JACC Vol. 28, No. 5
November 1, 1996:1161–7

Deficiency in Nitric Oxide Bioactivity in Epicardial Coronary Arteries of Cigarette Smokers

KIYOTAKA KUGIYAMA, MD, HIROFUMI YASUE, MD, MASAMICHI OHGUSHI, MD, TAKESHI MOTOYAMA, MD, HIROAKI KAWANO, MD, YOSHITO INOBE, MD, OSAMU HIRASHIMA, MD, SEIGO SUGIYAMA, MD

Kumamoto, Japan

Objectives. **This study sought to examine nitric oxide-mediated regulation of epicardial coronary arterial tone in cigarette smokers.**

Background. **Cigarette smoking is a major risk factor for coronary artery disease and is highly prevalent in patients with coronary spastic angina. Long-term exposure to cigarette smoking has been recently reported to suppress endothelium-dependent arterial relaxation in vivo humans.**

Methods. **Responses of epicardial coronary artery diameter to single or combined infusion of acetylcholine and N^G-monomethyl-L-arginine (L-NMMA) into the left main coronary artery were examined in 11 current smokers and 17 nonsmokers using quantitative coronary angiography.**

Results. **Acetylcholine dilated one-third of the proximal segments and most of the distal segments of coronary arteries in nonsmokers, whereas it constricted most of the proximal and distal segments in smokers. L-NMMA decreased the basal diameter of coronary arteries in nonsmokers but had minimal effect on the basal diameter in smokers. L-NMMA abolished the dilator response to acetylcholine in the coronary arteries of nonsmokers but had minimal effect on the constrictor response to acetylcholine in the arteries of smokers. The dilator response to nitroglycerin was significantly increased in the coronary arteries of smokers compared with in those of nonsmokers. The constrictor response to L-NMMA at rest was significantly correlated with the dilator response to nitroglycerin and with the diameter changes to acetylcholine in both smokers and nonsmokers.**

Conclusions. **Nitric oxide bioactivity at rest and at acetylcholine-stimulated conditions in smokers was decreased, leading to the supersensitivity of the artery to the dilator effect of nitroglycerin as well as the constrictor effect of acetylcholine in smokers. Cigarette smoking affects nitric oxide-mediated regulation of coronary artery tone.**

(J Am Coll Cardiol 1996;28:1161–7)

Risk factors for coronary artery disease have been shown (1–4) to be associated with an impairment of endothelium-dependent vasodilation and an increase in coronary artery tone. Cigarette smoking is a major risk factor for coronary artery disease and atherosclerosis (5–10), and we have recently demonstrated (11) that cigarette smoke extract inactivates endothelium-derived nitric oxide and increases arterial tone in the isolated porcine coronary arteries. Recently, long-term cigarette exposure has been reported (12) to suppress endothelium-dependent dilation of coronary arteries in vivo humans, but nitric oxide-mediated regulation of coronary artery tone remains undetermined in smokers.

Nitric oxide is an important dilator that is synthesized by the endothelial cells using the amino acid L-arginine as a substrate in a process catalyzed by the enzyme nitric oxide synthase (13–15). Analogues of L-arginine, such as N^G-monomethyl-L-arginine (L-NMMA), competitively inhibit nitric oxide production and have been widely used to examine the nitric oxide pathway in animals and humans (4,16–19). The constitutive nitric oxide synthase in the arterial endothelium continuously generates nitric oxide, which has been shown to maintain basal vascular tone in animals and humans (4,16–19). Moncada et al. (19) showed that removal of basal nitric oxide-mediated vasodilation in the vascular system leads to supersensitivity to an exogenous nitrovasodilator in experimental animals. Lefroy et al. (18) further confirmed this phenomenon in coronary arteries of in vivo humans. Thus, a deficiency or decrease in the endogenous nitrovasodilator system could lead to increased basal arterial tone and an enhanced dilator response to nitroglycerin. Nitric oxide release from vascular endothelium is also stimulated by several agonists acting on different endothelial surface receptors and using distinct intracellular signal transduction pathways (20–23). We have shown (24,25) that intracoronary injection of acetylcholine, one of the agonists, induces coronary spasm in patients with coronary spastic angina. The arterial response to acetylcholine is determined by the balance between the dilator action of endothelium-derived substances, including nitric oxide, and the direct constrictor

From the Division of Cardiology, Kumamoto University School of Medicine, Kumamoto, Japan. This study was supported in part by Grants-in-Aid C07670793 from the Ministry of Education, Science, and Culture, Japan and the Smoking Research Foundation Grant for Biomedical Research, Tokyo, Japan and the ONO Medical Research Foundation, Osaka, Japan.

Manuscript received April 15, 1996; revised manuscript received June 27, 1996, accepted July 1, 1996.

