Journal of the American College of Cardiology
© 2001 by the American College of Cardiology
Published by Elsevier Science Inc.

Vol. 38, No. 2, 2001
ISSN 0735-1097/01/$20.00
PII S0735-1097(01)01380-8

Endothelium-Dependent Vasodilation Is Independent of the Plasma L-Arginine/ADMA Ratio in Men With Stable Angina

Lack of Effect of Oral L-Arginine on Endothelial Function, Oxidative Stress and Exercise Performance

Hamish A. Walker, MRCP,* Elaine McGing,* Ian Fisher, BSc,† Rainer H. Böger, MD,‡ Stefanie M. Bode-Böger, MD,§ Graham Jackson, FRCP,* James M. Ritter, FRCP,† Philip J. Chowienczyk, FRCP†

London, United Kingdom; and Hamburg and Hannover, Germany

OBJECTIVES This study was designed to determine the effect of two weeks' treatment with L-arginine on the ratio of plasma L-arginine to asymmetric dimethylarginine (ADMA), oxidative stress, endothelium-dependent vasodilatation to acetylcholine, exercise performance and heart rate variability in men with stable angina.

BACKGROUND The ratio of plasma L-arginine:ADMA has been proposed as a determinant of endothelium-dependent dilation; dietary supplementation with L-arginine has been shown to improve endothelium-dependent vasodilation and symptoms in some conditions.

METHODS Men (n = 40) with stable angina, at least one epicardial coronary artery with a stenosis >50% and a positive exercise test were randomized to receive L-arginine (15 g daily) or placebo for two weeks according to a double-blind parallel-group design. Plasma L-arginine, ADMA, 8-epi-prostaglandin $F_{2\alpha}$ (a marker of oxidative stress) and forearm vasodilator responses to brachial artery infusion of nitroprusside and acetylcholine (±L-arginine) were measured. A standard Bruce protocol exercise test was performed before and at the end of the treatment period.

RESULTS Plasma L-arginine increased after oral L-arginine, whereas ADMA remained unchanged, leading to an increase in the L-arginine/ADMA ratio of 62 ± 11% (mean ± SE, $p < 0.01$). Despite a significant enhancement in acetylcholine response by intra-arterial L-arginine at baseline, this response remained unchanged after oral L-arginine. Measures of oxidative stress and exercise performance after L-arginine/placebo were similar in placebo and active groups.

CONCLUSIONS In men with stable angina, an increase in plasma L-arginine/ADMA ratio after two weeks' oral supplementation with L-arginine is not associated with an improvement in endothelium-dependent vasodilatation, oxidative stress or exercise performance. (J Am Coll Cardiol 2001; 38:499–505) © 2001 by the American College of Cardiology

L-arginine, the physiological substrate for nitric oxide (NO) synthesis (1), improves endothelium-dependent vasodilation in hypercholesterolemic humans (2–4) and, in animal models, has anti-atherogenic actions reducing oxidative stress (5), platelet aggregation (6,7), monocyte adhesion (8,9) and the formation of intimal lesions (10,11). Decreased platelet aggregation has also been observed in humans (12). Such actions suggest a potential therapeutic role for L-arginine. Short-term administration of L-arginine produces vasodilation in patients with critical limb ischemia (13). When given orally, or as repeated parenteral infusions, L-arginine improves symptoms of claudication in patients with peripheral vascular disease (14,15). In patients with nonobstructive coronary artery disease (CAD), endothelium-dependent vasodilator responses are improved, with a reduction in symptoms and an improved exercise tolerance (16).

Beneficial effects of L-arginine are thought to result from increased NO production (17). The mechanism by which this occurs is controversial, however, because intracellular concentrations of L-arginine greatly exceed the Michaelis-Menton rate constant of endothelial NO synthase (NOS) (18). One possibility is that endothelial dysfunction is associated with an increase in asymmetric dimethylarginine (ADMA) (19) and possibly other endogenous inhibitors of NOS (20). Such inhibitors may compete with L-arginine as a substrate for NOS, increase oxidative stress (possibly via uncoupling of electron transport between NOS and L-arginine) and decrease the production/availability of endothelium-derived NO (20). As a result, endothelium-dependent vasodilation may be dependent on the plasma L-arginine/ADMA ratio (21).

The purpose of the present study was to determine whether endothelium-dependent vasodilation in men with stable angina that was caused by obstructive CAD correlates

From the *Cardiothoracic Centre, St. Thomas' Hospital, London, United Kingdom; †Department of Clinical Pharmacology, Centre for Cardiovascular Medicine and Biology, King's College, London, St. Thomas' Hospital, London, United Kingdom; ‡Department of Pharmacology, University Hospital, Hamburg-Eppendorf, Hamburg, Germany; §Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany. This work was supported by the British Heart Foundation.

