

## Nitric oxide activity in the human coronary circulation. Impact of risk factors for coronary atherosclerosis.

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**Research Article**

The bioavailability of nitric oxide (NO) in the human coronary circulation at rest and after acetylcholine (ACH)-induced vasodilation was investigated in 32 patients with angiographically normal coronary arteries. The effects of intracoronary L-NG monomethyl arginine (L-NMMA) were investigated at rest and after ACH, sodium nitroprusside, and adenosine. L-NMMA (64  $\mu$ mol/min) increased resting coronary vascular resistance by 22% ( $P < 0.001$ ), reduced distal epicardial coronary artery diameter by 12.6% ( $P < 0.001$ ), and inhibited ACH-induced coronary epicardial and microvascular vasodilation. These effects were reversed with intracoronary L-arginine. L-NMMA did not inhibit dilation in response to sodium nitroprusside and adenosine. 23 patients were exposed to one or more coronary risk factors. The vasoconstrictor effect of L-NMMA on the epicardial and microvessels was greater in patients free of risk factors: Coronary vascular resistance was 36% higher in patients without risks, compared to 17% higher in patients with risks ( $P < 0.05$ ). Both epicardial and microvascular dilator effects of ACH were greater in patients without risk factors, and the inhibition of these effects by L-NMMA was also greater in patients without risk factors. Thus: (a) NO contributes importantly to resting epicardial and coronary microvascular tone, (b) coronary vascular dilation in response to ACH is predominantly due to increased production of NO, and (c) despite the absence of angiographic evidence of atherosclerosis, exposure to coronary [...]

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# Nitric Oxide Activity in the Human Coronary Circulation

## Impact of Risk Factors for Coronary Atherosclerosis

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### Abstract

The bioavailability of nitric oxide (NO) in the human coronary circulation at rest and after acetylcholine (ACH)-induced vasodilation was investigated in 32 patients with angiographically normal coronary arteries. The effects of intracoronary L-N<sup>G</sup> monomethyl arginine (L-NMMA) were investigated at rest and after ACH, sodium nitroprusside, and adenosine. L-NMMA (64 μmol/min) increased resting coronary vascular resistance by 22% ( $P < 0.001$ ), reduced distal epicardial coronary artery diameter by 12.6% ( $P < 0.001$ ), and inhibited ACH-induced coronary epicardial and microvascular vasodilation. These effects were reversed with intracoronary L-arginine. L-NMMA did not inhibit dilation in response to sodium nitroprusside and adenosine.

23 patients were exposed to one or more coronary risk factors. The vasoconstrictor effect of L-NMMA on the epicardial and microvessels was greater in patients free of risk factors: Coronary vascular resistance was 36% higher in patients without risks, compared to 17% higher in patients with risks ( $P < 0.05$ ). Both epicardial and microvascular dilator effects of ACH were greater in patients without risk factors, and the inhibition of these effects by L-NMMA was also greater in patients without risk factors.

Thus: (a) NO contributes importantly to resting epicardial and coronary microvascular tone, (b) coronary vascular dilation in response to ACH is predominantly due to increased production of NO, and (c) despite the absence of angiographic evidence of atherosclerosis, exposure to coronary risk factors is associated with reduced resting and stimulated bioavailability of NO from the human coronary circulation. (*J. Clin. Invest.* 1995; 95:1747–1755.) Key words: nitric oxide • coronary circulation • endothelium-dependent vasodilation • coronary risk factors

### Introduction

The vascular endothelium plays an important role in maintaining blood vessel tone by releasing different dilator and constrictor substances (1–4). Endothelium-derived relaxing factors

are produced in response to a variety of receptor-dependent and -independent pharmacologic probes, including acetylcholine (ACH),<sup>1</sup> substance P, bradykinin, ADP, and calcium ionophore, and also in response to physiologic phenomena such as increases in shear stress (1–9). One important endothelium-derived relaxing factor has been characterized as nitric oxide (NO), or a compound closely related to NO (10–12). The capacity of blood vessels to release NO at rest or with stimulation is variable between organs and species (13–22). Studies of human coronary endothelial function have largely relied on stimulating production of endothelium-derived relaxing factor with ACH (8, 9, 23–31). In these investigations, abnormal endothelial function is considered to be present when epicardial coronary arteries constrict and blood flow increase is attenuated with intracoronary ACH, but whether this is an accurate reflection of the state of the endothelium in general, and of NO bioactivity in particular, is unknown. There are sparse data on the basal activity of NO in human coronary vasculature *in vivo*, and doubts have been raised in a recent study as to whether ACH causes release of NO from the human coronary vasculature (32). Moreover, although the effect of ACH is depressed in the presence of risk factors for coronary atherosclerosis (33, 34), it is not known whether risk factors adversely affect basal or stimulated production of NO from the human coronary circulation *in vivo*.

We therefore designed this study to: (a) investigate the role of NO in modulating resting coronary vascular tone, (b) study whether ACH releases NO from the human coronary vasculature, and (c) elucidate the effects of exposure to risk factors for coronary atherosclerosis on NO production from the human coronary arteries.