Address for correspondence: Dr. Kiyotaka Kugiyama, Division of Cardiology, Kumamoto University School of Medicine, Honjo 1-1-1, Kumamoto City, Japan 860. E-mail: kiyo@gpo.kumamoto-u.ac.jp.

0735-1097/96/$15.00
PII S0735-1097(96)00325-7

JACC Vol. 28, No. 5
November 1, 1996:1161–7

Abbreviations and Acronyms
ECG = electrocardiogram
L-NMMA = N^G-monomethyl-L-arginine

action of acetylcholine on smooth muscle (1–4,20–28). Quyyumi et al. (4,28) recently showed that the response of the arterial diameter to intracoronary injection of acetylcholine after L-NMMA infusion was comparable in subjects showing coronary dilation to acetylcholine and those showing coronary constriction to acetylcholine. These results from previous reports suggest that nitric oxide may be the most important determinant of the response of the coronary artery tone to acetylcholine. Cigarette smoking is highly prevalent in the Japanese versus the U.S. population, although the incidence of coronary artery disease is paradoxically lower in the Japanese than the U.S. population (5–9,29–31). The prevalence of smoking history is also higher in Japanese patients and white premenopausal women with coronary spastic angina and normal results on coronary angiograms (32,33). This epidemiologic evidence suggests that smoking may impair the dilator response to acetylcholine and cause coronary spasm through the mechanism independent of its promoting effect of atherosclerosis. The present study thus examined nitric oxide-mediated regulation of coronary artery tone in cigarette smokers versus nonsmokers.

Methods

Study patients. The study included 11 current smokers (mean age 61 years, range 46 to 75; 7 men, 4 women) and 17 life-long nonsmokers (mean age 60 years, range 43 to 78; 11 men, 6 women), all of whom underwent diagnostic cardiac catheterization for evaluation of atypical chest pain. All current smokers had smoked at least 20 cigarettes/day for >10 years. The smokers and nonsmokers included in the study had angiographically normal coronary arteries and had no other coronary risk factors, such as hypercholesterolemia (≥240 mg/dl), hypertension (≥140/90 mm Hg or antihypertensive medication), diabetes mellitus, low high density lipoprotein cholesterol levels (<35 mg/dl) and a family history of premature coronary artery disease, except for advancing age. Clinical characteristics of the study patients are shown in Table 1. No study subject had a clinical history suggestive of variant angina, and all showed coronary artery spasm after the intracoronary injection of acetylcholine. All medications were withdrawn at least 3 days before the study. No study subject had a previous myocardial infarction, congestive heart failure, valvular heart disease, left ventricular hypertrophy or other serious diseases. Written informed consent was obtained from all patients before the study. The study was approved by the ethics committee at our institution.

Cigarette smoke is reported to contain various chemicals, such as nicotine, nitric oxide-derived free radicals, carbon monoxide and large amounts of oxygen free radicals (34–36), which directly or indirectly cause short-term effects on coronary artery tone and hemodynamic variables (11,37,38). In the present study, because we aimed to determine the intrinsic functional integrity of coronary arteries, smokers refrained from cigarette smoking for at least 2 days before the study to avoid the possible effects of the vasoactive substances contained in cigarette smoke.

Table 1. Clinical Characteristics of Study Patients and Baseline Coronary Artery Diameters

	Smokers (n = 11)	Nonsmokers (n = 17)	p Value
Age (yr)	61 ± 8	60 ± 9	NS
Female/male	4/7	6/11	NS
Total serum cholesterol (mg/dl)	182 ± 22	177 ± 27	NS
LDL cholesterol (mg/dl)	99 ± 33	109 ± 25	NS
HDL cholesterol (mg/dl)	50 ± 11	59 ± 15	NS
Mean arterial pressure (mm Hg)	98 ± 20	103 ± 11	NS
Hypercholesterolemia	0	0	
Hypertension	0	0	
Diabetes mellitus	0	0	
Baseline coronary artery diameter (mm)			
LAD			
Proximal segment	2.9 ± 0.5	3.1 ± 0.6	NS
Distal segment	1.7 ± 0.4	1.9 ± 0.3	NS
LCx			
Proximal segment	2.8 ± 0.4	3.0 ± 0.4	NS
Distal segment	1.7 ± 0.5	1.8 ± 0.5	NS

Data presented are mean value ± SD or number of patients. HDL = high density lipoprotein; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; LDL = low density lipoprotein.