Manuscript received December 14, 2000; revised manuscript received March 29, 2001, accepted April 10, 2001.

JACC Vol. 38, No. 2, 2001
August 2001:499–505

Abbreviations and Acronyms

ADMA = asymmetric dimethylarginine
AUC = area under forearm blood flow dose response curve
CAD = coronary artery disease
8-epi-$PGF_{2\alpha}$ = 8-epi-prostaglandin-$F_{2\alpha}$
LDL = low-density lipoprotein
NO = nitric oxide
NOS = nitric oxide synthase
SDMA = symmetric dimethylarginine

with the L-arginine/ADMA ratio and whether oral supplementation with L-arginine decreases oxidative stress and improves endothelium-dependent vasodilation. Further end points were to determine whether oral L-arginine supplementation improves myocardial ischemia or heart rate variability, a risk factor that may be influenced by L-arginine (22).

METHODS

Subjects. Men with stable angina (n = 40) fulfilling the study's inclusion and exclusion criteria were consecutively recruited after coronary angiography for investigation of angina. The study was approved by the St. Thomas' Hospital Research Ethics Committee, and all subjects gave written informed consent. Inclusion criteria comprised stable angina, at least one epicardial coronary artery with a stenosis >50% and a positive exercise test, defined as either 1 mm ST depression in two or more leads and/or anginal symptoms during the test. Exclusion criteria comprised an acute coronary syndrome within the previous three months, triple-vessel or left main stem disease requiring urgent revascularization, prior coronary artery bypass grafting, left ventricular failure with an ejection fraction (estimated from ventriculography) <30%, arrhythmias requiring treatment, diabetes mellitus, clinically significant renal, liver or systemic disease or anticoagulation or anti-oxidant treatment. Lipid-lowering medication was stopped for four weeks before the first visit and for the duration of the study. Baseline characteristics obtained after this period are shown in Table 1.

Study design. Subjects were randomized to receive either 5 g L-arginine (Martindale Pharmaceuticals, Romford, United Kingdom) or lactose placebo three times a day for two weeks according to a parallel-group double-blind placebo-controlled design. Investigations before and at the end of the supplementation period were performed over two days. Oral nitrates were withheld 24 h before each visit until completion of the investigations on the second day. On the first day of each visit, fasting blood samples were collected for L-arginine, ADMA, symmetric dimethylarginine (SMDA), 8-epi $PGF_{2\alpha}$, total and high density lipoprotein cholesterol, triglycerides, and renal and liver function tests. Forearm blood flow responses were determined, followed by attachment of a 24-h Holter recorder. Subjects returned on the second day for removal of the Holter recorder and to perform an exercise treadmill test.

Table 1. Subject Characteristics

	L-Arginine (n = 21)	Placebo (n = 19)
Age (yrs)	60 ± 2	63 ± 2
Smokers	6 (29%)	1 (5%)*
BMI (kg/m^2)	27 ± 1	26 ± 1
Total cholesterol (mmol/l)	5.7 ± 0.2	6.5 ± 0.2*
LDL cholesterol (mmol/l)	3.7 ± 0.2	4.2 ± 0.2
HDL cholesterol (mmol/l)	1.3 ± 0.1	1.2 ± 0.0
Triglycerides (mmol/l)	1.6 ± 0.2	2.3 ± 0.3*
Blood pressure (mm Hg)	143/78 ± 4/2	145/78 ± 4/2
Anatomy—1-vessel	5 (24%)	4 (21%)
Anatomy—2-vessel	3 (14%)	5 (26%)
Anatomy—3-vessel	13 (62%)	10 (53%)
Myocardial infarction	11 (52%)	10 (53%)
History of hypertension	12 (57%)	9 (47%)
Medication—ACE inhibitors	2 (10%)	3 (16%)
Medication—beta-blockers	18 (86%)	11 (58%)*
Medication—Ca^{++} antagonists	6 (29%)	14 (74%)*
Medication—nitrates	2 (10%)	8 (42%)*
Medication—nicorandil	1 (5%)	1 (5%)

* $p < 0.05$ compared with L-arginine group. Values are means ± SE or %.
ACE = angiotensin-converting enzyme; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Plasma L-arginine, ADMA, SDMA and 8-epi-$PGF_{2\alpha}$. Plasma concentrations of L-arginine, ADMA and SDMA were measured by high-performance liquid chromatography and precolumn derivatization with o-phthaldialdehyde as previously described (21). Plasma concentrations of 8-epi-$PGF_{2\alpha}$, a nonenzymic oxidation product of arachidonic acid, were used as a marker of oxidative stress (23). The compound 8-epi-$PGF_{2\alpha}$ was determined by negative-ion chemical ionization mass spectrometry using tetradeuterated 8-epi-$PGF_{2\alpha}$ as internal standard (24).