### Methods

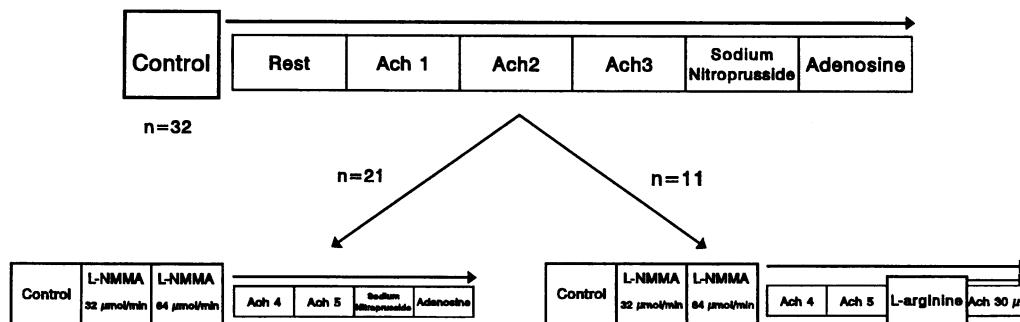
**Patients.** We studied 32 patients with angiographically normal coronary arteries who were undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. Patients with previous myocardial infarction or valvular heart disease were excluded. There were 15 (47%) men and 17 women with a mean age of 46±11 yr. 10 patients were hypertensive (BP > 140/90), but none had echocardiographic evidence of left-ventricular hypertrophy. Hypercholesterolemia (total cholesterol > 260 mg/dl) was present in 7 patients, 10 were current smokers, 6 had diabetes, and 5 patients were older than 60 yrs of age. 23 patients were exposed to one or more of the aforementioned risk factors, and 9 had none. All cardiac medications were with-

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1. Abbreviations used in this paper: ACH, acetylcholine; CVR, coronary vascular resistance; L-NMMA, L-N<sup>G</sup> monomethyl arginine; NO, nitric oxide.



**Figure 1.** Protocol design. ACH 1, 2, and 3 denote the dose-response curve with ACH. ACH 4 and 5 denote the two highest vasodilating doses of ACH that were reinfused after L-NMMA.

drawn at least 48 h before the study. Aspirin was discontinued for at least 7 d, and cholesterol-lowering medications were stopped 1 mo before the study in the one patient on this therapy. The study was approved by the National Heart, Lung, and Blood Investigational Review Board, and informed written consent was obtained from all patients.

**Protocol.** After completion of diagnostic coronary arteriography, a 6-French guide catheter was introduced into the left main coronary artery, and blood flow velocity was measured using a 0.018-inch wire equipped with a Doppler crystal at its tip (Cardiometrics Flowire; Cardiometrics, Inc., Sunnyvale, CA) (35, 36). The Doppler flow wire was advanced into either the left main ( $n = 6$ ) or the proximal segment of a major epicardial coronary artery (left anterior descending coronary artery in 22 patients and the circumflex coronary artery in 4 patients). The wire tip was carefully positioned in a segment of the vessel that was straight, was free of any branches 1 cm from the tip, produced an adequate flow velocity signal, and could be imaged without overlap from other vessels, thus allowing for quantitative measurements of the coronary artery diameter. All drugs were infused directly into the left main coronary artery via the guide catheter at infusion rates ranging from 1 to 2 ml/min. After a 5-min infusion of dextrose 5% at 1 ml/min, baseline coronary blood flow velocity and coronary angiography were performed. A 7-French multipurpose A2 catheter was inserted via the right internal jugular vein into the midcoronary sinus for blood sampling. Oxygen saturation of arterial and coronary sinus venous blood was measured using an oximeter in 23 patients at baseline and after infusions of L-N<sup>G</sup> monomethyl arginine (L-NMMA; Calbiochem-Novabiochem Corp., La Jolla, CA).

Endothelium-dependent vasodilation was estimated by performing a dose-response curve with incremental infusions of intracoronary ACH starting at 3  $\mu\text{g}/\text{min}$  (Fig. 1). After 2 min, blood flow velocity and coronary angiography were performed. This was followed by 2-min infusions of 30, 100, and 300  $\mu\text{g}/\text{min}$  of intracoronary ACH with measurement of Doppler flow velocity and angiography after each increment. ACH dose was not increased further once an infusion either reduced blood flow velocity or severely (> 50%) narrowed the epicardial coronary tree. Thus, all patients received the 30- $\mu\text{g}/\text{min}$  dose, 18 received doses up to 100  $\mu\text{g}/\text{min}$ , and 13 received doses up to 300  $\mu\text{g}/\text{min}$ . The peak flow response with ACH was achieved at the 30- $\mu\text{g}/\text{min}$  dose in 18 patients, at the 100- $\mu\text{g}/\text{min}$  dose in 12 patients, and at the 300- $\mu\text{g}/\text{min}$  dose in 2 patients.

5 min after performing the ACH dose-response curve, endothelium-independent function was estimated with sodium nitroprusside and adenosine (Fig. 1). Intracoronary sodium nitroprusside was given at 40  $\mu\text{g}/\text{min}$  for 3 min, followed by measurement of blood flow velocity and coronary angiography. This was followed by administration of intracoronary adenosine at 2.2 mg/min for 2 min. Peak flow velocity measurement and angiography were repeated.

After a 10-min interval, while continuing dextrose 5% infusion, repeat baseline measurement of flow velocity, oxygen saturations, and angiography were made (Fig. 1). These were followed by infusion of

L-NMMA, a specific inhibitor of NO synthesis from L-arginine (37). L-NMMA was infused at 32  $\mu\text{mol}/\text{min}$  (0.5 ml/min) for 5 min and then increased to 64  $\mu\text{mol}/\text{min}$  (1 ml/min) for another 5 min. Blood flow velocity, coronary angiography, and arteriovenous oxygen saturations were measured at each stage.

While continuing the infusion of L-NMMA at 64  $\mu\text{mol}/\text{min}$ , ACH was readministered in 30 patients at the two vasodilating doses that produced the highest increase in flow velocity. 21 patients had repeat infusion of 40  $\mu\text{g}/\text{min}$  sodium nitroprusside for 3 min, and adenosine 2.2 mg/min was reinfused in 15 patients (Fig. 1). Blood flow velocity was measured and coronary angiography was performed after each intervention. In the remaining 11 patients, L-arginine was administered to test whether the effects of L-NMMA were reversible (Fig. 1). 10 min after intracoronary L-arginine (160  $\mu\text{mol}/\text{min}$  at 1 ml/min), repeat measurements of blood flow velocity and coronary angiography were performed. ACH was reinfused at 30  $\mu\text{g}/\text{min}$  for 2 min in 10 patients.

