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Effect of Oral L- arginine on Blood Pressure and Symptoms and Endothelial Function in Patients With Systemic Hypertension, Positive Exercise Tests, and Normal Coronary Arteries

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Thirteen hypertensive patients with microvascular angina were studied before and after receiving oral L-arginine (4 weeks, 2 g, 3 times daily). L-arginine significantly improved angina class, systolic blood pressure at rest, and quality of life. Maximal forearm blood flow, plasma L-arginine, L-arginine:asymmetric dimethyl arginine ratio, and cyclic guanylate monophosphate increased significantly after treatment. In medically treated hypertensive patients with microvascular angina, oral L-arginine may represent a useful therapeutic option. ©2004 by Excerpta Medica, Inc.

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The present study investigated the effects of medium long-term oral administration of L-arginine on control of blood pressure and endothelial function in patients with hypertension, angina, positive exercise tests, and normal coronary arteries.

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Thirteen consecutive patients with grade 2 to 3 hypertension (6 women; age 65 ± 8 years; range 57 to 77), angina, positive exercise tests, and angiographically smooth coronary arteries, who were using antihypertensive and antianginal therapy, were recruited from the Division of Clinical Cardiology at the Instituto Scientifico San Raffaele. All patients gave informed consent to participate in this study. All patients were screened by

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TABLE 1 Data at Baseline and After L-Arginine Treatment*

	Baseline	L-Arginine
SBP at rest (mm Hg)	166 ± 27	146 ± 12*
DBP at rest (mm Hg)	84 ± 17	77 ± 10
Basal FBF (ml/min/100 ml)	2.5 ± 0.8	2.8 ± 0.5
Maximal post-ischemic FBF (ml/min 100 ml)	22 ± 5	31 ± 11*
Nitric oxide (μmol/L)	36 ± 22	35 ± 36
Homocysteine (μmol/L)	17 ± 8	13 ± 4
Cyclic guanylate monophosphate (pmol/ml)	2.30 ± 0.73	2.68 ± 0.86*
Asymmetric dimethyl arginine (μmol/L)	0.57 ± 0.12	0.64 ± 0.13
Arginine (μmol/L)	54 ± 17.4	71.7 ± 19.7*
Arginine: asymmetric dimethyl arginine	95 ± 22	113 ± 30*
Endothelin-1 (pg/ml)	6.44 ± 0.23	6.32 ± 0.38

Data are mean ± SD.
*p <0.05 versus control values.
DBP = diastolic blood pressure; SBP = systolic blood pressure.

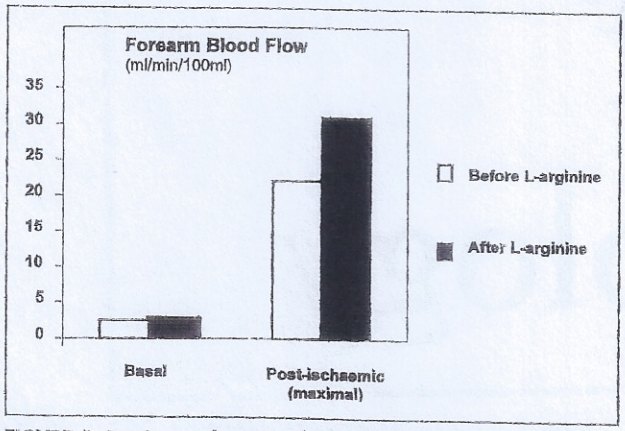

FIGURE 1. Resting and maximal FBF after ischemia (p <0.01).