Forearm blood flow studies. Forearm blood flow measurements were performed in a quiet clinical laboratory (temperature 24°C). Blood flow was measured in both forearms using venous occlusion plethysmography with electrically calibrated strain gauges (25,26). Wrist occlusion pressure was 180 mm Hg, and collecting cuff pressure 40 mm Hg. An unmounted 27-gauge steel needle (Cooper's Needleworks, Birmingham, United Kingdom) was sealed by dental wax to an epidural catheter and inserted into the left brachial artery under sterile conditions using <1 ml of 1% lignocaine hydrochloride to provide local anesthesia. After cannulation of the brachial artery, saline was infused at 1 ml/min for 20 min using a constant-rate infusion pump. A cumulative rising dose of nitroprusside (David Bull Laboratories, Victoria, Australia, 3, 10 and 30 nmol/min, each dose for 5 min) was infused and, after a 15 min washout period was followed by acetylcholine (CIBA Vision Opthalmics, Southampton, United Kingdom, 40, 80 and 160 nmol/min, each dose for 5 min). Finally, after a further 20 min washout period, L-arginine (Torbay Pharmacy, Torbay Hospital, United Kingdom, 50 μmol/min) was infused alone and then co-infused during a second identical

Table 2. Plasma Concentrations of Arginine, Methylarginines, 8-epi-$PGF_{2\alpha}$, Lipid Profiles and Blood Pressure Before (Visit 1) and at the End (Visit 2) of Treatment With Oral Arginine/Placebo

	L-Arginine		Placebo	
	Visit 1	Visit 2	Visit 1	Visit 2
L-arginine (μmol/l)	80 ± 2	117 ± 4*	83 ± 4	89 ± 5
ADMA (μmol/l)	5.6 ± 0.3	5.4 ± 0.4	4.9 ± 0.3	4.8 ± 0.3
SDMA (μmol/l)	0.59 ± 0.05	0.79 ± 0.10	0.60 ± 0.06	0.61 ± 0.06
L-arg/ADMA	15.1 ± 1.0	23.6 ± 1.5*	17.6 ± 1.1	20.1 ± 1.6
8-epi-$PGF_{2\alpha}$ (nmol/l)	0.30 ± 0.05	0.22 ± 0.03	0.25 ± 0.04	0.24 ± 0.04
LDL-chol (mmol/l)	3.7 ± 0.2	3.9 ± 0.2	4.2 ± 0.2	4.5 ± 0.2
HDL-chol (mmol/l)	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Triglycerides (mmol/l)	1.6 ± 0.2	1.5 ± 0.1	2.3 ± 0.3	2.5 ± 0.4
Blood pressure (mm Hg)	143/78 ± 4/2	140/79 ± 4/2	145/75 ± 4/2	141/76 ± 4/2

* $p < 0.01$ compared with visit 1.
ADMA = asymmetric dimethylarginine; HDL-chol = high density lipoprotein cholesterol; L-arg/ADMA = ratio of L-arginine to ADMA: LDL-chol = low density lipoprotein cholesterol.

cumulative rising dose infusion of acetylcholine. Forearm blood flow was recorded during the last 3 min of each infusion period using 10-s venous occlusions every 20 s. Blood flow was calculated from the mean of the last five venous inflations and expressed in units of ml/min per 100 ml of forearm volume (25). Blood pressure was measured by mercury sphygmomanometry at the end of the study. Area under dose response curve (AUC, arbitrary units) was calculated using values of absolute blood flow (27) for blood flow responses to nitroprusside, acetylcholine and acetylcholine in the presence of L-arginine.

Exercise treadmill test. Symptom-limited standard Bruce protocol exercise tests were performed by the same investigators, blind to conditions. In one patient the test was terminated because of development of ST depression >4 mm. In addition to total exercise time, other parameters studied included time to 1 mm ST depression (for a minimum of 10 consecutive beats), time to onset of symptoms and rate pressure product.