**Estimation of coronary blood flow and diameter.** Coronary blood flow was estimated from measurement of coronary blood flow velocity and diameter measurements using the formula ( $\pi \times \text{average peak velocity} \times 0.125 \times \text{diameter}^2$ ). Coronary vascular resistance (CVR) was calculated as mean arterial pressure divided by coronary blood flow.

For calculating flow, coronary artery diameter was measured in a 0.5-cm segment of vessel beginning 0.25 cm beyond the tip of the flow wire. Coronary angiograms were recorded using a cineangiographic system (Toshiba American Medical Systems Inc., Malvern, PA). Quantitative angiography was performed with the ARTEK software (Quantim 2001, StatVIEW; ImageComm Systems, Inc., Sunnyvale, CA). In addition to measurement of the diameter at the level of the Doppler flow wire, 0.5–1-cm segments of the proximal and distal segments of the epicardial coronary arteries were also measured by quantitative coronary angiography.

**Statistical analysis.** Data are expressed as mean  $\pm$  SD in the text and mean  $\pm$  SE in figures. Differences between means were compared by paired or unpaired Student's *t* test, as appropriate. The effect of L-NMMA on the two doses of ACH, and the effects of two doses of L-NMMA on patients with and without risk factors, were compared by ANOVA for repeated measures using a multiple regression model that included dummy variables to correct for between-subject variability (38). The differences between the effects of L-NMMA in patients with and without risk factors were compared using the percent change from baseline for all parameters because of the baseline differences in regional diameters and flow in the two subgroups. All *P* values are two tailed, and a value  $< 0.05$  is considered of statistical significance. Where the effect of two doses of L-NMMA on coronary hemodynamics is compared with baseline, a paired Student's *t* test was performed using a Bonferroni adjustment to alpha.

## Results

**Coronary vascular response to inhibition of NO synthesis with L-NMMA.** Epicardial coronary arteries progressively constricted

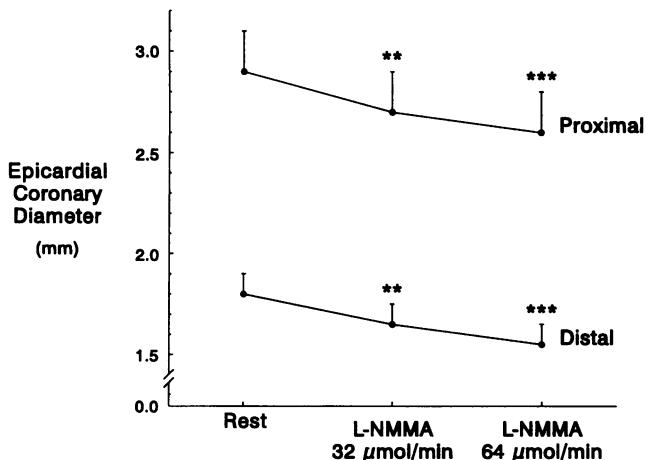


Figure 2. Proximal and distal epicardial coronary artery diameter changes with L-NMMA. Data represent mean $\pm$ SEM. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared to rest.

with increasing doses of L-NMMA (Fig. 2). Proximal and distal segments of the epicardial coronary arteries constricted by 5.3 and 7.4%, respectively, with the lower dose of L-NMMA, and by 9.2 and 12.6%, respectively, at the 64  $\mu\text{mol}/\text{min}$  dose, demonstrating tonic basal release of NO from the coronary epicardial vessels. The differences between proximal and distal segments were not significant.

CVR also progressively increased with L-NMMA (22% with the 64  $\mu\text{mol}/\text{min}$  dose), indicating tonic release of NO from the coronary microvessels under resting conditions (Fig. 3). Arterial pressure increased by a mean of 2.9% at the lower dose and 6.8% at the higher dose of L-NMMA. Arterio-venous

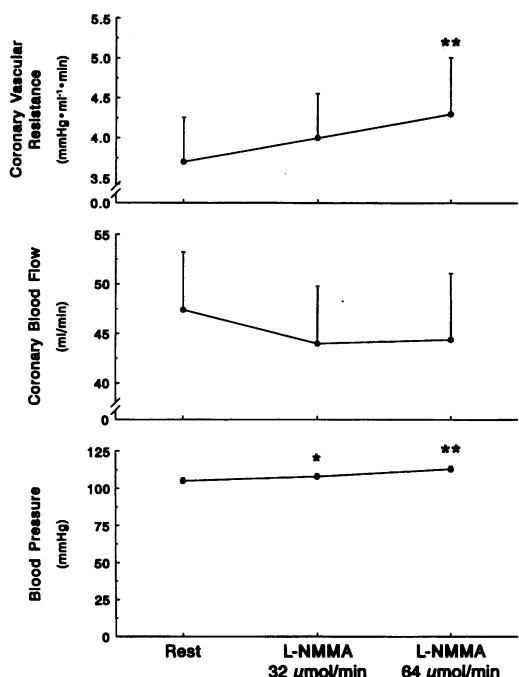


Figure 3. Changes in coronary vascular resistance, blood flow, and mean arterial pressure with increasing concentrations of L-NMMA. Data represent mean $\pm$ SEM. \* $P < 0.025$ , \*\* $P < 0.01$  compared to rest.