Quantitative coronary angiography. Coronary angiographic studies were performed with the Judkins technique using contrast material (Ioxaglate, Guerbet S.A.) in the morning when the patients were fasting. Coronary angiograms were taken in the right and left anterior oblique positions with adequate angulations to allow clear visualization of the left main and right coronary arteries, respectively, during the study period. Focal spot, patient and height of the image tube were kept constant during the angiographic study. When acetylcholine was injected into the coronary artery, a tripolar electrode catheter (USCI) was placed in the right ventricular apex through the right femoral vein and was connected to a temporary pacemaker set at a rate of 50 beats/min. Measurement of the lumen diameter of the coronary artery was performed quantitatively with the use of the computer-assisted coronary angiographic analysis system by two observers (M.O., S.S.) with no knowledge of the study protocol. End-diastolic cine films most clearly visualizing the coronary segments were videodigitized and stored in a cardiac image analysis system (Cardio 500, Kontron Instruments). Automated contour detection was performed by the geometric edge differentiation technique; this technique has been described and validated elsewhere (3,25,39). A Judkins catheter was used to calibrate the arterial diameter (in mm). The left anterior descending and left circumflex coronary artery trunk was divided into

Table 2. Systemic Hemodynamic Variables

	Baseline	ACh (50 μg)	L-NMMA 25 μmol/min	L-NMMA 50 μmol/min	ACh (50 μg) + L-NMMA (50 μmol/min)	Nitroglycerin
Heart rate (beats/min)						
Smoker	69 ± 17	71 ± 16	67 ± 15	69 ± 15	66 ± 16	70 ± 18
Nonsmoker	72 ± 10	73 ± 9	72 ± 9	68 ± 8	65 ± 6	77 ± 13
Mean blood pressure (mm Hg)						
Smoker	98 ± 20	98 ± 15	103 ± 14	104 ± 20	104 ± 19	90 ± 17*
Nonsmoker	103 ± 11	103 ± 16	103 ± 12	109 ± 12	109 ± 11	91 ± 14*

*$p < 0.01$ versus baseline. Data presented are mean value ± SD. ACh = acetylcholine; L-NMMA = N^G-monomethyl-L-arginine.

proximal and distal segments of equal lengths. The lumenal diameter at the center of each segment was measured, and special care was taken to measure the diameter at the same site for each study condition with use of anatomic references. Coronary segments ~10 mm long were measured at three points, and the diameters measured were averaged within each segment. The arterial diameters at proximal and distal segments in both the left anterior descending and left circumflex coronary arteries were evaluated before and after each intracoronary injection. Analysis of intraobserver and interobserver variability for measurement of coronary artery diameter showed high reproducibility ($r = 0.99$, SEE = 0.05 mm, $p < 0.001$; $r = 0.99$, SEE = 0.04 mm, $p < 0.001$, respectively). A 12-lead electrocardiogram (ECG) and arterial pressure were continuously monitored during the study period.

Study protocols. After measurements of heart rate and blood pressure, baseline angiography of the left main and right coronary arteries was performed. Then, incremental doses of acetylcholine were directly injected into the left main coronary artery (50 and 100 μg) and subsequently into the right coronary artery (50 μg). The method of injecting acetylcholine has been reported in detail elsewhere (1–4,24,25). Hemodynamic measurements and coronary angiography were repeated at each of the acetylcholine injections. Fifteen minutes after completion of the intracoronary injection of acetylcholine, the systemic hemodynamic variables and angiographic coronary artery diameter had returned to baseline levels, and control angiography of the left main coronary artery was performed. Thereafter, incremental doses of L-NMMA (25 and 50 μmol/min, each for 4 min) were infused into the left main coronary artery. L-NMMA infusion was performed at a rate of 2 ml/min, and measurement of systemic hemodynamic variables and coronary angiography were repeated during the last 30 s of each infusion. Subsequently, L-NMMA (50 μmol/min) was infused for an additional 5 min into the left main coronary artery, and at the last 1 min of the L-NMMA infusion, 50 μg of acetylcholine was simultaneously injected into the left main coronary artery in the same manner as before the L-NMMA infusion, and measurement of systemic hemodynamic variables and coronary angiography were performed at the end of the combined infusion of L-NMMA and acetylcholine. After an additional 15 min, an intravenous bolus injection of nitroglycerin (250 μg) was given, and 2 min thereafter coronary angiography was performed in all study patients in multiple projections. The response of the coronary artery diameter to the drugs was expressed as the percent change in the baseline coronary diameter.

Drugs. L-NMMA was obtained from Wako Chemicals (Osaka, Japan) and was dissolved in physiologic saline and then sterilely filtered before use. All drug solutions were kept at 37°C.

Statistical analysis. Data are expressed as mean value ± SE, unless otherwise indicated. For comparison of the dose–response of the coronary lumen diameters to L-NMMA between smokers and nonsmokers, two-way analysis of variance for repeated measures, followed by the Bonferroni multiple comparison test, was used. Serial changes in coronary diameters in response to L-NMMA and changes in systemic hemodynamic variables were compared using one-way analysis of variance. Differences between two mean values were compared by the paired or unpaired Student *t* test. Correlations between percent changes in coronary diameters in response to L-NMMA versus nitroglycerin and acetylcholine were examined using linear regression analysis; $p < 0.05$ was considered statistically significant.