24-h electrocardiographic recording. Heart rate variability was determined from 24-h ambulatory recording of the electrocardiograph using two-channel Oxford Tracker 2 recorders (Reynolds Medical Ltd., Hertford, United Kingdom). Tape recordings free of ectopic beats and artifact were analyzed for heart rate variability in the time domain using Reynolds Professional and Century 3000 analysis software (Reynolds Medical Ltd., Hertford, United Kingdom) as recommended by the 1996 European Task Force Guidelines (28).

Statistical power and analysis. Power calculations were based upon the previously assessed reproducibility of the blood flow responses to acetylcholine measured by forearm plethysmography (27). A sample size of 40 was estimated to allow a 20% difference in the AUC to acetylcholine to be determined with a power of >90%. Baseline characteristics were compared using chi-squared or Student *t* test. Correlations were sought using least-squares regression analysis and analysis of covariance where appropriate. Outcome measures were compared between groups using two-way analysis of variance. Forearm blood flow responses were assumed to be log normally distributed (27), and statistical analysis was performed on log-transformed data, with $p < 0.05$ (two-sided) being taken as significant.

RESULTS

One subject in the L-arginine group (n = 21) developed unstable angina and was admitted for percutaneous angioplasty; a second subject in the placebo group (n = 19) was unable to complete the study, because of a viral illness. Analysis was thus performed on 20 subjects who received L-arginine and 18 who received placebo. Blood pressure and lipid profiles remained similar before and after supplementation in both groups (Table 2).

Plasma L-arginine, ADMA, SDMA and 8-epi $PGF_{2\alpha}$. Baseline values of L-arginine were similar to those previously obtained in control subjects, but those of ADMA were higher, with the ratio of L-arginine/ADMA <50% of that seen in historical controls and similar to values obtained in hypercholesterolemic subjects (21). After supplementation with L-arginine, plasma L-arginine increased from 80 ± 2 μmol/l to 117 ± 4 μmol/l ($p < 0.001$), whereas in the placebo group, L-arginine remained unchanged. Plasma ADMA, SDMA and 8-epi-$PGF_{2\alpha}$ remained similar before and after supplementation in both groups (Table 2). The mean increase in L-arginine/ADMA ratio in the L-arginine group was 62 ± 11% ($p < 0.01$).

Forearm blood flow responses. Forearm blood flow responses to brachial artery infusion of nitroprusside and acetylcholine are shown in Figure 1. Responses to acetylcholine were impaired by 20% relative to age-matched historical controls. In the placebo group blood-flow responses to nitroprusside and acetylcholine did not change significantly after supplementation (AUC for nitroprusside at baseline: 21.5 ± 1.4 vs. 22.6 ± 1.4 arbitrary units after placebo; AUC for acetylcholine at baseline: 24.8 ± 3.4 vs. 25.8 ± 2.6 after placebo). After L-arginine supplementation