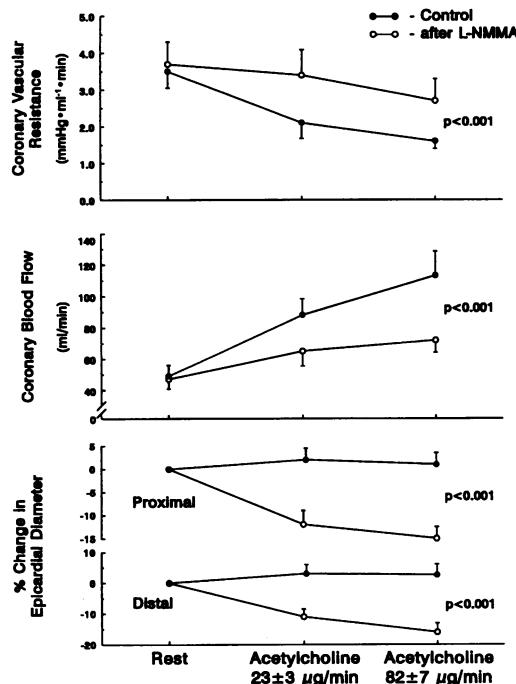


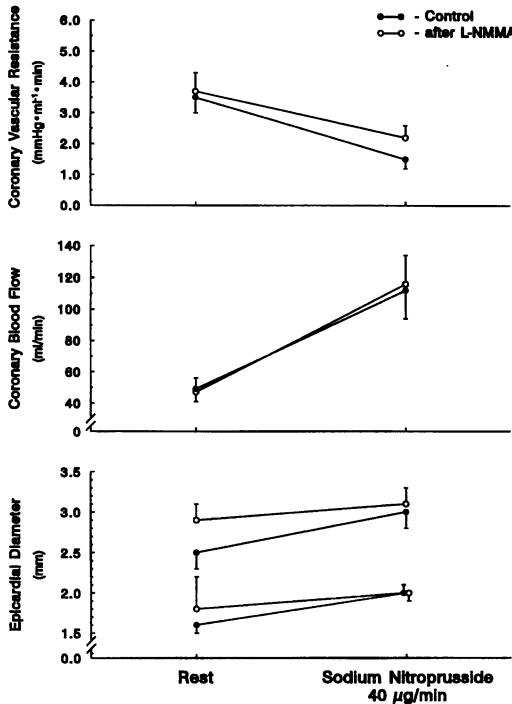
Figure 4. Effects of increasing doses of intracoronary ACH on coronary vascular resistance, flow, and the percent change in proximal and distal epicardial coronary artery diameters before (solid circles) and after (open circles) L-NMMA. Two vasodilating doses of ACH were reinfused after L-NMMA, and the  $P$  values denote results of ANOVA comparing the response to ACH before vs after L-NMMA. Data represent mean $\pm$ SEM.

oxygen difference widened from  $56\pm 7$  to  $59\pm 6$  ( $P = 0.003$ ) with the higher dose of L-NMMA, while the rate-pressure product remained unchanged (8,736 before to 8,958  $\text{mmHg} \cdot \text{bpm}^{-1}$  after L-NMMA).

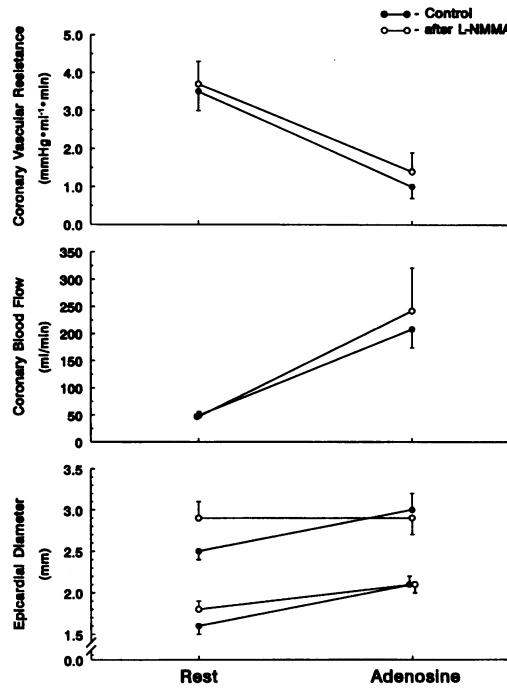
*Effect of L-NMMA on the response to ACH.* ACH infusions produced graded increases in coronary blood flow and reduction in coronary vascular resistance. At the peak vasodilating dose of ACH, coronary blood flow increased by 161% and CVR decreased by 52%. Coronary artery diameter changes were heterogeneous, but in the group as a whole there was no significant alteration in either the proximal or the distal coronary artery diameters.

When ACH was reinfused at the two highest vasodilating doses, there was significant inhibition of ACH-induced epicardial and microvascular dilation (Fig. 4). Thus, at the lower concentration of ACH, flow was 26% lower and CVR 61% higher, and, at the higher concentration, flow was 28% lower and CVR 85% higher after L-NMMA ( $P < 0.003$ ).

An insignificant change in epicardial coronary artery diameter with ACH alone was converted to a net reduction after L-NMMA in the proximal and distal segments (Fig. 4). Because the coronary artery diameters during the initial baseline study tended to be lower compared to the pre-L-NMMA baseline ( $P = \text{NS}$ ), diameter changes with ACH were compared as percent change from baseline before and after L-NMMA. Thus, at the higher dose of ACH, proximal and distal segments of epicardial coronary arteries dilated by 1.2 and 2.6%, respectively. After L-NMMA, the same dose of ACH produced 15 and 16% ( $P < 0.001$ ) reduction in diameter, respectively.



**Figure 5.** Effect of L-NMMA on the coronary vascular resistance, flow, and proximal and distal epicardial coronary artery diameters with intra-coronary sodium nitroprusside. Control study is indicated by solid circles, and post-L-NMMA study by open circles. Differences were not statistically significant. Data represent mean  $\pm$  SEM.