Results

Baseline variables. As shown in Tables 1 and 2, baseline heart rate, mean blood pressure and coronary artery diameters were not significantly different between smokers and nonsmokers.

Response to acetylcholine. Acetylcholine injection at the 50-μg dose dilated only 2 (9%) of 22 distal segments of the coronary arteries (left anterior descending coronary artery plus left circumflex coronary artery) in smokers but constricted all the remaining proximal and distal coronary segments in smokers. In contrast, the 50-μg dose of acetylcholine dilated 10 (29%) of 34 proximal segments and 26 (76%) of 34 distal segments of the coronary arteries in nonsmokers and constricted the remaining coronary segments in nonsmokers. As a whole, the coronary arteries of the nonsmokers showed no significant alteration in diameter at the proximal segments but showed significant dilation at the distal segments ($p < 0.05$ vs. baseline) (Fig. 1). In contrast, the coronary arteries of smokers showed significant constriction at both segments (both $p <$

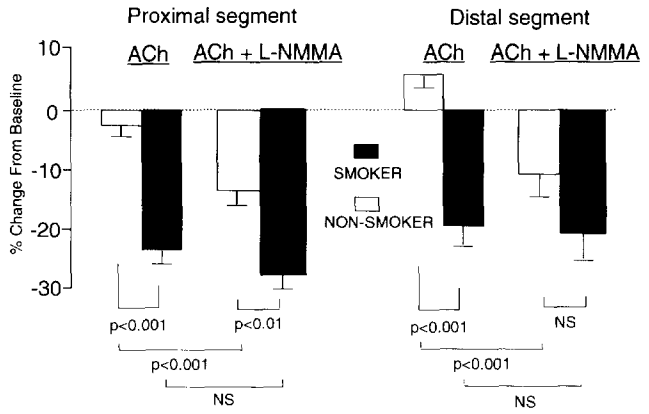


Figure 1. Percent changes (mean ± SE) in lumen diameters from baseline values in proximal and distal segments in response to intracoronary injection of acetylcholine (ACh) alone (50 μg) and combined infusion of acetylcholine (50 μg) and L-NMMA (50 μmol/min over 4 min) in coronary arteries of smokers and nonsmokers.

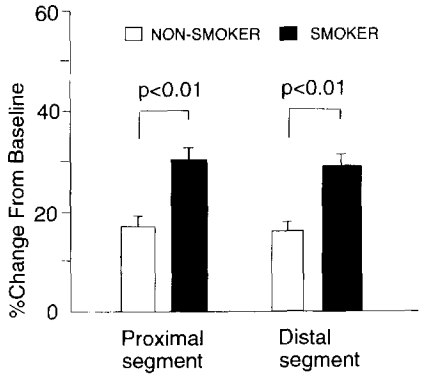
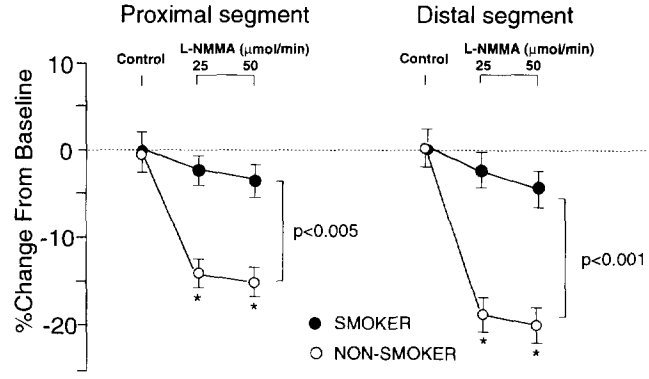


Figure 3. Percent change (mean ± SEM) from baseline values in coronary artery lumen diameters at proximal and distal segments in response to nitroglycerin in smokers and nonsmokers.

0.01 vs. respective baseline values), as shown in Figure 1. Figure 1 shows a significantly greater constriction of the coronary arteries in response to acetylcholine injection either at the proximal or distal segments in smokers than in nonsmokers. In any coronary arteries of the study subjects, acetylcholine injection up to 100 μg did not induce coronary spasm, defined as total or subtotal occlusion of the epicardial coronary arteries associated with signs of myocardial ischemia, such as chest pain and ischemic ST segment changes on the ECG.

Response of coronary diameter to L-NMMA. L-NMMA infusion decreased the basal coronary diameter in both the proximal and distal segments of coronary arteries in nonsmokers (Fig. 2). In contrast, L-NMMA infusion showed minimal effect on the basal coronary diameter either at the proximal or distal segments in smokers. The simultaneous infusion of L-NMMA with acetylcholine converted the dilator response to 50 μg of acetylcholine to constriction in 30 (83%) of the 36 coronary segments and enhanced the constrictor response to acetylcholine in the remaining 32 coronary segments in nonsmokers (Fig. 1). In contrast, the simultaneous infusion of L-NMMA had no significant effect on the constrictor response to 50 μg of acetylcholine in the coronary arteries either at the proximal or distal segments in smokers. However, the constrictor response of the coronary artery diameter to acetylcholine injection with L-NMMA was still much greater at the proximal segments in smokers than in nonsmokers (Fig. 1). The intracoronary infusion of L-NMMA showed no appreciable effects on heart rate and mean blood pressure in both groups (Table 2).