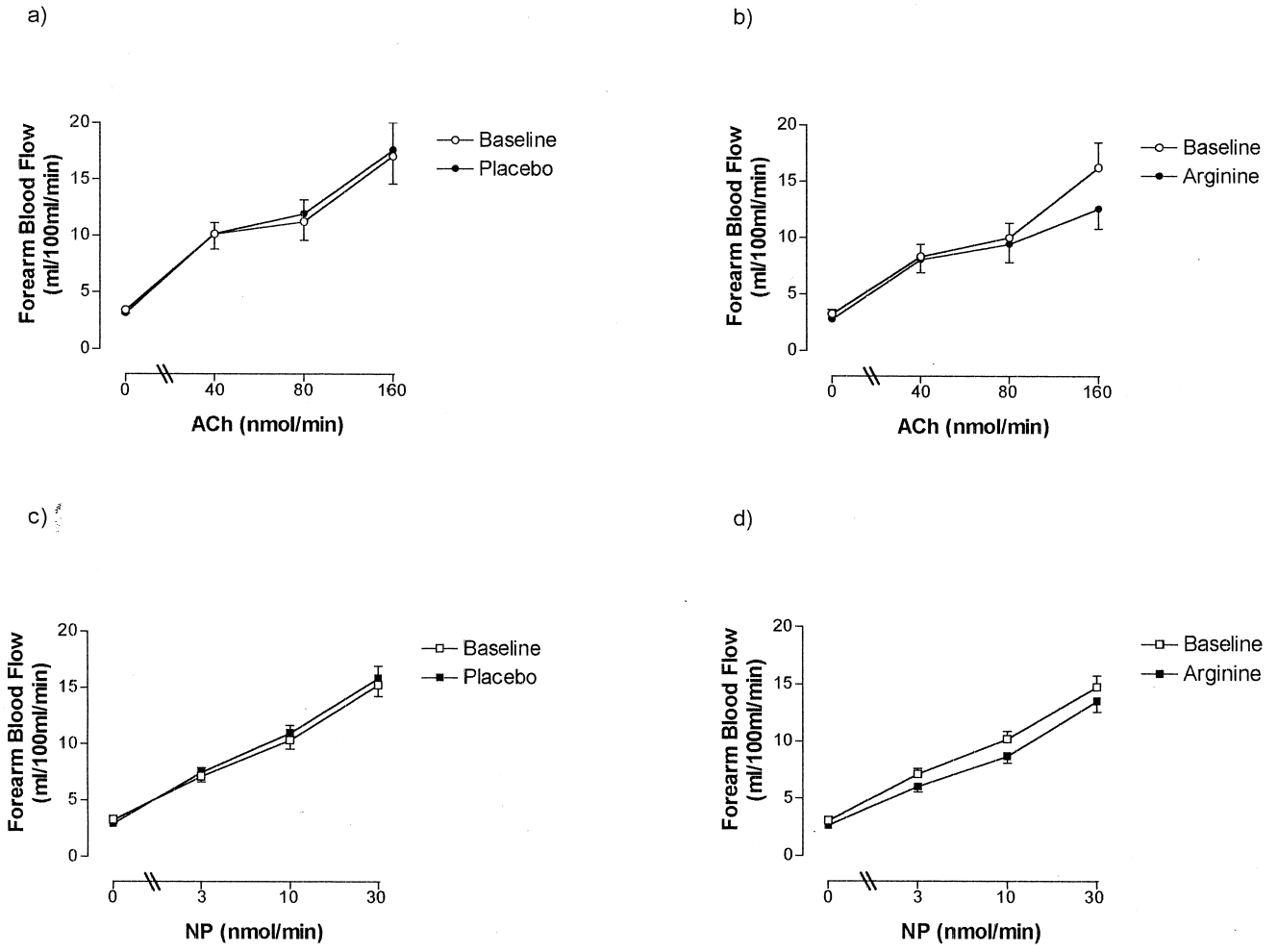

Figure 1. Forearm blood flow responses to **a)** acetylcholine (ACh) at baseline and after placebo; **b)** ACh at baseline and after L-arginine; **c)** nitroprusside (NP) at baseline and after placebo; **d)** nitroprusside at baseline and after L-arginine. Responses to NP decreased significantly ($p < 0.01$) after L-arginine compared with placebo by two-way analysis of variance. Those to ACh tended to decrease but the difference did not reach significance ($p < 0.1$).

there was a small but significant decrease in the response to nitroprusside (AUC 21.1 ± 1.4 at baseline compared with 18.5 ± 1.2 after supplementation, $p < 0.01$ for the change relative to that in the placebo group). There was a nonsignificant trend to a lower response to acetylcholine after L-arginine (22.3 ± 2.8 vs.19.7 ± 2.9, $p < 0.1$ for the change relative to that in the placebo group). The upper 95% confidence limit for an improvement in blood flow response (AUC) to acetylcholine after oral L-arginine (relative to placebo) was 3%. There was no correlation between AUC for acetylcholine and the L-arginine/ADMA ratio at baseline ($r = 0.28$, $p = 0.10$). The within-subject coefficient of variation for the AUC response for acetylcholine calculated from the baseline and placebo visits was 28%.

Effects of intra-arterial L-arginine. At baseline, intra-arterial L-arginine produced a similar increase in the AUC response to acetylcholine in the L-arginine group (18.3 ± 1.8% increase) and in the placebo group (19.5 ± 8.5% increase, each $p < 0.01$). After supplementation, the corresponding values were 26 ± 9.8% and 33.3 ± 12.4% in the L-arginine and placebo groups, respectively (p = ns compared with baseline). There was no correlation between the percent increase in forearm blood flow response to acetylcholine in the presence of intrabrachial L-arginine with the plasma L-arginine/ADMA ratio at baseline ($r = -0.06$, $p = 0.6$). During short-term intra-arterial infusion of L-arginine, plasma concentrations of L-arginine in the infused arm were estimated (from the rate of drug infusion and forearm blood flow) to be approximately 500 μmol/L, more than fivefold higher than baseline values, producing an increase in the L-arginine/ADMA ratio of approximately 500%.