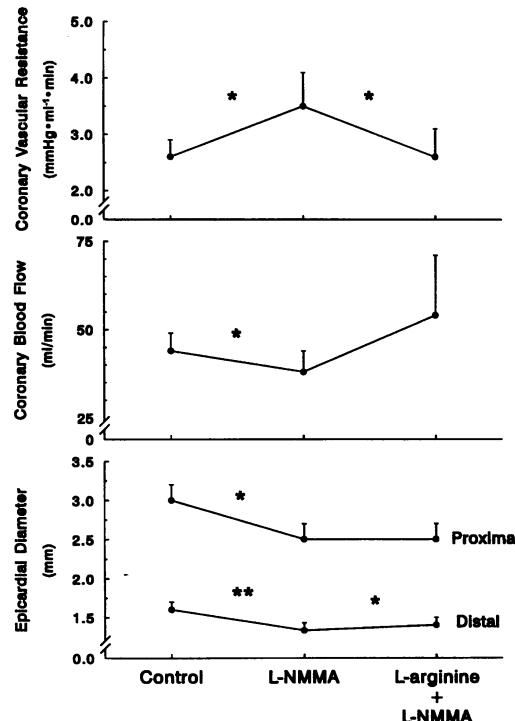
**Figure 6.** Effect of L-NMMA on the coronary vascular resistance, flow, and proximal and distal epicardial coronary artery diameters with intra-coronary adenosine. Control study is indicated by solid circles, post-L-NMMA study by open circles. Differences were not statistically significant. Data represent mean  $\pm$  SEM.

**Effect of L-NMMA on the response to sodium nitroprusside.** Coronary blood flow increased by 137%, CVR was reduced by 56%, and blood pressure fell by a mean of 10 mmHg with 40 µg/min infusion of sodium nitroprusside during the control study. After L-NMMA, the increase in blood flow and reduction in CVR with sodium nitroprusside were similar; 137 and -56% change, respectively,  $P = \text{NS}$  (Fig. 5). Thus, CVR with sodium nitroprusside was similar before and after L-NMMA. Similarly, epicardial coronary artery diameters were similar in the proximal and distal segments with sodium nitroprusside before and after L-NMMA (Fig. 5).

**Effect of L-NMMA on the response to adenosine.** Coronary blood flow increased by 396% and CVR decreased by 76% during the control study, and by 376 and 72%, respectively, after L-NMMA ( $P = \text{NS}$ ) (Fig. 6). Coronary diameter increased to similar levels with adenosine before and after L-NMMA in the proximal and distal segments (Fig. 6).

**Effect of L-arginine on the response to L-NMMA at rest.** The results of concomitant administration of L-arginine and L-NMMA on resting coronary vascular hemodynamics are illustrated in Fig. 7. The L-NMMA-induced increase in resting CVR and reduction in distal coronary artery diameter were reversed by L-arginine.

**Effect of L-arginine on the response to L-NMMA and ACH.** ACH at 30 µg/min produced a 47% reduction in the coronary vascular resistance before, but only a 9% reduction after L-NMMA (Fig. 8). After L-arginine, this inhibitory effect of L-NMMA on endothelium-dependent vasodilation with ACH was reversed (CVR reduced by 48%) (Fig. 8). Similarly, epicardial coronary arteries, particularly in the distal coronary segments,



**Figure 7.** The effect of L-arginine on the coronary vascular effects of L-NMMA at rest. Coronary vascular resistance, flow, and proximal and distal epicardial coronary diameters are compared at rest (control), after L-NMMA, and after L-NMMA and L-arginine. Results are expressed as mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ .

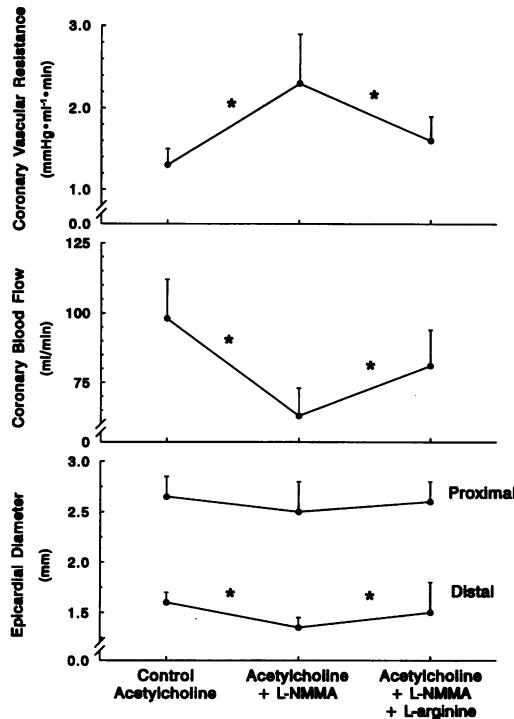


Figure 8. The effect of L-arginine on the response to ACH before and after L-NMMA. Coronary vascular resistance, flow, and proximal and distal epicardial coronary diameters with ACH are compared under resting conditions (control), after L-NMMA, and after L-arginine and L-NMMA. Results are expressed as mean $\pm$ SEM. \* $P < 0.05$ .

were more constricted when ACH was administered with L-NMMA compared with the control study. L-arginine counteracted this constrictive effect of L-NMMA.

*The impact of coronary risk factors on the response to L-NMMA at rest.* The responses of epicardial coronary arteries and of the coronary microvasculature to L-NMMA under resting conditions were heterogeneous, ranging from a decrease in CVR of 29% to an increase of 99% at the higher dose of L-NMMA, suggesting variability in the basal activity of NO. To investigate whether this heterogeneity was related to the presence of risk factors for coronary atherosclerosis, the responses in patients without risk factors were compared to the responses in patients with risk factors for coronary atherosclerosis. Risk factors for coronary atherosclerosis were defined as serum cholesterol  $> 260$  mg/dl, hypertension  $> 140/90$  mmHg, age  $> 60$  yr, history of current smoking, or diabetes. 9 patients were exposed to none of these risk factors, whereas the remaining 23 had exposure to one or more risk factors.