Figure 2. Percent changes (mean ± SE) in lumen diameters from baseline values after intracoronary injection of L-NMMA at rest in coronary arteries of smokers and nonsmokers. The p values for comparison of the two curves by two-way analysis of variance for repeated measures are shown. Control = change from baseline values in coronary diameters just before L-NMMA infusion. *p < 0.01 versus respective control values.



Response to nitroglycerin. Nitroglycerin increased the coronary diameter of all segments in both smokers and nonsmokers. The percent increase in coronary diameter after nitroglycerin was significantly greater in smokers at both segments than in nonsmokers (Fig. 3).

Correlation between percent change in coronary lumen diameter in response to L-NMMA at rest versus nitroglycerin and acetylcholine. There was a significant positive correlation between the percent change in coronary diameters from baseline in response to L-NMMA at rest versus nitroglycerin in smokers and nonsmokers (Fig. 4). Also, there was a significant inverse correlation between the percent change in coronary diameters from baseline in response to L-NMMA at rest versus acetylcholine in both groups (Fig. 5). These effects indicate that the dilator response of the coronary artery to nitroglycerin and the constrictor response to acetylcholine are both related to the endogenous release of nitric oxide from the artery in smokers and nonsmokers.

Discussion

The present study showed that the coronary artery diameter response to L-NMMA infusion at rest and at intracoronary injection of acetylcholine was reduced in cigarette smokers compared with nonsmokers, and the vasodilator response to nitroglycerin was enhanced in cigarette smokers as compared

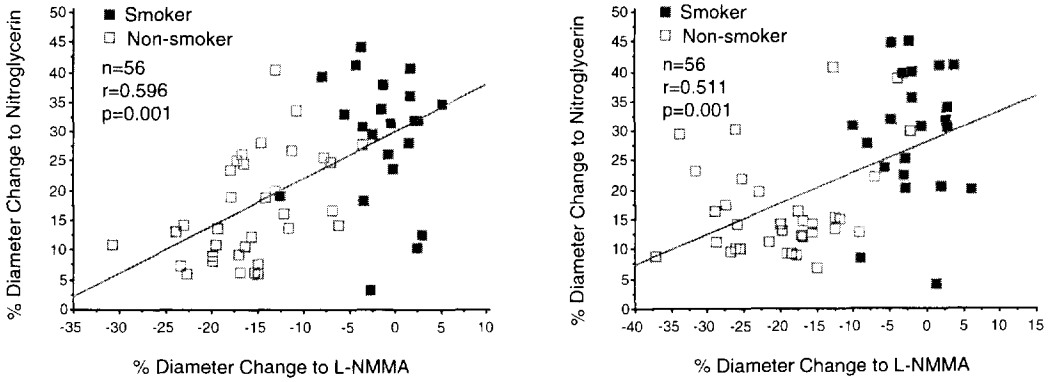
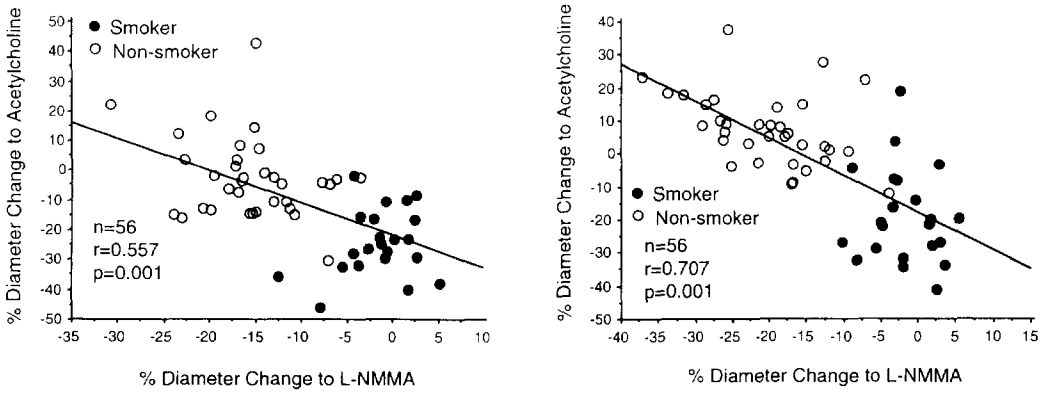


Figure 4. Correlation between percent changes from baseline values in lumen diameters in response to 25 μmol/min of L-NMMA at rest versus nitroglycerin in the proximal (**left**) and distal segments (**right**) of coronary arteries of smokers and nonsmokers.

