JACC Vol. 32, No. 1
July 1998:63–8

Increased Release of Nitric Oxide in Ischemic Hearts After Exercise in Patients With Effort Angina

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Objectives. **The aim of this study was to determine whether the release of nitric oxide (NO) from the ischemic heart increases during exercise in patients with effort angina.**

Background. **Myocardial ischemia increases NO production in the canine heart, but no such increase has been demonstrated in the ischemic human heart.**

Methods. **Fifteen patients with effort angina underwent supine ergometer exercise tests. All patients had severe proximal stenosis (>90%) in the left anterior descending coronary artery. The control group consisted of 17 subjects without coronary artery disease or systemic hemodynamic abnormalities.**

Results. **Neither the lactate extraction ratio (LER) nor the difference in NO concentration between coronary venous and arterial blood (ΔVA[NO]) was affected by exercise in the control subjects. In patients with effort angina, neither variable differed from that in the control group at rest; however, exercise markedly decreased LER and significantly increased ΔVA(NO) (from 4.7 ± 0.3 to 16.5 ± 1.6 μmol/liter, $p < 0.001$) in the patient group. The extent of decrease in LER was significantly correlated with the extent of increase in ΔVA(NO) in the patients with effort angina ($r^2 = -0.837$, $p < 0.001$).**

Conclusions. **Provocation of myocardial ischemia by exercise stress increases NO production in the hearts of patients with effort angina.**

(J Am Coll Cardiol 1998;32:63–8)

Recent studies in animals and humans have demonstrated that the vascular endothelium plays an important role in the regulation of regional blood flow by releasing endothelium-derived relaxing factor (1–8), a major component of which is nitric oxide (NO). NO is produced in the endothelium (9–14) and other tissues of the heart, where it acts as a paracrine and autocrine autacoid. In human hearts with restricted coronary circulation, increases in myocardial oxygen demand relative to oxygen supply cause angina pectoris and the release of lactate into the coronary venous blood. In this condition, endogenous vasodilators such as NO may be released to increase oxygen supply (15–17). NO is present at low basal concentrations in the well oxygenated myocardium, but its abundance increases during ischemia, which may contribute to the improvement of myocardial metabolic dysfunction (18,19). Although we detected NO in coronary venous blood after transient ischemia in dogs (20), a similar increase in NO has not been demonstrated during provocation of myocardial ischemia in humans. During metabolic stimulation of the human heart, released NO contributes to coronary vasodilation (4,6). This contribution of NO is reduced in patients exposed to risk factors for coronary atherosclerosis, with a consequent reduction in vasodilation during stress (21–23). Little is known of the in vivo metabolism of NO during myocardial ischemia in humans. Therefore, we have investigated whether NO is released during an angina attack induced by an exercise stress test in patients with coronary artery disease (CAD).

Methods

All study protocols were approved by the local Human Investigations Committee, and study subjects gave written informed consent. The study was performed while subjects were in the supine position in a quiet room maintained at a constant temperature of 22 to 24°C.

Study subjects. The experimental group comprised 15 patients (7 men and 8 women) who had CAD and a history of stable exertional angina pectoris. Patients were selected during admission to Osaka University Hospital from May 1994 to August 1996, according to the following inclusion criteria: 1) a history of stable effort-related angina pectoris; 2) coronary

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Manuscript received October 3, 1996; revised manuscript received March 11, 1998, accepted March 20, 1998.

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0735-1097/98/$19.00
PII S0735-1097(98)00196-X

Abbreviations and Acronyms

CAD	=	coronary artery disease
ECG	=	electrocardiogram, electrocardiographic
LAD	=	left anterior descending coronary artery
LER	=	lactate extraction ratio
NO	=	nitric oxide
ΔVA(NO)	=	difference in nitric oxide concentration between coronary venous and arterial blood