Coronary microvascular and epicardial vessel changes in response to L-NMMA at rest in patients with and without risk factors for coronary atherosclerosis are shown in Fig. 9. The inhibitory effect of L-NMMA on the coronary microvasculature was greater in patients without risk factors. The mean 36% increase in CVR and 18% reduction in coronary blood flow (both  $P < 0.01$  compared to baseline) with the higher dose of L-NMMA in patients without risk factors was significantly greater compared to patients with risk factors, who had a 17% increase in CVR ( $P = 0.08$  compared to baseline) and 3% fall ( $P = \text{NS}$  compared to baseline) in blood flow. Although significant epicardial vessel constriction occurred with L-NMMA in both

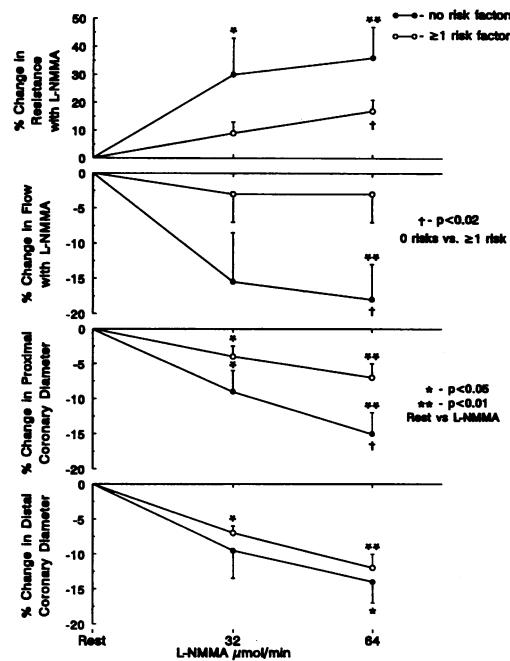


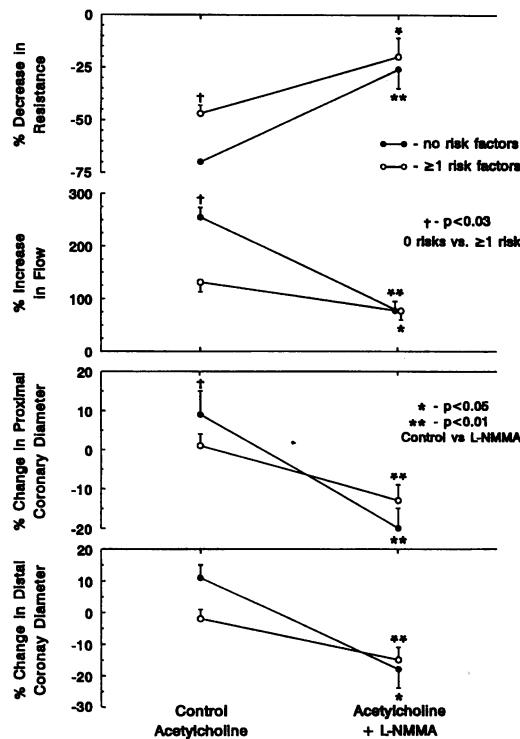
Figure 9. The effect of risk factors for coronary atherosclerosis on the change in resting coronary vascular tone with L-NMMA. The percent change in the coronary vascular resistance, flow, and proximal and distal epicardial diameters with L-NMMA are compared in patients with  $\geq 1$  risk factor (open circles) and in patients without risk factors (solid circles). Results are expressed as mean $\pm$ SEM.  $^{\dagger}P < 0.001$ , and denotes differences in the response to L-NMMA by ANOVA between patients with and without risk factors. \* $P < 0.05$ , and \*\* $P < 0.01$  denote differences between rest and post-L-NMMA measurements in each group.

groups, the magnitude of constriction appeared to be greater in patients without risk factors and reached statistical significance in the proximal coronary artery segments ( $P < 0.02$ ), but not in the distal segments ( $P = 0.07$ ) (Fig. 9).

*Effect of L-NMMA on the response to ACH in patients with risk factors.* The peak microvascular dilator response with ACH (69% fall in CVR) was significantly higher in patients without risk factors than in those with one or more risk factors (47% fall in CVR) (Fig. 10). At this concentration of ACH, epicardial coronary arteries dilated in patients without risk factors, whereas there was constriction in patients with risk factors. To investigate whether these differences represented differences in stimulated release of NO in these patients, the effect of L-NMMA was studied.

L-NMMA inhibited the microvascular dilation in response to ACH in both groups of patients, but the magnitude of inhibition was significantly greater in patients without risk factors: CVR increased by 116% in patients without risk factors compared with a 70% increase in those with risk factors ( $P < 0.02$ ). Similarly, distal epicardial diameter decreased by 18% in patients without risks compared with a 5% decrease in those with risk factors ( $P < 0.01$ ). Thus, after L-NMMA, the percent increase in flow, the reduction in resistance, and the reduction in epicardial diameter with ACH were similar in both groups (Fig. 10).

*Effect of L-NMMA on the responses to sodium nitroprusside and adenosine in patients with risk factors.* Baseline microvas-



**Figure 10.** The impact of risk factors for coronary atherosclerosis on the vascular effects of ACH before and after L-NMMA. The percent change in coronary vascular resistance, flow, and proximal and distal epicardial coronary diameters with ACH in patients with  $\geq 1$  risk factor (open circles) is compared to that in patients without risk factors (solid circles). Responses to the peak dose of ACH are compared before and after L-NMMA in the two groups. Results are expressed as mean  $\pm$  SEM.  $^{\dagger}P < 0.03$ , and denotes differences in the response to ACH between patients with and without risk factors under control conditions.  $*P < 0.05$  and  $^{**}P < 0.01$  denote differences between control and post-L-NMMA measurements in each group.

cular vasodilation with sodium nitroprusside was similar in patients with and without risk factors: a  $58 \pm 20\%$  reduction in CVR in patients without vs a  $59 \pm 16\%$  decrease in patients with risk factors. Coronary diameter increases were also similar:  $23 \pm 10$  vs  $27 \pm 19\%$  increase in distal epicardial diameter in patients without and with risk factors, respectively. After L-NMMA, epicardial diameter and CVR with sodium nitroprusside remained unchanged in both groups ( $P = \text{NS}$ ).