with nonsmokers. Furthermore, the present results showed that there was a significant positive correlation between percent changes in coronary diameter from baseline in response to L-NMMA at rest versus nitroglycerin. These results indicate that the basal and stimulated release of nitric oxide was decreased, leading to supersensitivity of the coronary arteries to the vasodilator effect of exogenous nitric oxide in smokers. Acetylcholine did not dilate but constricted both segments of coronary arteries in most smokers, whereas it dilated one-third of the proximal and most of the distal segments and constricted the remaining coronary segments in nonsmokers, with a lower magnitude of constriction than in smokers. Furthermore, the present results showed that there was a significant inverse correlation between diameter responses to acetylcholine and to L-NMMA. Thus, the enhanced constrictor response to acetylcholine in smokers may be at least partly explained by decreased nitric oxide bioactivity during acetylcholine stimulation. The supersensitivity of smooth muscle to the constrictor effect of acetylcholine and the reduced release of other endothelium-derived relaxing factors, such as hyperpolarizing factor and prostacyclin, may also contribute to the enhanced constrictor response to acetylcholine in smokers because the constrictor response of the proximal coronary segments to acetylcholine with L-NMMA was still greater in smokers than in nonsmokers. It is also possible that increases of endogenous vasoconstrictors, such as endothelins or endothelium-dependent constricting factors, may participate in the alteration of the coronary reactivity.

Previous studies. Recent reports (12,40) have shown that flow-mediated endothelium-dependent dilation of coronary and peripheral arteries was impaired in smokers, a phenomenon that is compatible with our results; however, one report (12) showed that the dilator response of the coronary arteries to nitroglycerin was decreased in smokers, which contrasts with the present results. However, most of the cigarette smokers studied in that report (12) had hypercholesterolemia (mean serum cholesterol level 231 ± 23 mg/dl) and coronary lumen irregularities, whereas all the smokers in the present study had normocholesterolemia (182 ± 22 mg/dl) and normal coronary angiographic findings. Therefore, it is possible that atheroscle-

Figure 5. Correlation between percent changes from baseline values in coronary lumen diameters in response to 25 μmol/min of L-NMMA at rest versus acetylcholine (50 μg) in proximal (**left**) and distal segments (**right**) of coronary arteries in smokers and nonsmokers.



rotic changes in coronary arteries might be highly developed in the smokers in the previous report compared with those in the present study. The possible development of coronary atherosclerosis in the previous report may have caused a lack of supersensitive dilator response to nitroglycerin because the dilator response to nitroglycerin is reported (41) to be also impaired in atherosclerotic coronary arteries in humans in vivo. Furthermore, cigarette smoke is known to contain various chemicals, such as nicotine, nitric oxide-derived free radicals and other large amounts of oxygen free radicals (34–36), that directly or indirectly cause coronary constriction and dilation (11,37,38). These acute effects of cigarette smoking on coronary artery tone may complicate the interpretation of the intrinsic integrity of coronary endothelium and smooth muscle functions. In the present study, we aimed to determine the intrinsic integrity of coronary vascular functions so that smoking was ceased >2 days before the study in the smokers to avoid the possible influence of the vasoactive substances contained in cigarette smoke. However, it remains unknown whether abrupt withdrawal may affect coronary reactivity.

Cigarette smoking as a risk factor for coronary artery disease and coronary artery spasm. Several studies have identified cigarette smoking as a primary risk factor for coronary artery disease and atherosclerosis (5–10). Because nitric oxide has been shown (42,43) to inhibit the several processes involved in the development of the atherosclerosis, cigarette smoking could promote the atherosclerotic process by decrease in nitric oxide bioactivity, as shown in the present study. However, it is unknown why the prevalence of coronary artery disease is low in the Japanese population where cigarette smoking is highly prevalent compared with the U.S. population (5,6,8,9,29–31). It is well known that hypercholesterolemia acts synergistically with cigarette smoking to increase the risk of coronary artery disease (44,45). It is thus possible that low rate of hypercholesterolemia in the Japanese population may result in a lower prevalence of coronary artery disease in Japanese smokers compared with smokers in the U.S. population (6,8,9,30,31). Cigarette smoking is also a risk factor for coronary spastic angina in Japanese patients and white premenopausal women (32,33). The precise mechanisms by which coronary spasm occurs remain unclear, but the present results indicate the possibility that the deficiency in nitric oxide bioactivity associated with cigarette smoking may contribute to the genesis of coronary spasm.

Possible mechanisms. The precise mechanism of the suppressive effect of cigarette smoking on nitric oxide bioactivity also remained undetermined in the present study. Several basic experiments have shown that tobacco smoke is capable of inducing endothelial toxicity (46,47). We and others have demonstrated (11,34–37,48,49) that free radicals such as superoxide anions, contained in cigarette smoke, degrade nitric oxide released from endothelium and impair endothelial function. In fact, natural antioxidants such as vitamins E and C and beta-carotene, present in the plasma, are reported (50,51) to be decreased in smokers. Therefore, it should be determined whether the intake of these antioxidants improves nitric oxide bioactivity in the coronary arteries of cigarette smokers.