Exercise tests. No significant differences were observed in the incidences of ST depression and symptoms in either group before or after supplementation (Table 3). Total exercise time increased within both groups, from 452 ± 30 s to 497 ± 31 s in the L-arginine group, and from 467 ± 36 s to 520 ± 37 s in the placebo group (each $p < 0.01$). However, there was no significant difference between groups ($p = 0.7$). Similarly, changes in rate pressure product, time to 1 mm ST depression or time to onset of symptoms in L-arginine and placebo groups were similar.

Table 3. Exercise Tolerance and Ischemia Before (Visit 1) and at the End (Visit 2) of Treatment With Oral Arginine/Placebo

	L-Arginine		Placebo	
	Visit 1	Visit 2	Visit 1	Visit 2
Total exercise time(s)	452 ± 30	497 ± 31*	467 ± 36	520 ± 37*
Rate pressure product (mm Hg/s)	215 ± 12	218 ± 13	223 ± 12	216 ± 11
Time to 1 mm ST depression(s)	352 ± 42	339 ± 36	325 ± 63	306 ± 55
Time to symptoms (s)	348 ± 32	404 ± 40	314 ± 36	305 ± 38

*$p < 0.05$ compared with visit 1.

24-h Holter monitoring and heart rate variability. There were no significant changes in indices of overall, short- and long-term heart rate variability: standard deviation of R-R intervals, standard deviation of mean R-R intervals calculated over 5-min intervals or root mean square of differences between successive R-R intervals in either group (data not shown).

DISCUSSION

Effects of short-term intra-arterial L-arginine on endothelium-dependent vasodilation. L-arginine has received much attention as a potential treatment for conditions associated with endothelial dysfunction (17), its effects being attributed to an increase in the plasma L-arginine/ADMA ratio (29). Short-term intra-arterial administration, producing an increase of approximately 500% in the L-arginine/ADMA ratio, improved vascular responses to brachial artery infusion of acetylcholine in the men with stable angina in this study. This observation is consistent with those of other investigations (2,3,30,31). However, the blood-flow response to acetylcholine was not significantly correlated with the plasma L-arginine/ADMA ratio. Furthermore, the improvement in blood flow obtained by short-term intra-arterial infusion of L-arginine did not correlate significantly with the plasma L-arginine/ADMA ratio. This suggests that the plasma L-arginine/ADMA ratio is not a major determinant of endothelium-dependent vasodilation in these patients.

Lack of effect of oral L-arginine on endothelial function. Oral supplementation with L-arginine resulted in a 60% increase in the L-arginine/ADMA ratio. However, we did not observe any improvement in vascular function. Indeed, there was a quantitatively small but statistically significant decrease in response to the NO donor nitroprusside and a tendency for a decreased response to acetylcholine. Thus, although the variability of the response to acetylcholine was greater than in previous studies (27), the upper 95% confidence limit for the improvement in blood flow response (AUC) to acetylcholine after oral L-arginine (relative to placebo) was only 3%. We cannot exclude the possibility that the failure to produce an improvement in the acetylcholine response was due to the relatively modest increase in L-arginine/ADMA ratio (compared with that achieved with intra-arterial L-arginine). However, these observations, taken together with the lack of correlation between the acetylcholine response and the L-arginine/ADMA ratio, argue against an important influence of the arginine/ADMA ratio in determining endothelium-dependent vasodilation in patients with symptomatic atherosclerotic CAD. The dose of L-arginine used is close to the maximum tolerated (32). Thus, even if the L-arginine/ADMA ratio is an important determinant of the acetylcholine response, the present findings suggest that oral L-arginine is unlikely to elevate this enough to improve endothelial function in these patients.

Lack of effect of L-arginine on oxidative stress. L-arginine reduces the production of superoxide anion in endothelial cells incubated with low-density lipoprotein (LDL) (33) and reduces urinary 8-epi-$PGF_{2\alpha}$ in cholesterol-fed rabbits (5). Our study is the first, to our knowledge, to assess the effects of oral L-arginine supplementation on the plasma marker of oxidative stress 8-epi-$PGF_{2\alpha}$. Although 8-epi-$PGF_{2\alpha}$ cannot be regarded as a universal marker of oxidant stress (for example, lipid peroxidation may not occur, despite increased oxidant stress, because of protection by anti-oxidants), it is a more sensitive marker of oxidative injury in endothelial cells than other indices such as concentrations of thiobarbituric acid reactive substances (34). Furthermore, we have previously demonstrated changes in 8-epi-$PGF_{2\alpha}$ to parallel those in endothelium-dependent dilation (35). Lack of effect of oral L-arginine on plasma 8-epi-$PGF_{2\alpha}$ suggests that the results observed in hypercholesterolemic rabbits cannot necessarily be extrapolated to patients with CAD.