Vasodilation in response to adenosine was also similar in both groups: CVR  $80 \pm 2\%$  lower in patients without compared with  $77 \pm 8\%$  in patients with risk factors ( $P = \text{NS}$ ). Coronary diameter increases with adenosine were also similar:  $28 \pm 5$  vs  $29 \pm 19\%$  increase in distal epicardial diameter in patients without and with risk factors, respectively. After L-NMMA, epicardial diameter and CVR with adenosine remained unchanged in both groups ( $P = \text{NS}$ ).

## Discussion

### NO and basal epicardial coronary artery tone

There was progressive constriction of epicardial coronary arteries with increasing concentrations of intracoronary L-NMMA. In patients with normal coronary arteries who were not exposed

to risk factors for atherosclerosis, segments of proximal and distal epicardial coronary arteries constricted by 9–10% at the lower dose and between 14 and 15% at the higher dose of L-NMMA (Fig. 9). There did not appear to be any quantitative difference in the effect of L-NMMA in the proximal compared to the distal sections of the normal epicardial coronary tree. These data provide evidence for tonic release of NO from coronary epicardial vessels under resting conditions in humans. Since L-NMMA is a competitive antagonist and therefore may not have completely blocked all NO production, the results indicate that at least 15% of resting epicardial coronary vasodilator tone is due to NO.

### NO and basal coronary microvascular tone

The progressive reduction in coronary blood flow and increase in CVR with incremental concentrations of L-NMMA demonstrate that there is also tonic release of NO from the coronary microvasculature under resting conditions. Thus, in patients without coronary risk factors for atherosclerosis, coronary blood flow was 18% lower and CVR 36% higher after the higher dose of L-NMMA, suggesting that a significant proportion of resting coronary microvascular tone is influenced by tonic release of NO (Fig. 9). That this effect of L-NMMA was specific for inhibiting L-arginine metabolism to NO was suggested by the fact that its effects were reversed by L-arginine at a concentration that does not have intrinsic vasodilator effects (Fig. 7) (39, 40).

Since CVR is believed to be determined predominantly by arterioles  $< 200 \mu\text{m}$  in diameter in a setting of normal epicardial coronary arteries (41), and because CVR is inversely related to the fourth power of the radius of these resistance vessels, it can be estimated from our results that the diameter of these vessels would need to be reduced by  $\sim 7.5\%$  to produce a 36% increase in CVR. Thus, the magnitude of effect in terms of change in diameter appears to be lower in the resistance vessels compared to the epicardial vessels, suggesting that the contribution of NO to vascular tone in the normal human coronary circulation is dependent on the size of the coronary vessels.

Compared to the increase in microvascular forearm vascular resistance observed in normal individuals, which has been reported to vary between 40 and 50% (42–45), the 36% increase in CVR observed in patients without risk factors appears to be similar and indicates equivalent contribution of NO to resting blood vessel tone in the coronary and forearm vascular beds in humans.

### ACH releases NO from the human coronary vasculature

**Epicardial coronary arteries.** Several investigators have interpreted the effects of ACH on human coronary arteries as indicators of endothelial function, but the role of ACH in releasing NO from human coronary vessels remains controversial (32). Our study unequivocally demonstrates that coronary epicardial vasodilation with ACH is in large part due to release of NO. In patients without risk factors, ACH-induced epicardial vasodilation was abolished and converted to vasoconstriction in all sections of the coronary epicardial tree after L-NMMA, suggesting that ACH-induced dilation of normal coronary arteries is predominantly due to release of NO. Additionally, it appears that, in the absence of endothelium, the direct effect of ACH on human coronary epicardial vessels is constriction, as indicated by the response of ACH after L-NMMA. Our data also indicate that the epicardial coronary arteries of patients with

risk factors release NO with ACH, as evidenced by the significant further constriction that occurred with ACH after L-NMMA. The bioavailability of NO, however, is lower in patients with risk factors compared to those free of risk factors.

**Coronary microvessels.** Coronary microvascular dilation due to ACH in patients free of risk factors was significantly inhibited by L-NMMA, suggesting that ACH also promotes release of NO from human coronary microvessels. However, lack of complete inhibition of ACH-induced dilation of the microvessels suggests, but does not confirm, a non-NO component of ACH-induced vasodilation in human coronary vessels. Whether this is due to release of endothelium-derived hyperpolarizing factor (4) needs to be further investigated. An alternative explanation for persistent vasodilation of the coronary microvasculature with ACH after L-NMMA is that there is incomplete inhibition of NO synthase by the dose of L-NMMA employed in this study.

#### *Specificity of inhibition of NO synthesis by L-NMMA*

The effect of L-NMMA on the coronary microvasculature was specific for inhibiting endothelium-dependent effects of ACH because the responses of coronary microvessels to sodium nitroprusside, a direct donor of NO, and to adenosine, both endothelium-independent dilators, were unaffected by L-NMMA (Figs. 5 and 6). Moreover, the effect of L-NMMA on vascular tone was reversed by L-arginine, suggesting that the effect of the L-arginine analogue was specifically due to competitive inhibition of the L-arginine-NO synthase interaction.

The epicardial diameters with adenosine and sodium nitroprusside before and after L-NMMA were similar (Figs. 5 and 6), suggesting that the effect of L-NMMA on epicardial vessels is specific for endothelium-dependent vasodilation, a finding supported by the restoration of the baseline constriction with L-NMMA by L-arginine in the distal coronary segments (Fig. 7). However, when the diameters are considered as percent change from baseline, the increase was lower after L-NMMA with both vasodilators, a manifestation of the slightly higher baseline epicardial diameter before L-NMMA was given compared to the baseline at the beginning of the study. There are two possible explanations for this finding. One is that both adenosine and sodium nitroprusside cause maximal or near-maximal epicardial dilation before and after L-NMMA, and the lower percent increase after L-NMMA was merely due to a higher baseline diameter before the second study. Alternatively, the lower percent increase in diameter after L-NMMA could be due to inhibition of flow-mediated vasodilation by L-NMMA (6–9).