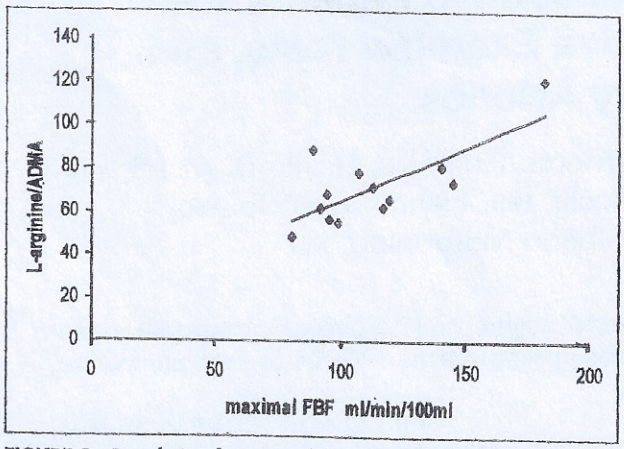

FIGURE 2. Correlation between the ratio of L-arginine to asymmetric dimethyl arginine (ADMA) and maximal FBF (r = 0.75, p <0.005).

clinical history (mean hypertension duration was 16 ± 8 years) and physical examination. Fifteen percent of patients were smokers, and another 15% were ex-smokers. Inclusion criteria were essential hypertension, angina pectoris and positive exercise tests, angiographically normal coronary arteries without inducible coronary spasm (ergonovine and hyperventilation), no prior myocardial infarction, absence of diabetes mellitus or other comorbid systemic metabolic disease, and normal lipid profile. The patients were on full antihypertensive and antianginal therapy titrated to optimal control of blood pressure. All patients were studied before and after 4 weeks of oral L-arginine (20-ml phials, 2 g, 3 phials daily) therapy.

All studies were performed in the same quiet room at room temperature, after an overnight fast, with the subject lying supine. Two days before testing, patients consumed a standard diet without ingredients that could influence endogenous nitric oxide synthesis (sausages, ham, and derivatives). Each patient rested 30 minutes before the study. At the beginning of each test, a plastic cannula (Abbocath, Abbott Laboratories, Chicago, Illinois) was inserted into a dorsal vein of 1 hand in the retrograde position, and the hand was placed in a Plexiglas box and maintained at 55°C for intermittent sampling (arterialized). To obtain the best possible conditions, it was necessary to arterialize venous blood by heating the hand. Blood samples were drawn at the beginning of the test. Proximal forearm blood flow (FBF; milliliters per minute per 100 ml) was measured by venous occlusion plethysmography at baseline and 5 minutes after induction of ischemia. FBF was measured at baseline immediately after blood sampling by venous occlusion plethysmography. Two cuffs were inflated simultaneously to obtain a collecting pressure of 60 mm Hg and a wrist occlusion pressure of 220 mm Hg. Changes in forearm volume were measured by a temperature-compensated rubber strain gauge placed distally to the tip of the cannula, as previously reported.[1] FBF was expressed as milliliters per minute per 100 ml of forearm tissue volume. Forearm ischemia was obtained by systolic blood pressure plus 50 mm Hg of cuff pressure exertion at arm level for 5 minutes. Maximal post-ischemic FBF was measured according to the method of Capaldo et al.[2] In addition, blood pressure was monitored throughout the study at 2-minute intervals. Endothelin-1 samples were measured with a commercial radioimmunoassay kit (NEN Life Science Products, Boston, Massachusetts). Specifically, to enrich the peptide from the plasma sample to measurable values, endothelin-1 was extracted on a SepPack C18 (Amprep, Amersham International Buckinghamshire, United Kingdom) and the eluate was evaporated in a Speedvac concentrator (Savant Instruments, Inc., Farmingdale, New York). The samples were then reconstituted in 250 μl of radioimmunoassay buffer and assayed. In the radioimmunoassay kit, the antiserum was a rabbit antiendothelin-1 antibody and the tracer was endothelin-1 labeled with iodine-125. Intra- and interassay coefficients of variation were 3.0% and 11.9%, respectively. Nitric oxide levels were measured by using metabolic end prod-