Summary. The present study shows that 1) L-NMMA decreased the basal diameter of coronary arteries in nonsmokers, whereas it had minimal effect on the basal diameter in smokers; 2) acetylcholine dilated one-third of proximal segments and most of distal segments of coronary arteries in nonsmokers, whereas it constricted most of the proximal and distal segments in smokers; 3) L-NMMA abolished the dilator response to acetylcholine and enhanced the constrictor response to acetylcholine in coronary arteries of nonsmokers, whereas it had minimal effect on the constrictor response to acetylcholine in the arteries of smokers; 4) the dilator response to nitroglycerin was significantly increased in the arteries of smokers compared with in those of nonsmokers; 5) the constrictor response to L-NMMA at rest was significantly correlated with the dilator response to nitroglycerin and with the diameter changes in response to acetylcholine in both smokers and nonsmokers.

Conclusions. There is a deficiency in both basal and stimulated nitric oxide bioactivity in the coronary arteries of cigarette smokers, leading to the supersensitivity of the arteries to the dilator effect of nitroglycerin as well as the constrictor effect of acetylcholine. Thus, cigarette smoking causes the impairment of nitric oxide-mediated regulation of coronary artery tone.

References

1. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomoter response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–7.
2. Zeiher AM, Drexler H, Saurbrier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. J Clin Invest 1993;92:652–62.
3. Yasue H, Matsuyama K, Matsuyama K, Okumura K, Morikami Y, Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment; possible role of early coronary atherosclerosis. Circulation 1990;81:482–90.
4. Quyyumi AA, Dakak N, Andrews NP, et al. Nitric oxide activity in the human coronary circulation: impact of risk factors for coronary atherosclerosis. J Clin Invest 1995;95:1747–55.
5. Wolf PA, D'Agostino RB, Kannel WB, Bonita R, Belanger AJ. Cigarette smoking as a risk factor for stroke: the Framingham Study. JAMA 1988;259: 1025–9.
6. Wilson PWF, Christiansen JC, Anderson KM, Kannel WB. Impact of national guidelines for cholesterol risk factor screening: the Framingham Offspring Study. JAMA 1989;262:41–4.
7. Jonas MA, Oates JA, Ockene JK, Hennekens CH. Statement on smoking and cardiovascular disease for health care professionals. Circulation 1992; 86:1664–9.
8. Konishi M, Iida M, Naito Y, et al. The trend of coronary heart disease and its risk factors based on epidemiological investigations. Jpn Circ J 1987;51: 319–24.
9. Dawber TR. The Framingham Study: The Epidemiology of Atherosclerotic Disease. Cambridge (MA): Harvard University Press, 1980:172–89.
10. Tell GS, Polak JF, Ward BJ, Kittner SJ, Savage PJ, Robbins J, the Cardiovascular Health Study (CHS) Collaborative Research Group. Relation of smoking with carotid artery wall thickness and stenosis in older adults: the Cardiovascular Health Study. Circulation 1994;90:2905–8.
11. Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Yasue H. Cigarette smoke extract contracts isolated porcine coronary arteries by superoxide anion-mediated degradation of EDRF. Am J Physiol 1994;266:H874–80.