angiographic evidence of >90% stenosis (diameter reduction) of the proximal left anterior descending coronary artery (LAD); 3) absence of >50% stenosis of the left main, left circumflex or right coronary artery; 4) presence of conditions facilitating catheterization of the great cardiac vein; 5) presence of sinus rhythm; and 6) a normal contraction pattern in the anterior and septal left ventricular wall and a normal ejection fraction as assessed by biplane cineangiography. Left ventricular end-systolic pressure was within normal limits (<12 mm Hg). No patient showed either ST segment elevation during an upright bicycle exercise test before catheterization and while receiving their usual medical regimen or angiographic or echocardiographic evidence of left ventricular hypertrophy. Patients with valvular heart disease, a history of angina at rest, clinical evidence of a previous myocardial infarction, hyperlipidemia, hypertension, chronic heart failure, renal dysfunction or diabetes mellitus were excluded. None of the patients showed vasospasm of the epicardial coronary arteries in response to either ergonovine or acetylcholine. The control group comprised 17 subjects (9 men and 8 women) who did not exhibit either CAD or systemic hemodynamic abnormalities.

Cardiac catheterization. Subjects underwent right and left heart catheterization in the fasting state. After completion of diagnostic coronary arteriography, either a 7F or 8F coronary sinus flow catheter (Wilton-Webster) was advanced under fluoroscopic guidance into the great cardiac vein through the femoral vein (24). The catheter tip was positioned at the junction of the anterior interventricular and great cardiac veins. Coronary venous drainage at this site is thought to originate mainly from the myocardium perfused through the LAD (25). Aortic blood pressure was measured with a Judkins catheter introduced through the right or left femoral artery.

Blood sampling. Either a 7F or 8F thermodilution coronary blood flow catheter was positioned at the great cardiac vein. Paired blood samples were obtained from the great cardiac vein and descending aorta at rest and immediately after the onset of symptom-limited submaximal cycling exercise in the supine position and were placed in an ice bath immediately after collection. After centrifugation of the blood sample, the plasma was packed in dry ice for subsequent determination of NO and lactate concentrations.

Measurement of NO and lactate levels. NO was measured as described previously (26). Briefly, 1.5 ml of blood was collected into heparinized tubes within 30 s and centrifuged for 5 min at 2,000*g*. The plasma fraction was diluted 1:1 with nitrite-free and nitrate-free distilled water, and 400 μl of the diluted sample was centrifuged at 2,000*g* in an ultrafree microcentrifuge device to remove substances >10 kDa. The filtrate was passed through a copper-plated cadmium column to reduce nitrate to nitrite and then reacted with Griess reagents. Absorbance at 540 nm was measured and represents the total amount of plasma NO end products (nitrate plus nitrite). The coronary arteriovenous difference in the concentration of nitrate plus nitrite reflects the amount of NO released from the myocardium. To measure the plasma lactate level, blood (1 ml) was rapidly sampled and centrifuged, and the lactate concentration in 0.2 ml of the plasma supernatant was measured by enzyme assay (27). Lactate extraction ratio (LER) was obtained by dividing the coronary arteriovenous difference in lactate concentration by the arterial lactate concentration and multiplying by 100%.

Exercise protocol. All antianginal medications were discontinued at least 24 h before the study. Patients and control subjects underwent bicycle ergometer exercise tests in the supine position. Each subject began to exercise at a work load of 25 W, which was increased every 2 min in stepwise increments of 25 W. For all patients, the exercise was discontinued when anginal pain occurred. Hemodynamic variables were measured every minute. Blood was sampled simultaneously from the great cardiac vein and descending aorta at rest and at the end of exercise. A standard 12-lead electrocardiogram (ECG) was recorded at rest and during exercise, and the data were compared at the same exercise time and at equivalent work loads.

Respiratory gas exchange variables. Gas exchange data were collected throughout exercise with a breath-by-breath respirometer system (Minato, model RM-200), which was calibrated immediately before each study with a 2-liter calibration syringe and a gas mixture of 14.93% oxygen, 5.00% carbon dioxide and 80.07% nitrogen. Oxygen uptake, carbon dioxide output, minute ventilation and the respiratory gas exchange ratio were obtained every 10 s during exercise.

Hemodynamic variables. Heart rate was measured by continuous ECG monitoring. Significant ST segment depression on exercise testing was defined as >0.1 mV of horizontal or downsloping ST segment depression measured 60 to 80 ms after the J point and present on at least one lead.