ucts, i.e., nitrite and nitrate, and enzymatic catalysis coupled with Griess reaction, as previously reported.[3] Cyclic guanylate monophosphate was assayed with a radioimmunoassay kit (Amersham International, Buckinghamshire, United Kingdom). Specifically, plasma samples were centrifuged at 4°C after the addition of cold 6% trichloroacetic acid. Supernatants were washed with 5 volumes of water-saturated diethyl ether and dried under a nitrogen stream at 60°C. The dried extracts were dissolved in 0.5 mol/L of acetate buffer, pH 5.8, and acetylated by a mixture of acetic acid anhydride and triethylamine and then assayed. Asymmetric dimethyl arginine was extracted from plasma samples using cation-exchange Strata SCX 100 mg columns (Phenomenex, Torrance, California) and assayed by high-performance liquid chromatography.[4] Homocysteine was measured with a microparticle enzyme immunoassay (IMX, Abbott Laboratories, Abbott Park, Illinois). At study entry and after treatment, all patients underwent assessment of anginal function based on the classification of the Canadian Cardiovascular Society,[5] which was performed by physicians not involved in the study and who were blinded to patients' treatment. Patients were also evaluated for the weekly number of angina episodes and the number of nitroglycerin tablets consumed (the week before study entry and the last week of L-arginine supplementation). Self-rated quality of life was assessed according to a visual analog scale, with a range of 0 to 100.

Data were elaborated with Excel 2000 (Microsoft, Redmond, Washington) and are presented as mean ± SD. A normality test was applied for the sample in the study. The paired 2-tailed Student's *t* test was used for data analysis. Statistical significance was defined as $p < 0.05$. Standard (Pearson's) correlation was performed to evaluate the correlation of biochemical and plethysmographic data.

Oral L-arginine was well tolerated by all subjects, and no side effects related to the drug were reported. There was no significant correlation between FBF and age, gender, total cholesterol, high-density lipoproteins, low-density lipoproteins, triglycerides, glycemia, homocysteine, asymmetric dimethyl arginine, L-arginine, or history of smoking. Table 1 lists the detailed results of the study. Compared with baseline values, arginine treatment resulted in a significant decrease in mean systolic blood pressure at rest (from 166 ± 27 to 146 ± 12 mm Hg; $p < 0.005$). Oral L-arginine did not influence basal FBF. Conversely, maximal FBF after 5 minutes of induced ischemia increased from 22 ± 5 to 31 ± 11 ml/min/100 ml ($p < 0.01$) when on arginine (Figure 1). Levels of nitric oxide and endothelin-1 remained unchanged, whereas homocysteine decreased slightly and cyclic guanylate monophosphate levels increased significantly after oral L-arginine treatment (from 2.30 ± 0.73 to 2.68 ± 0.86 pmol/ml; $p < 0.04$). Plasma L-arginine levels increased significantly after 4 weeks of supplementation (from 54 ± 17 to 72 ± 20 μmol/L; $p < 0.03$). Asymmetric dimethyl arginine concentrations after arginine supplementation did not differ from baseline values. However, the ratio of L-arginine to asymmetric dimethyl arginine increased significantly after L-arginine treatment (from 95 ± 22 to 113 ± 30 μmol/L; $p < 0.04$). Further, the ratio of L-arginine to asymmetric dimethyl arginine correlated positively with maximal FBF ($r = 0.75$, $p < 0.005$; Figure 2). Angina class, as determined by the Canadian Cardiovascular Society, decreased from 3.5 ± 1.1 to 1.8 ± 0.8, frequency of anginal attacks decreased from 12 ± 3 to 4 ± 1 per week, nitroglycerin intake decreased from 9 ± 2 to 3 ± 1 a week, and the overall self-rated quality of life increased from 24 ± 19 to 72 ± 27 (all p values < 0.001).

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The present study shows that endothelial function in hypertensive patients with microvascular angina can be improved by supplemental oral administration of L-arginine. Endothelial function improved after 4 weeks of supplementation with oral L-arginine, the physiologic precursor of nitric oxide, suggesting that this agent can restore endothelial function, probably by interfering with the pathway of L-arginine and nitric oxide.[6] This mechanism also might be responsible for the observed amelioration of symptoms and better overall quality of life as self-measured by the patients. These results are consistent with previous studies showing a beneficial effect of L-arginine supplementation in normotensive patients with anginaand normal coronary arteries (syndrome X),[7,8] suggesting a common pathogenetic mechanism.[9]

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