Lack of effect of L-arginine on exercise performance and heart rate variability. Short-term administration of L-arginine improves coronary microvascular function in hypercholesterolemic subjects (3) and therefore might be expected to improve myocardial ischemia in patients with stable angina. In the present study, however, we observed no greater improvement in exercise tolerance than in the placebo group. These findings do not, therefore, support a role for L-arginine as an anti-anginal agent. The possibility that L-arginine modulates parasympathetic tone, which is a major determinant of heart rate variability, was raised in an earlier study (22), but the present study does not support this conclusion in men with stable angina.

Comparison with other studies of L-arginine supplementation. The lack of effect of increasing the L-arginine/ADMA ratio on endothelial function and on oxidative

stress in the present study contradicts findings in animals and in some but not all of the clinical studies. Oral L-arginine increases flow-mediated dilation (FMD) of the brachial artery in young hypercholesterolemic subjects with no evidence of CAD (4) and has also been shown to improve FMD in young men with CAD (36). In this study, L-arginine 7 g three times a day led to a marked improvement in flow-mediated brachial artery dilation within three days. Although mean LDL cholesterol was similar to that in the present study, the subjects were younger (41 ± 2 years) and were selected on the basis of poor endothelial function. A dose of 3 g three times a day improved endothelial function and symptoms in patients with nonobstructive CAD (16). However, subjects with heart failure did not benefit from a four-week course of L-arginine 20 g daily (37). In a recent study in a group of 29 patients with similar characteristics to those in the present study, L-arginine 9 g per day for 28 days produced no effect on nitrogen oxides or FMD of the brachial artery, or on the cell adhesion molecules E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (38). In that study, as in the present study, patients continued on medical therapy. It is possible that conventional treatment has a beneficial effect on endothelial function (which in the present study was only modestly impaired), masking effects of oral L-arginine. For L-arginine to be of therapeutic value, however, effects over and above conventional therapy would be necessary.

An alternative possibility is that improvement with dietary L-arginine may depend upon the predominant mechanism underlying the endothelial dysfunction. Young patients with marked hypercholesterolemia or patients with nonobstructive CAD, for example, may benefit from oral L-arginine to a greater extent than older patients with symptomatic coronary atherosclerosis. It is possible that, in younger patients with uncomplicated hypercholesterolemia, the arginine/ADMA ratio is a more important determinant of endothelial function, as in the hypercholesterolemic rabbit.

Conclusion. In men with stable angina and mild hypercholesterolemia, elevating the L-arginine/ADMA ratio with oral L-arginine does not improve endothelium-dependent vasodilation. A decreased L-arginine/ADMA ratio may not be the predominant mechanism underlying endothelial dysfunction in this group of patients. Furthermore, L-arginine does not possess significant anti-oxidant or anti-anginal activities in such patients.

Reprint requests and correspondence: Dr. P.J. Chowienczyk, Department of Clinical Pharmacology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, United Kingdom. E-mail: phil.chowienczyk@kcl.ac.uk.