Statistical analysis. Data are expressed as the mean value ± SEM. The analyses were performed using the SAS system. The normality of the distribution and homogeneity of variance had been assessed by using the Shapiro-Wilks test and the *F* test, respectively. Comparison of descriptive characteristics and performance and hemodynamic variables between control subjects and patients was done using the unpaired *t* test, the Welch *t* test or the Mann-Whitney *U* test, as appropriate. Comparison of NO in the great cardiac vein and aortic root, the difference in NO concentration between coronary venous and arterial blood (ΔVA[NO]) and LER before and after exercise was done using the paired *t* test. Comparison of NO in the great cardiac vein and aortic root, ΔVA(NO) and

Table 1. Clinical Characteristics of Control Subjects and Patients With Effort Angina

Characteristic	Control (n = 17)	Patients (n = 15)
Gender (M/F)	9/8	7/8
Age (years)	60 ± 4	61 ± 3
Range	45–72	47–76
Obesity	2	3
Body mass index (kg/m^2)	24 ± 2	26 ± 2
Serum cholesterol (mg/dl)	176 ± 6	191 ± 7
Serum triglycerides (mg/dl)	132 ± 18	139 ± 14
Ejection fraction (%)	72 ± 5	69 ± 4
Medication used previously		
Beta-blockers	0	2
Long-acting nitrates	0	0
Calcium channel blockers	0	3
Aspirin	0	0
ACE inhibitors	0	0

Data presented are mean value ± SEM or a number of subjects. ACE = angiotensin-converting enzyme; F = female; M = male.

Table 2. Performance and Hemodynamic Variables at Baseline and Maximal Exercise for Patients and Control Subjects

Variable	Control Subjects	Patients
Work load (W)	117 ± 11	83 ± 4*
Percent predicted work capacity	87 ± 3	65 ± 4
Heart rate (beats/min)		
Baseline	67 ± 5	71 ± 4
Maximal exercise	138 ± 12	128 ± 7
Blood pressure (mm Hg)		
Systolic		
Baseline	122 ± 18	126 ± 14
Maximal exercise	188 ± 12	169 ± 6
Diastolic		
Baseline	70 ± 5	72 ± 6
Maximal exercise	102 ± 5	94 ± 3
RPP (10^2 mm Hg/min)		
Baseline	82 ± 4	89 ± 6
Maximal exercise	259 ± 15	216 ± 12
Exercise duration (min)	7.1 ± 1.8	5.3 ± 1.2*
Peak $\dot{V}O_2$ (ml/min per kg)	22.4 ± 2.1	20.1 ± 1.9
Respiratory gas exchange ratio	1.1 ± 0.06	1.0 ± 0.04

*$p < 0.05$ compared with control subjects (analysis of variance). Data presented are mean value ± SEM. RPP = rate–pressure product; $\dot{V}O_2$ = oxygen consumption.

LER after exercise between the control group and patient group was done using the Welch *t* test. Linear regression analysis was used to correlate the changes in LER and those in ΔVA(NO). A p value <0.05 was considered statistically significant.

Results

The clinical characteristics of the patients with effort angina and the control subjects are shown in Table 1. There were no significant differences between the two groups with regard to age, gender, body mass index, serum cholesterol, serum triglycerides or ejection fraction. Performance and hemodynamic variables at peak exercise for patients and control subjects are shown in Table 2. The work load at maximal exercise and exercise duration were significantly greater for the control subjects than for the patients. All patients experienced chest pain during exercise and developed ST-T segment changes of the ischemic type on the one-lead ECG (0.21 ± 0.03 mV). Neither LER nor ΔVA(NO) was affected by exercise in the control group. In patients at rest these variables were similar to those of the control subjects. However, LER decreased significantly and ΔVA(NO) increased significantly in patients during exercise (Table 3 and Fig. 1). Although we did not obtain the total NO release because we did not measure coronary blood flow, total cardiac release was also increased in patients during exercise as compared with control subjects, when we estimated coronary blood flow using the rate–pressure product (28). The extent of the decrease in LER correlated significantly with the extent of the increase in ΔVA(NO) (Fig. 2).