The responses of L-NMMA at rest and after ACH were reversed by a 10-min infusion of 160  $\mu\text{mol}/\text{min}$  of L-arginine. However, this reversal was not significant with respect to the proximal coronary diameter (Figs. 7 and 8). This incomplete reversal may have occurred because L-NMMA was administered for at least 20 min, whereas L-arginine was only given for 10 min before measurements were made. This may not have allowed sufficient time to overcome all the inhibitory effects of L-NMMA.

#### *Comparison with previous studies*

Our results are compatible with some animal studies that have examined the role of NO on basal coronary vascular tone (14, 15, 20) but differ from other studies (18, 21, 40). The contribution of NO to coronary epicardial and microvascular tone in

humans in this study appears to be substantially higher than that estimated by Lefroy et al. (32). This difference might be explained either by the smaller concentration of L-NMMA employed, or because coronary blood flow was not directly measured in their study.

Whereas some animal experiments using analogues of L-arginine to block NO synthesis have demonstrated reduced vasodilation of epicardial and coronary microvessels with ACH (17–19, 21, 46), others have failed to demonstrate inhibition of ACH-induced vasodilation with L-NMMA (20). A study using L-NMMA in human coronary arteries (32) demonstrated inhibition of ACH-induced dilation of distal epicardial coronary arteries only, but no effect was observed in either the proximal epicardial vessels or the coronary microvessels. Our results unequivocally demonstrate that ACH stimulates release of NO from the human epicardial and microvascular coronary circulation. In view of the wide diversity in NO bioavailability that has been reported in different species, it was important in this study to quantitate the role of NO and ACH in the human coronary circulation.

#### *Effect of risk factors on NO release from the coronary vasculature*

This study demonstrates, for the first time in the human coronary circulation, that the presence of risk factors for atherosclerosis is associated with a reduced basal activity of NO in both the epicardial coronary arteries and the coronary microvessels. This was demonstrated as a reduced vasoconstrictor action of L-NMMA in patients with risk factors. Our study nevertheless does not elucidate the mechanism for the depressed bioavailability of NO in these patients. This may be due to a lower rate of synthesis of NO, which may in turn result from substrate deficiency (39, 40), or it may result from a defect in the signal transduction pathways (47) or in the enzyme itself. Alternatively, the depressed bioavailability of NO may be secondary to increased breakdown of normally produced NO by superoxide anions (48, 49) in patients with risk factors. Also, we are unable to determine whether there are differences in the magnitude of reduction of the bioavailability of NO with the presence of specific risk factors such as hypertension, hypercholesterolemia, diabetes, smoking, or aging, because of the relatively small number of patients with single risk factors enrolled in this study.

The dilator responses to ACH from the epicardial and microvascular circulation were diminished in patients with risk factors, a finding that is similar to other reports (33, 34, 50–52). We have additionally confirmed, using the L-arginine analogue, that, in the presence of risk factors, not only is the basal effect of NO reduced, but the stimulated release of NO (in response to ACH) is also depressed. Because the effect of ACH after L-NMMA is similar in patients with and without risk factors (Fig. 10), it appears that the attenuated response to ACH in patients with risk factors is due specifically to reduced availability of NO, and not due to an abnormality of other non-NO endothelium-derived relaxing factors or constricting factors.

The epicardial and microvascular dilator responses to sodium nitroprusside and adenosine were similar in patients with and without risk factors, indicating that the blunted ACH response observed in the latter group is indeed an expression of impaired endothelium-dependent relaxation and not a consequence of reduced responsiveness of the vascular smooth muscle, either to NO-donating dilators like sodium nitroprusside or to non-NO-producing vasodilators such as adenosine. These

findings are in agreement with other reports demonstrating depressed ACH responses and normal smooth muscle vasodilation in patients with atherosclerosis (27, 31, 33, 50–52).

It is also important to recognize that abnormalities in the activity of NO in the coronary epicardial vasculature were observed in the absence of any clear angiographic evidence of atherosclerosis. Intimal thickening, observed by intravascular ultrasound, may be present in patients with risk factors despite the absence of angiographic irregularities (53, 54); this is associated, *in vitro*, with reduced production of NO (51) and, *in vivo*, with an abnormal ACH response (53). Moreover, coronary microvessels that do not develop structural changes in atherosclerosis also have reduced bioavailability of NO in the presence of risk factors. These findings are compatible with *in vitro* and animal studies (55–58). They are also in agreement with human studies examining endothelium-derived NO activity in the peripheral microvasculature, where reduced bioavailability of NO in patients with hypertension and hypercholesterolemia was demonstrated (43, 44). Combined with these observations, our findings suggest that risk factors for atherosclerosis are associated with, and may be a cause of, a generalized abnormality of the vascular endothelium-dependent NO system.

### Summary

In summary, this study demonstrates that constitutive release of NO contributes substantially to resting human coronary vascular tone. ACH-induced coronary vasodilation is largely due to release of NO from the coronary epicardial and microvascular tree. In the presence of risk factors, and despite absence of angiographic evidence for atherosclerosis, there is reduced basal bioavailability of NO from both the coronary epicardial arteries and microvessels. Moreover, patients with risk factors also have reduced stimulated release of NO with ACH.

Our findings imply that patients with risk factors may have increased resting conductance and resistance vessel tone as a result of reduced NO activity, and that because of reduced stimulated release of NO, vasodilation may also be limited during physiologic stresses (27) and therefore contribute to myocardial ischemia in patients exposed to multiple risk factors. Depressed bioavailability of NO may also result in reduced platelet-inhibitory effects of NO (59) and predispose these patients to thrombotic vascular events. Over time, because of the reduced anti-smooth muscle cell proliferative effects of NO, these individuals may also succumb to more rapid progression of atherosclerosis (60–62).

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