12. Zeiher AM, Schächinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. Circulation 1995;92:1094–100.
13. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109–42.
14. Nishida K, Harrison DG, Navas JP, et al. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest 1992;90:2092–6.
15. Lamas S, Marsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci USA 1992;89:6348–52.
16. Chu A, Chambers DE, Lin C, et al. Effects of inhibition of nitric oxide formation on basal vasomotion and endothelium-dependent responses of the coronary arteries in awake dogs. J Clin Invest 1991;87:1964–8.
17. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989;2:997–1000.
18. Lefroy DC, Crake T, Uren NG, Davies GJ, Maseri A. Effect of inhibition of nitric oxide synthesis on epicardial coronary artery caliber and coronary blood flow in humans. Circulation 1993;88:43–54.
19. Moncada S, Rees DD, Schulz R, Palmer RMJ. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. Proc Natl Acad Sci USA 1991;88:2166–70.
20. Fuchgott RF. Role of endothelium in response of vascular smooth muscle. Circ Res 1983;53:557–73.
21. Ignarro LJ. Biological action and properties of endothelium-derived nitric oxide formed and released from artery and vein. Circ Res 1989;65:1–21.
22. Vanhoutte PM. The endothelium. Modulator of vascular smooth muscle tone. N Engl J Med 1988;319:512–3.
23. Bassenge E. Coronary vasomotor responses: role of endothelium and nitrovasodilators. Cardiovasc Drugs Ther 1994;8:600–12.
24. Yasue H, Horio Y, Kugiyama K, et al. Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. Circulation 1986;74:955–63.
25. Okumura K, Yasue H, Matsuyama K, et al. Effect of H_1 receptor stimulation on coronary artery diameter in patients with variant angina: comparison with effect of acetylcholine. J Am Coll Cardiol 1991;17:338–45.
26. Bossaller C, Habib GB, Yamamoto H, Williams C, Wells S, Henry PD. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5′-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. J Clin Invest 1987;79:170–4.
27. Maseri A, Davies G, Hackett D, Kaski JC. Coronary artery spasm and vasoconstriction. the case for a distinction. Circulation 1990;81:1983–91.
28. Quyyumi AA, Dakak N, Andrews NP, Gilligan DM, Panza JA, Cannon RO III. Contribution of nitric oxide to metabolic coronary vasodilation in the human heart. Circulation 1995;92:320–6.
29. Shimao T. Smoking and its control in Japan. Asian Med J 1988;31:195–200.
30. Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the seven countries study. Am J Epidemiol 1986;124:903–15.
31. Shimamoto T, Komachi Y, Inada H, et al. Trends for coronary heart disease and stroke and their risk factors in Japan. Circulation 1989;79:503–15.
32. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. Circulation 1993;87:76–9.
33. Caralis DG, Deligonul U, Kern MJ, Cohen JD. Smoking is a risk factor for coronary spasm in young women. Circulation 1992;85:905–9.
34. Hoffman D, Hecht SS, Ornaf RM, Wynder EL, Tso TC. Chemical studies on tobacco smoke. XLII. Nitrosonornicotine: presence in tobacco, formation and carcinogenicity. IARC Sci Publ 1976;14:307–20.
35. Bokhoven C, Niessen HJ. Amounts of oxides of nitrogen and carbon monoxide in cigarette smoke, with and without inhalation. Nature 1961;192:458–9.
36. Nakayama T, Kodama M. Generation of hydrogen peroxide and superoxide anion radical from cigarette smoke. Gann 1984;75:95–8.
37. Gruetter CA, Barry BK, Mcnamara DB, Kadowitz PJ, Ignarro LJ. Coronary arterial relaxation and guanylate cyclase activation by cigarette smoke, N′-nitrosonornicotine and nitric oxide. J Pharmacol Exp Ther 1980;214:9–15.
38. Winniford MD, Wheelan KR, Kremers MS, et al. Smoking-induced coronary vasoconstriction in patients with atherosclerotic coronary artery disease: evidence for adrenergically mediated alterations in coronary artery tone. Circulation 1986;73:662–7.
39. Nichols AB, Gabrieli CFO, Fenoglio JJ, Esser PD. Quantification of relative coronary arterial stenosis by videodensitometric analysis of the coronary angiograms. Circulation 1984;69:512–22.
40. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 1993;88(Pt 1):2149–55.
41. Schächinger V, Zeiher AM. Quantitative assessment of coronary vasoreactivity in humans in vivo. Importance of baseline vasomoter tone in atherosclerosis. Circulation 1995;92:2087–94.
42. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 1989;83:1774–7.
43. Cayatte AJ, Palacino JJ, Horten K, Cohen RA. Chronic inhibition of nitric oxide production accelerates neointima fromation and impairs endothelial function in hyperchlesterolemic rabbits. Arteriosclerosis Thrombosis 1994;14:753–9.
44. Willett WC, Green A, Stampfer MJ, et al. Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. N Engl J Med 1987;317:1303–9.
45. The Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: Final report of the pooling project. J Chronic Dis 1978;31:201–306.
46. Asmussen G, Kjeldsen K. Intimal ultrastructure of human umbilical arteries: observations on arteries from newborn children of smoking and nonsmoking mothers. Circ Res 1975;36:579–89.
47. Reinders JH, Brinkman HJ, van Mourik JA, de Groot PG. Cigarette smoke impairs endothelial cell prostacyclin production. Arteriosclerosis 1986;6:15–23.
48. Murohara T, Kugiyama K, Sugiyama S, Ohgushi M, Yasue H. Activated human polymorphonuclear leukocytes elicit endothelium-dependent contraction in isolated pig coronary arteries. J Cardiovasc Pharmacol. 1993;21:760–6.
49. White CR, Brock TA, Chang LY, et al. Superoxide and peroxynitrite in atherosclerosis. Proc Natl Acad Sci USA. 1994;91:1044–8.
50. Duthie GG, Wahle KWJ, James WPT. Oxidants, antioxidants and cardiovascular disease. Nutr Res Rev 1989;2:51–62.
51. Duthie GG, Wahle KWJ. Smoking, antioxidants essential fatty acids and coronary heart disease. Biochem Soc Trans 1990;18:1051–4.