Discussion

The endogenous nitrovasodilator NO is continuously synthesized from L-arginine by the action of NO synthase and released mainly from endothelial cells. NO release is stimulated by various local and circulating factors as well as by shear stress of the bloodstream acting on the endothelial lining (29,30). In the present study, exercise increased the NO concentration in systemic arterial blood in both patients and control subjects, suggesting that shear stress during exercise enhances the production of NO in normal subjects (31).

Myocardial ischemia and cardiac NO levels. We have previously shown that the concentration of NO end products in plasma increases during acute focal myocardial ischemia and reperfusion in dogs, and that this increase is proportional to the extent of ischemia (20). We have shown that induction of myocardial ischemia by exercise stress results in an increases in NO production in the hearts of patients with effort angina. Myocardial ischemia by itself has been shown to increase NO

Table 3. Effects of Exercise on Plasma Nitric Oxide (nitrate plus nitrite) Concentration and Lactate Extraction Ratio in Control Subjects and Patients

	Control Subjects		Patients	
Assay	Baseline	After Exercise	Baseline	After Exercise
NO (μmol/liter)				
Great cardiac vein	25.0 ± 1.3	43.5 ± 1.8*	27.2 ± 1.9	53.9 ± 2.5*
Aortic root	21.1 ± 1.8	39.1 ± 1.2*	22.5 ± 1.2	37.4 ± 2.1*
ΔVA(NO)	3.9 ± 0.2	4.4 ± 0.3	4.7 ± 0.3	16.5 ± 1.6†‡
LER (%)	24.2 ± 1.2	20.1 ± 1.1	21.0 ± 1.7	−4.4 ± 3.0*‡

*$p < 0.05$ and †$p < 0.001$ versus corresponding baseline value. ‡$p < 0.01$ versus corresponding control value. Data presented are mean value ± SEM. LER = lactate extraction ratio; NO = nitric oxide; ΔVA(NO) = coronary arteriovenous difference in NO.

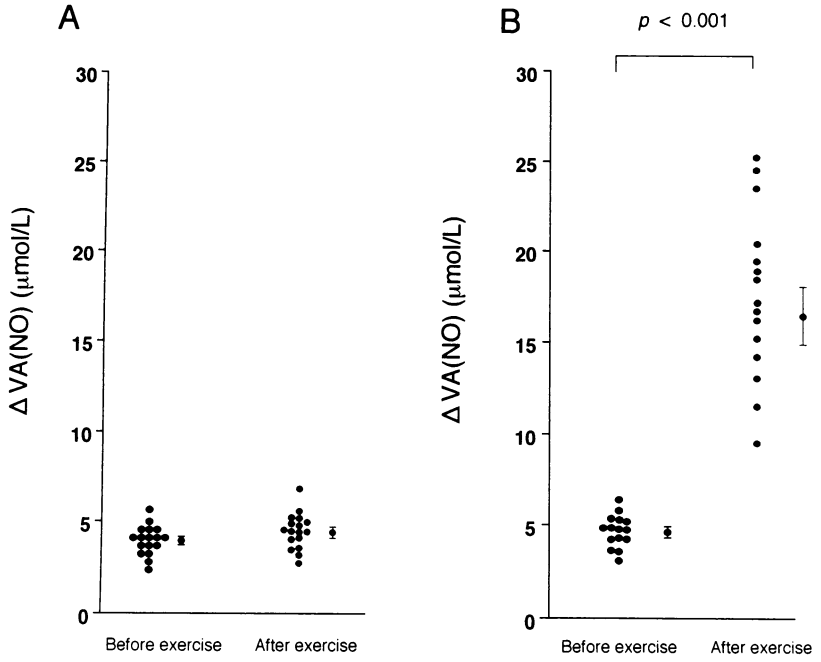
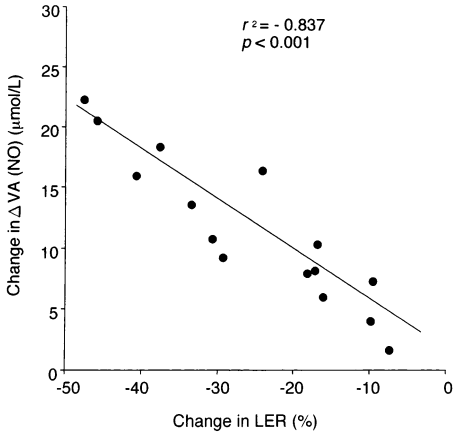