REFERENCES

1. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesise nitric oxide from L-arginine. Nature 1988;333:664–6.
2. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP. L-Arginine improves endothelium-dependent vasodilation in hypercholesterolaemic humans. J Clin Invest 1992;90:1248–53.
3. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in the coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 1991;338:1546–50.
4. Clarkson P, Adams MR, Powe AJ, et al. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolaemic young adults. J Clin Invest 1996;97:1989–94.
5. Boger RH, Bode-Boger SM, Phivthong-ngam L, et al. Dietary L-arginine and alpha-tocopherol reduce vascular oxidative stress and preserve endothelial function in hypercholesterolemic rabbits via different mechanisms. Atherosclerosis 1998;141:31–43.
6. Tsao PS, Theilmeier G, Singer AH, Leung LL, Cooke JP. L-arginine attenuates platelet reactivity in hypercholesterolemic rabbits. Arterioscler Thromb 1994;14:1529–33.
7. Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP. Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. Circulation 1994;89:2176–82.
8. Bode-Boger SM, Boger RH, Kienke S, et al. Chronic dietary supplementation with L-arginine inhibits platelet aggregation and thromboxane A2 synthesis in hypercholesterolaemic rabbits in vivo. Cardiovasc Res 1998;37:756–64.
9. Brandes RP, Brandes S, Boger RH, Bode-Boger SM, Mugge A. L-arginine supplementation in hypercholesterolemic rabbits normalizes leukocyte adhesion to non-endothelial matrix. Life Sci 2000;66:1519–24.
10. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolaemic rabbit. J Clin Invest 1992;90:1168–72.
11. 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.
12. 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 (Colch) 1994;87:303–10.
13. Bode-Boger SM, Boger RH, Alfke H, et al. L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. Circulation 1996;93:85–90.
14. Maxwell AJ, Anderson BE, Cooke JP. Nutritional therapy for peripheral arterial disease: a double-blind, placebo-controlled, randomized trial of HeartBar. Vasc Med 2000;5:11–9.
15. Boger RH, Bode-Boger SM, Thiele W, Creutzig A, Alexander K, Frolich JC. Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. J Am Coll Cardiol 1998;32:1336–44.
16. Lerman A, Burnett JC, Jr., Higano ST, McKinley LJ, Holmes DR, Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. Circulation 1998;97:2123–8.
17. Cooke JP, Tsao PS. Arginine: a new therapy for atherosclerosis? Circulation 1997;95:430–7.
18. Chowienczyk P, Ritter J. Arginine: NO more than a simple amino acid? Lancet 1997;350:901–2.
19. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 1999;99:3092–5.
20. Leiper J, Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. Cardiovasc Res 1999;43:542–8.
21. Boger RH, Bode-Boger SM, Szuba A, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842–7.
22. Nomura M, Nakaya Y, Nada T, et al. Effects of intravenous administration of L-arginine on autonomic nervous activities. Analysis of heart rate variability. Jpn Heart J 1998;39:331–8.
23. Morrow JD, Roberts LJ. The isoprostanes. Current knowledge and directions for future research. Biochem Pharmacol 1996;51:1–9.
24. Gopaul NK, Änggård EE, Mallet AI, Beteridge DJ, Wolff SP, Nourouz-Zadeh J. Plasma 8-epi-$PGF_{2\alpha}$ levels are elevated in individuals with non-insulin dependent diabetes mellitus. FEBS Lett 1995;368:225–9.
25. Whitney RJ. The measurement of volume changes in human limbs. J Physiol (London) 1953;121:1–27.
26. Hokanson DE, Sumner DS, Strandness DE, Jr. An electrically

calibrated plethysmograph for direct measurement of limb blood flow. IEEE Trans Biomed Eng 1975;22:25–9.
27. Walker HA, Jackson G, Ritter JM, Chowienczyk PJ. Assessment of forearm vasodilator responses to acetylcholine and albuterol by strain gauge plethysmography: reproducibility and influence of strain gauge placement. Br J Clin Pharmacol 2001;51:225–9.
28. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043–65.
29. Miyazaki H, Matsuoka H, Cooke JP, et al. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 1999;99:1141–6.
30. Chowienczyk PJ, Watts GF, Cockcroft JR, Brett SE, Ritter JM. Sex differences in endothelial function in normal and hypercholesterolaemic subjects. Lancet 1994;344:305–6.
31. Chowienczyk PJ, Kneale BJ, Brett SE, Paganga G, Jenkins BS, Ritter JM. Lack of effect of vitamin E on L-arginine-responsive endothelial dysfunction in patients with mild hypercholesterolaemia and coronary artery disease. Clin Sci (Colch) 1998;94:129–34.
32. Barbul A. Arginine: biochemistry, physiology, and therapeutic implications. J Parenter Enteral Nutr 1986;10:227–38.
33. Pritchard KA, Groszek L, Smalley DM, et al. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. Circ Res 1995;77:510–8.
34. Hart CM, Karman RJ, Blackburn TL, Gupta MP, Garcia JG, Mohler ER III. Role of 8-epi PGF2alpha, 8-isoprostane, in H2O2-induced derangements of pulmonary artery endothelial cell barrier function. Prostaglandins Leukot Essent Fatty Acids 1998;58:9–16.
35. Chowienczyk PJ, Brett SE, Gopaul NK et al. Oral treatment with an antioxidant (raxofelast) reduces oxidative stress and improves endothelial function in men with type II diabetes. Diabetologia 2000;43:974–7.
36. Adams MR, McCredie R, Jessup W, Robinson J, Sullivan D, Celermajer DS. Oral L-arginine improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease. Atherosclerosis 1997;129:261–9.
37. Chin-Dusting JP, Kaye DM, Lefkovits J, Wong J, Bergin P, Jennings GL. Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. J Am Coll Cardiol 1996;27:1207–13.
38. Blum A, Hathaway L, Mincemoyer R, et al. Oral L-arginine in patients with coronary artery disease on medical management. Circulation 2000;101:2160–4.