remain unknown. Therefore, a large-scaled study with longer treatment of L-arginine may need to confirm the results of present study.

Clinical implications

The present study has important clinical implications. Considering the clinical significance of endothelial dysfunction in the progression as well as long-term prognosis of atherosclerosis disease,^{1,2,4–6} reversing endothelial dysfunction may partly explain the beneficial effects of interventions proven to reduce cardiovascular risk.^{7,8,34} Several investigators have also shown that chronic oral administration of L-arginine improved clinical symptoms of vascular disease, including angina pectoris.^{13,14,16,17,34} The present study represents a relatively well-designed study demonstrating a beneficial effect of L-arginine consumption on vascular endothelial function independent of its

anti-inflammatory effects. These findings fit well with the growing appreciation that diet supplementation such as L-arginine and lifestyle modifications are important approaches to the prevention and treatment of CAD.

Conclusions

Oral consumption of L-arginine for 4 weeks improved endothelial function and reduced the susceptibility of LDL to oxidation in CAD patients with chronic stable angina. The mechanisms for the vascular protection effects of L-arginine might be multiple including the direct, NO-dependent anti-oxidant capacity and NO-independent pathways. It is then suggested that oral L-arginine supplement could act as an effective adjuvant therapy for clinical CAD.

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