Figure 1. Effects of exercise on the coronary arteriovenous difference in NO (ΔVA[NO]) in the control (**A**) and patient (**B**) groups.

production (18,20), although reperfusion after sustained ischemia is reported to impair basal NO synthesis (32). Because NO and lactate production were correlated in the present study, it is likely that the increase in NO synthesis during the exercise stress test is attributable to myocardial ischemia. Both cardiomyocytes (33) and endothelial cells (34) are thought to be potential sources of NO in the ischemic heart; it is not known which cell type was the major site of NO production in the present study.

We did not measure coronary blood flow during exercise. Because this variable may be reduced in patients with effort angina, the NO concentration may appear to be increased without any change in total production. Previous investigations have demonstrated that the extent of increase in coronary blood flow at rest and with mild exercise is identical in normal subjects and patients with CAD (35–37). However, it is reported that there were differences in coronary blood flow after heavy exercise between the control and angina groups (28,38). In the study of Holmberg et al. (38), because the maximal coronary flow after supine heavy exercise was 370% of rest coronary flow in the control group and 306% of rest coronary flow in the angina group, the difference in coronary flow during even heavy exercise could not account for the 240% increase in coronary arteriovenous difference in NO after exercise in patients with effort angina observed in the present study.

Figure 2. Correlation between the exercise-induced increase in ΔVA(NO) and the decrease in LER.



Possible sites of NO production. A decrease in NO-dependent vasodilator responses in the microcirculation has been observed in patients with atherosclerosis (39). Zeiher et al. (40) showed that impaired endothelium-dependent vasodilation of the coronary microcirculation is associated with exercise-induced myocardial ischemia. Furthermore, atherosclerosis of epicardial conductance vessels impairs the activity of endothelium-derived NO (22,23). Release of NO from endothelial cells in response to shear stress or acetylcholine is attenuated and induces vasoconstriction in such patients (22,23). Even when the protein level of NO synthase is decreased, decreases in pH or increases in intracellular Ca^{2+} concentration during myocardial ischemia trigger the production of NO through the activation of NO synthase. Because cardiomyocyte may also produce NO during hypoxia (33), the total amount of NO release during ischemia may increase by release from cardiomyocytes and surrounding intact endothelial cells of the ischemic zone.

Role of NO in coronary circulation. The contribution of NO to dilation of coronary resistance vessels is controversial. Inhibition of NO synthesis decreased the diameter of the epicardial coronary artery without affecting coronary blood flow or coronary vascular resistance in anesthetized dogs (41),

whereas inhibition of NO synthesis decreased the diameter of the basal distal LAD and decreased basal coronary blood flow in humans (6). Experimental studies revealed that administration of the NO synthase inhibitor reduced coronary blood flow during coronary hypoperfusion (1,20). Although we have shown that the human heart produces NO in response to ischemic stress, it remains unclear whether increased NO synthesis plays an important role in the regulation of the coronary circulation during myocardial ischemia in patients with CAD.

Possible mechanisms of increases in NO production. We did not investigate the mechanism of NO release during ischemia in the present study. We have previously shown that cellular acidosis (42) or alpha$_1$-adrenoceptor activation (43) during ischemia contributes to NO-induced coronary vasodilation in the canine myocardium. Exercise reduced the pH and LER as well as increased the concentration of noradrenaline in the coronary sinus of patients with effort angina (data not shown). These chemical and humoral factors may contribute to the release of NO.

Limitation of the present study. It has been suggested that the position of the catheter used to sample blood from the great cardiac vein may change during the course of an experiment, thereby introducing variability into repeated blood sampling (44). However, Magorien et al. (45) demonstrated a highly significant correlation between these two measurements during exercise. We confirmed that the position of the catheter did not change when blood was sampled.

We thank Kayoko Yoshida and Yukiyo Nomura for their technical assistance and Katsunori Shimada (STATZ Co., Tokyo) for advice on statistical analysis.

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